RESEARCH PAPER

Combinatorial Antioxidant, Cytoprotective, and Anti-Amyloid Activities of *Benkara Malabarica* and *Vicoa Indica* Ethanolic Extracts in Alzheimer's Disease

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ABSTRACT

The escalating global prevalence of Alzheimer's disease (AD) highlights the need for multi-target therapeutics capable of addressing its complex pathology. Traditional knowledge from Kerala attributes memory-enhancing properties to *Benkara malabarica* and *Vicoa indica*, yet their neuroprotective effects remain insufficiently validated. This study examined the phytochemical profiles and anti-Alzheimer's activities of their ethanolic extracts (ET-BM and ET-VI). Both extracts were rich in polyphenols, flavonoids, and other redox-active metabolites and exhibited strong antioxidant activity, as demonstrated by potent DPPH radical scavenging and elevated total antioxidant capacity.ET-BM and ET-VI also showed significant membrane-stabilising effects in RBC hemolysis assays, suggesting protection against peroxidative membrane damage. In SH-SY5Y neuronal cells, both extracts effectively attenuated $A\beta_{1-42}$-induced cytotoxicity and reduced malondialdehyde and nitric oxide levels, indicating mitigation of oxidative and nitrosative stress. These effects are consistent with known mechanisms of plant-derived antioxidants that preserve mitochondrial integrity and suppress inflammatory signalling. Overall, the extracts exhibited a multitarget protective profile of antioxidant, anti-inflammatory, cytoprotective, and anti-amyloidogenic, well aligned with therapeutic priorities in AD. The findings support the traditional medicinal use of *Benkara malabarica* and *Vicoa indica and* highlight their promise as candidates for further preclinical evaluation in Alzheimer's disease management.

Keywords: *Benkara Malabarica, Vicoa Indica*, Alzheimer's Disease, Neuroprotection, Phytochemicals, Cytotoxicity, Antioxidant Assay.

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INTRODUCTION

The exploration of natural sources for treating diseases dates back to ancient civilizations, with plants serving as primary therapeutic agents until the rise of modern medicine[1]. Over the past two decades, the use of herbal medicines and nutraceuticals phytochemical-rich functional foods that promote long-term health has expanded dramatically worldwide, as noted by the WHO[2]. These benefits mainly come from secondary metabolites produced by plants. Neuroprotection involves widely studied strategies aimed at safeguarding the central nervous system from both acute and chronic neurodegenerative insults, such as those seen in Alzheimer's disease (AD)[3]. Neurodegenerative disorders lead to progressive deficits in cognition, behavior, and motor function, resulting in irreversible neuronal loss. Among these, dementia most notably AD poses a significant global health challenge for the aging population[4]. Alzheimer's disease (AD) is a currently incurable neurodegenerative condition common in the elderly. According to the Alzheimer's Association, 13% of people over the age of 65 in developed countries suffer from this disease, which is the fifth leading cause of death in patients of this age. According to the World Health Organization (WHO), projections indicate that the global prevalence will quadruple in the coming decades, reaching 114 million patients by 2050[5]. AD is characterized by memory and cognitive decline, with complex causes including genetic

changes, amyloid beta and tau pathology, neurotrophic factor deficiency, mitochondrial dysfunction, and oxidative stress[6]. The hippocampus, a key site of neurodegeneration, is closely linked to these processes. Despite some therapeutic advances, such as cholinesterase inhibitors and NMDA antagonists, there remains no cure for AD. There is a pressing need for more effective treatments, which, however, do not prevent the underlying decline and death of brain cells. Conversely, herbal remedies are encouraged due to their specialized cholinesterase inhibitory activity and non-specific antioxidant and anti-inflammatory effects[7]. Plant-derived compounds have shown promise in alleviating AD symptoms, but clinical responses vary, emphasizing the ongoing need for new drug development. The plants *Benkara malabarica* and *Vicoa indica* have been widely reported to be used by the tribal healers in Kerala for memory-enhancing properties in many ethnobotanical studies[8]. These indigenous medicines have never been fully tested scientifically to prove and exploit their medicinal effects on brain cells remains a research gap.

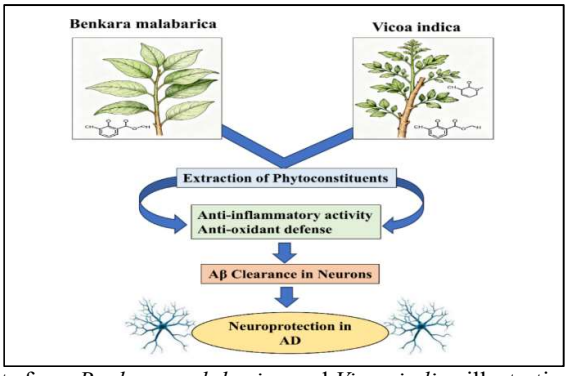

Figure 1: Phytoconstituents from *Benkara malabarica* and *Vicoa indica* illustrating potential mechanisms in Alzheimer's disease, including modulation of inflammation, enhancement of amyloid-β clearance, and neuroprotection in Alzheimer's disease

In light of the aforementioned, the current study was carried out to find naturally occurring substances that could be used as alternative treatment methods in the management and reversal of symptoms and conditions related to AD[9]. To comprehensively control the death of brain nerve cells, naturally occurring substances and compounds must be identified. Preliminary *in vitro* studies on brain cells were conducted to support the herbalist's reports[10]. The study will begin with the selection of plants based on ethnobotanical information and existing scientific literature, followed by the collection and authentication of *Benkara malabarica* and *Vicoa indica*. The collected plant materials will then undergo extraction using solvents of increasing polarity, and the resulting extracts will be subjected to phytochemical screening through both qualitative and quantitative analyses. After establishing the safety profile, the extracts will be evaluated for their anti-Alzheimer's potential using *in vitro* experimental models. The findings of this study may help identify promising herbal therapeutic candidates for Alzheimer's disease.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

The aerial parts of *Benkara malabarica* (Lam.) Tirveng. and the whole plant of *Vicoa indica* (L.) DC. were collected from the Palakkad region, Kerala. Taxonomic authentication was performed by expert botanists at Ottapalam. The plant materials were shade-dried to preserve thermolabile constituents, pulverized using a mechanical grinder, and sieved through a 40-mesh screen to obtain uniform powder for extraction[11].

Preparation of Extracts and determination of percentage yield

Dried plant powders were subjected to sequential Soxhlet extraction using solvents of increasing polarity: petroleum ether, acetone, ethyl acetate, ethanol, and water, to ensure comprehensive recovery of phytoconstituents. For each solvent, continuous refluxing was performed until exhaustive extraction. Solvents were removed under reduced pressure to obtain crude extracts for further analysis. The extraction yield was determined by calculating the percentage of crude extract obtained relative to the initial weight of the dried plant material. It was expressed using the formula: Percentage yield = (Weight of extract / Weight of dried plant material) × 100[12].

Preliminary Phytochemical Screening

Qualitative phytochemical analysis of the extracts was performed using standard protocols to detect major classes of secondary metabolites, including alkaloids, carbohydrates, glycosides, phytosterols, coumarins, flavonoids, tannins, phenolics, proteins, amino acids, saponins, and fixed oils depicted in Table.1.

Table 1: Preliminary Phytochemical Screening Was Performed Using Standard Qualitative Tests to Identify Major Secondary Metabolites

Secondary Metabolites	Tests
Alkaloids	Dragendorff's, Wagner's, Mayer's, and Hager's tests
Carbohydrates	Molisch's, Fehling's, and Benedict's tests
Glycosides	Legal, Baljet, Borntrager's, and Keller–Killiani tests
Phytosterols	Liebermann–Burchard and Salkowski tests
Coumarins	UV fluorescence after alkaline treatment
Flavonoids	Shinoda test
Tannins/Phenolics	Ferric chloride and gelatin tests
Protein/Amino acids	Biuret, Millon's, and ninhydrin tests
Saponins	Foam test
Fixed oils	Spot test and saponification test

Tests for phytochemical screening were performed according to standard protocols as described in references [13–22].

All tests were interpreted based on characteristic color formation or precipitate development indicative of the corresponding phytochemical class[23,24].

***In Vitro* Antioxidant and Cytoprotective Evaluation**

In vitro antioxidant activity of the extracts *Benkara malabarica* and *Vicoa indica* will be evaluated using free radical scavenging assays, reducing power estimation, and lipid peroxidation inhibition methods to determine their ability to neutralise oxidative species[25]. Cytoprotective effects will be assessed in neuronal cell lines exposed to oxidative stress, examining cell viability and intracellular ROS levels. Together, these assays will elucidate the extract's potential to mitigate oxidative damage, a key pathological feature in neurodegenerative conditions such as Alzheimer's disease.

RBC Hemolysis Assay

Anti-inflammatory activity was assessed using the RBC hemolysis assay. Fresh chicken blood was collected in EDTA tubes, centrifuged at 3000 rpm for 10 min, and the packed RBCs were washed thrice with 0.9% saline. A 10% RBC suspension was prepared and exposed to test samples, aspirin (standard), or saline (control)[26]. All mixtures were incubated at 56 °C for 30 min, and hemolysis was quantified by measuring absorbance at 620 nm. Percent protection was calculated as:

$$\%\text{Protection} = 100 - \left(\frac{\text{Abs}_{\text{test}}}{\text{Abs}_{\text{control}}} \times 100\right)$$

Total Antioxidant Capacity (TAC) Assay

TAC was determined using the phosphomolybdenum method. Samples (100 µL) were mixed with TAC reagent (sulfuric acid, sodium sulphate, and ammonium molybdate) and incubated before measuring absorbance at 695 nm. Antioxidant activity was expressed as ascorbic acid equivalents (AAE)[27]. Fold increase and percent activity were calculated using:

$$\text{Fold Increase} = \frac{\text{TAC}_{\text{test}}}{\text{TAC}_{\text{control}}}$$

$$\%\text{Activity} = \frac{\text{TAC}_{\text{test}}}{\text{TAC}_{\text{standard}}} \times 100$$

DPPH Radical Scavenging Assay

Free radical scavenging activity of the extracts was assessed using a 0.1 mM DPPH assay. Serial dilutions of the extracts and ascorbic acid (standard) were mixed with DPPH solution and incubated in the dark for 30 minutes, after which absorbance was recorded at 517 nm[28].

The percentage inhibition of DPPH radicals was calculated using the formula:

$$\%\text{DPPH Scavenging} = \frac{A_{\text{control}} - A_{\text{test}}}{A_{\text{control}}} \times 100$$

Anticholinesterase Inhibition Assay

The acetylcholinesterase (AChE) inhibition assay was performed using a modified Ellman's colorimetric method, in which acetylthiocholine iodide (ATCI) is hydrolyzed by AChE to produce thiocholine that reacts with 5,5-dithiobis-2-nitrobenzoate (DTNB) to form a yellow chromophore measured at 415 nm.

$$\%\text{ Inhibition } = \frac{A\ control - A\ test}{A\ control}\ X\ 100$$

Cytotoxicity Assessment by MTT Assay

Human neuroblastoma SH-SY5Y cells (NCCS, Pune) were cultured in DMEM supplemented with 10% FBS and antibiotics at 37 °C in 5% CO_2. Cells (5×10^4/well) were seeded in 96-well plates, pre-exposed to β-amyloid to induce toxicity, and treated with serial dilutions of extracts. After 24 h, MTT solution was added and incubated for 4 h; resulting formazan crystals were solubilized with DMSO. Absorbance was measured at 540 nm[29]. Cell viability was calculated as:

$$\%\text{Viability} = \frac{\text{OD}_{\text{test}}}{\text{OD}_{\text{control}}} \times 100$$

IC_{50} values were determined by plotting concentration–response curves, where the percentage inhibition at each tested dose was fitted to a nonlinear regression model. The extract concentration required to produce 50% inhibition

(IC_{50}) was then extrapolated from the curve, providing a quantitative measure of antioxidant potency.

Cell Culture and Treatment

Human neuroblastoma SH-SY5Y cells were cultured in DMEM/F12 medium. This medium was enriched with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin. The cells were maintained at 37°C in a humidified atmosphere containing 5% CO_2 atmosphere. For all subsequent experiments, cells were initially plated at the required densities and then treated according to specific protocols devised for the experimental design[30].

Preparation of Cell Lysates

After the treatment period, culture medium was aspirated and cells were washed with ice-cold PBS. The cells were after scraped and harvested then pelleted by centrifugation at 5000 rpm for 5 min at 4°C. The resulting pellets were resuspended in 2 ml of lysis buffer containing: 10 mM Tris-HCl (pH 7.5), 50 mM NaCl, 1% Triton X-100, 5 mM EDTA, 50 mM NaF, 100 µM Na_3VO_4, 1 mM PMSF, 10 µg/ml leupeptin, and 10 µg/ml aprotinin. The suspension was incubated on ice for 30 min and clarified by centrifugation at 12,000 rpm for 20 min at 4°C. Supernatants (cell lysates) were collected and stored at −20°C for the later analysis[31].

Lipid Peroxidation (LPO)

LPO levels were determined by measuring malondialdehyde (MDA) content using the thiobarbituric acid (TBA) assay. Cell lysate (0.2 ml) was mixed with 0.8 ml saline, 0.5 ml BHT, and 3.5 ml of 0.8% TBA reagent. Samples were incubated at 60°C, cooled, and centrifuged at 2000 rpm for 10 min. Absorbance of the supernatant was measured at 532 nm[32].

Nitric Oxide (NO)

NO levels were quantified using the Griess reaction. A 0.2 ml portion of the culture medium was combined with 1.8 ml saline and 0.4 ml of 35 % sulphosalicylic acid to precipitate proteins. After centrifugation at 4000 rpm for 10 min, 1 ml of the supernatant was combined with 2 ml of Griess reagent. The mixture was incubated for 20 min in darkness, and the absorbance was subsequently recorded at 540 nm. A standard curve was generated using sodium nitrite (200 – 1000 ng) for quantitative analysis[33].

Estimation of Reduced Glutathione (GSH)

GSH levels were quantified by cell lysate (0.25 ml) was mixed with equal volume of ice-cold 5% TCA and followed by centrifugation at 4000 rpm for 10 mi. To 1 ml of the resulting supernatant, 0.25 ml of 0.2 M phosphate buffer (pH 8.0) and 0.5 ml of DTNB (0.6 mM) were added. Absorbance was recorded at 412 nm using a UV spectrophotometer[34].

Superoxide Dismutase (SOD) Activity

SOD activity was determined using a specific reaction mixture. This mixture contained 0.3 ml sodium pyrophosphate buffer (0.025 M, pH 8.3) and 0.05 ml cell lysate. PMS (0.025 ml; 186 µM) and NBT (0.075 ml; 300 µM) were added, and the reaction was initiated by adding NADH (0.075 ml). After incubation at 30°C for 90 s, the reaction was terminated with 0.25 ml glacial acetic acid. The final mixture was extracted with 2 ml n-butanol Following centrifuged at 4000 rpm for 1 min, and absorbance was read at 560 nm against an n-butanol blank[35].

Catalase (CAT) Activity

The enzymatic activity of Catalase (CAT) was measured. A 100 µl aliquot of cell lysate was incubated with 2.25 ml of 65 mM potassium phosphate buffer (pH 7.8) for 30 min at 25 °C. Hydrogen peroxide (650 µl of 7.5 mM) was then added to initiate the reaction. The resulting decrease in absorbance, indicative of H_2O_2 breakdown, was tracked for 2–3 min at 240 nm using a UV spectrophotometer[36].

Protein Estimation

Total protein content within the cell lysates was quantified by the established Lowry method. This procedure relies on the interaction of proteins with alkaline copper tartrate, followed by the subsequent reduction of the Folin–Ciocalteu reagent, which generates a measurable blue chromophore. Spectrophotometric measurement of the colour intensity was performed at 660 nm. Bovine serum albumin (BSA) was used as the reference to create the standard calibration curve. All enzyme activity results were standardized and reported as units per mg of protein[37].

Estimation of Pro-inflammatory Cytokines (IL-1β and TNF-α)

IL-1β and TNF-α levels were quantified in cell lysates using commercial ELISA kits (Invitrogen, R&D Systems, and Alpha Diagnostics, USA) as per the manufacturer's instructions. Briefly, standards and samples (50 µl sample + 100 µl distilled water) were dispensed in duplicate wells and incubated for 3h at room temperature. Wells were washed three times with wash buffer before adding 100 µl TMB substrate and incubating for 15 min. The reaction was stopped with 100 µl stop solution, and absorbance was measured at 410 nm using an ELISA reader[38].

Statistical Analysis

All data gathered from the experiments were expressed as the mean ± SEM. Statistical comparisons between the different treatment groups were carried out using one-way ANOVA, followed by Tukey's post-hoc test to identify specific significant differences. The IC_{50} values were derived by fitting the percentage inhibition data to a non-linear regression model of the dose–response curves. A probability value (p-value) of less than

0.05 ($p < 0.05$) was considered to be statistically significant[39].

Instruments and Equipment

All experimental procedures were carried out using standard laboratory instruments. Plant extraction was performed using a Soxhlet apparatus (Borosil®, India) with a heating mantle (Remi, India), and extracts were concentrated using a rotary vacuum evaporator (Rotavapor® R-300, BUCHI, Switzerland) coupled with a vacuum pump (V-300, BUCHI). Dried samples were processed using a mechanical grinder (Cyclotec™ 1093, Foss, Denmark) and weighed using an analytical balance (Entris® II, Sartorius, Germany). Phytochemical screening and biochemical estimations were conducted using a UV–Visible spectrophotometer (Shimadzu UV-1900i/UV-2600, Japan), pH meter (Mettler Toledo, Switzerland), centrifuge (Remi R-8C, India), and water bath (Memmert, Germany). Microplate-based assays, including DPPH, TAC, AChE inhibition, and RBC hemolysis, were carried out using a multimode microplate reader (SpectraMax® i3x, Molecular Devices, USA) and ELISA reader (BioTek® ELx800, Agilent, USA). Cell culture and MTT assays were performed in a CO_2 incubator (Heracell™ VIOS 160i, Thermo Fisher Scientific, USA) within a Class II biosafety cabinet (NuAire, USA), and observed under an inverted microscope (Olympus CKX53, Japan). Enzymatic assays (SOD, CAT, GSH, LPO, NO) were conducted using refrigerated centrifuges (Eppendorf, Germany) and standard spectrophotometric methods. Cytokine estimation was performed using an ELISA plate reader (Multiskan™ FC, Thermo Fisher Scientific, USA). All instruments were calibrated prior to use according to manufacturer instructions.

RESULTS

Preparation of Extracts and the Calculation of Percentage Yield

The powdered aerial parts of *Benkara malabarica* and whole plant of *Vicoa indica* were subjected to sequential extraction using solvents of increasing polarity-petroleum ether, acetone, ethyl acetate, ethanol, and water. Each extraction was carried out by using Soxhlet apparatus until exhaustive extraction was achieved. The resulting extracts were filtered and concentrated under reduced pressure using a rotary evaporator, followed by drying in a vacuum desiccator to obtain dry extracts.

The percentage yield of each extract was calculated to evaluate the efficiency of the extraction process and solvent effectiveness. The extracts were stored in screw cap vials until further use. The percentage yields of *B.malabarica* and *V.indica* were shown in table 2 and 3

The extraction yields of different solvents from the aerial parts of *Benkara malabarica* and the whole plant of *Vicoa indica* were investigated using continuous hot percolation. Both plants demonstrated solvent-dependent variations in extractable components, reflecting the complexity and polarity range of their phytoconstituents.

In both species, petroleum ether yielded the lowest quantity (3.02 g in *Benkara malabarica*, 4.13 g in *Vicoa indica*), consistent with its non-polar nature extracting fewer non-polar phytochemicals. Intermediate polarity solvents like acetone and ethyl acetate provided moderate yields-13.55 g and 17.85 g for *Benkara malabarica*, and 15.24 g and 17.37 g for *Vicoa indica*, indicating efficient extraction of moderately polar compounds such as flavonoids, terpenoids, and some alkaloids.

Table 2: Percentage Yield of the Aerial Parts of *B.malabarica*

Plant Name	Parts Used	Method of Extraction	Solvent System	Yield of Extract	Percentage Yield
Benkara malabarica	Aerial parts	Continuous Hot Percolation	Pet ether	3.02 gm	3%
			Acetone	13.55 gm	13.5%
			Ethyl acetate	17.85 gm	18%
			ethanol	17.25 gm	17%
			water	37.1 gm	37%

Table 3: Percentage Yield of the Whole Plant of V. Indica

Plant Name	Parts Used	Method of Extraction	Solvent System	Yield of Extract	Percentage Yield
Vicoa indica	whole plant	Continuous Hot Percolation	Pet ether	4.13 gm	4%
			Acetone	15.24 gm	15%
			Ethyl acetate	17.37 gm	17%
			ethanol	21.77 gm	22%
			water	34.69 gm	35%

Ethanol, a polar organic solvent, gave similarly high yields in both plants (17.25 g in *Benkara malabarica*, 21.77 g in *Vicoa indica*), signifying its effectiveness in extracting polar bioactive

constituents including phenolics and glycosides. The aqueous extract produced the highest yield in both cases (37.1 g for *Benkara malabarica*, 34.69 g for *Vicoa indica*), reflecting the abundance of water-soluble phytochemicals like tannins, saponins, and polysaccharides within these plants.

The consistent trend of increasing yield with solvent polarity across both species underscores the importance of solvent selection in maximizing extract recovery from plant matrices. The continuous hot percolation method used ensured efficient solute-solvent interaction, facilitating exhaustive extraction.

These findings provide practical insights for future phytochemical and pharmacological investigations, suggesting that polar solvents such as ethanol and water are preferable for comprehensive extraction of bioactive constituents from both *Benkara malabarica* and *Vicoa indica.*

Phytochemical Screening of *Vicoa indica*

The different extracts of *Vicoa indica* were subjected to screening for its phytochemical constituents. The phytochemical screening results are presented in Table 4 Preliminary phytochemical evaluation of the petroleum ether, chloroform, ethyl acetate, ethanol and aqueous extracts of *Vicoa indica* revealed distinct differences in the distribution of secondary metabolites.

Alkaloids were absent in the non-polar extracts but appeared consistently in the moderately polar and polar extracts (EA-VI, ET-VI and AQ-VI). This pattern indicates that the alkaloidal constituents of *V. indica* possess higher solubility in solvents of intermediate to high polarity. Carbohydrates and glycosides followed a similar trend, with clear positivity in the ethyl acetate, ethanol and aqueous fractions. This suggests the presence of water-soluble carbohydrate derivatives and glycosidic linkages that partition preferentially into hydrophilic solvents.

Phytosterols were detected only in the petroleum ether and chloroform extracts, which is expected considering their lipophilic nature. Their absence in the remaining extracts confirms good specificity of the tests employed and reflects a clean solvent-based separation of sterol components. Flavonoids were predominantly found in the more polar extracts (EA-VI, ET-VI and AQ-VI), aligning with the typical solubility characteristics of polyphenolic compounds. Phenolic compounds and tannins were also strongly present in these same extracts. This is consistent with traditional phytochemical behaviour, as phenolics generally require solvents with hydrogen-bonding capability.

Proteins and amino acids were detected only in the polar extracts, which is logical due to their hydrophilic nature and insolubility in organic solvents. Saponins were identified in all extracts except the petroleum ether fraction, indicating a moderate polarity range for these constituents. Fixed oils and fats were detected solely in the petroleum ether and chloroform extracts, confirming the presence of non-polar lipids and validating the extraction efficiency of low-polarity solvents.

Overall, *Vicoa indica* exhibited a rich profile of phenolics, flavonoids, saponins and alkaloids, especially in the ethyl acetate, ethanol and aqueous fractions, suggesting these extracts may hold significant pharmacological potential.

Table 4: Phytochemical Screening of Various Extracts of *Vicoa Indica*

S. No	Chemical test	PE-VI	AC-VI	EA-VI	ET-VI	AQ-VI
1	Alkaloids	N	N	P	P	P
2	Carbohydrate & glycosides	N	N	P	P	P
3	Phytosterols	P	P	N	N	N
4	Flavonoids	N	N	P	P	P
5	Phenolic compounds and tannins	N	N	P	P	P
6	Protein and amino acid	N	P	P	P	P
7	Saponins	N	P	P	P	P
8	Fixed oil and fats	P	P	N	N	N

Screening result denoted as P for Positive (presence) and N for Negative (absence)

Phytochemical Screening of *Benkara Malabarica*

The different extracts of *Benkara malabarica* were subjected to screening for its phytochemical constituents. The phytochemical screening results are presented in Table 5 The phytochemical profile of *Benkara malabarica* displayed a pattern comparable in some respects to *V. indica*, although notable differences were also observed.

Alkaloids were absent in the petroleum ether and chloroform extracts but were clearly detected in the ethyl acetate, ethanol and aqueous extracts, reflecting a polar-oriented distribution similar to *V.*

indica. Carbohydrates and glycosides also appeared exclusively in the polar extracts, indicating the presence of hydrophilic constituents. As with *V. indica*, phytosterols were restricted to the petroleum ether and chloroform fractions, reinforcing their lipophilic nature.

In contrast to *V. indica*, flavonoids appeared in the chloroform, ethyl acetate, ethanol and aqueous fractions of *B. malabarica*, but not in the petroleum ether extract. This broader distribution suggests the presence of flavonoids with varying polarity, reflecting a more diverse flavonoid population in this plant.

Phenolic compounds and tannins were strongly present in all polar extracts, suggesting *B. malabarica* is particularly rich in polyphenolic constituents. Proteins and amino acids were detected in EA-BM, ET-BM and AQ-BM, indicating the presence of water-soluble nitrogenous metabolites. Saponins were consistently observed in every extract except the non-polar petroleum ether fraction, similar to the results obtained for *V. indica.*

Fixed oils and fats were limited to the petroleum ether and chloroform extracts, confirming the selective isolation of lipid components. The presence of phytosterols and fixed oils in the non-polar fractions validates the efficiency of sequential extraction and the integrity of the extraction methodology.

Table 5: Phytochemical Screening of Various Extracts of *Benkara Malabarica*

S. No	Chemical test	PE-BM	AC-BM	EA-BM	ET-BM	AQ-BM
1	Alkaloids	N	N	P	P	P
2	Carbohydrates and glycosides	N	N	P	P	P
3	Phytosterols	P	P	N	N	N
4	Flavonoids	N	P	P	P	P
5	Phenolic compounds and tannins	N	N	P	P	P
6	Protein and amino acid	N	N	P	P	P
7	Saponins	P	P	P	P	P
8	Fixed oil and fats	P	P	N	N	N

Screening result denoted as P for Positive (presence) and N for Negative (absence)

Across all screening, the ethanolic extract was prioritized due to its higher extraction yield, enriched polyphenolic and flavonoid content as indicated by preliminary phytochemical screening, and superior solubility of neuroactive compounds.

RBC Hemolysis Assay

The RBC hemolysis assay was performed to evaluate the membrane-stabilizing potential of the test samples. The use of chicken erythrocytes in the RBC hemolysis assay was primarily based on their ready availability, ease of collection without invasive procedures, and compatibility with established experimental protocols. Avian erythrocytes are nucleated and structurally stable, making them suitable for reproducible membrane stability assessments in preliminary screening studies. Moreover, several validated studies have successfully employed chicken RBCs for evaluating hemolytic and membrane-protective effects, supporting their reliability as a model system. In the control group, the absorbance value recorded for heat-induced hemolysis was 0.094, indicating the baseline level of erythrocyte lysis under experimental conditions. This value served as the reference point for assessing the protective effects of the treated samples. Any reduction in absorbance relative to the control was considered indicative of membrane stabilization and inhibition of hemolysis.

Table 6: Absorbance and Percentage Protection of *Benkara malabarica* and *Vicoa indica* Extracts in Human RBC Membrane Stabilization Assay

S. No	Sample	Absorbance	% Protection
Benkara malabarica			
1	AQ-BM	0.045±0.003	52.13 ± 2.4^{b}
2	PE-BM	0.085±0.006	9.57 ± 1.2^{f}

3	ET-BM	0.039±0.002	58.51±3.1[a]
4	EA-BM	0.063±0.004	32.98±2.8[d]
5	AC-BM	0.041±0.002	56.38±2.5[a]
Vicoa indica			
1	AQ-VI	0.068±0.004	27.66±1.9[e]
2	PE-VI	0.057±0.003	39.36±2.1[c]
3	ET-VI	0.050±0.005	46.81±3.4[b]
4	EA-VI	0.072±0.005	23.40±1.5[e]
5	AC-VI	0.078±0.006	17.02±1.8[f]

Protective efficacy of various solvent extracts of *Benkara malabarica* and *Vicoa indica*. Data are expressed as Mean ± SEM (n=3). Statistical significance was evaluated using a One-Way ANOVA followed by Tukey's post-hoc multiple comparison test. Values in the '% Protection' column sharing different superscript letters ([a], [b], [c], etc.) are significantly different from each other at $p < 0.05$.

Total Anti-Oxidant Capacity

The total antioxidant capacity of the samples was assessed using the phosphomolybdate reduction method. The control group exhibited a TAC value of 0.088, reflecting the basal non-enzymatic antioxidant defence under experimental conditions. In contrast, the standard antioxidant showed a markedly higher TAC value of 0.8571, demonstrating strong electron-donating and free-radical quenching ability. This substantial difference between the control and standard highlights the sensitivity of the assay and provides a reference framework for evaluating the antioxidant potential of the test samples.

Table 7: Total Antioxidant Capacity (TAC) Values, Fold Increase vs Control, and % Activity vs Standard for Extracts of *Vicoa indica* and *Benkara malabarica*.

S. No	Sample	Tac Value	Fold Increase Vs Control	% Activity Vs Standard
Vincoa indica				
1	AQ-VI	0.138	1.568	16.10
2	PE-VI	0.122	1.386	14.23
3	ET-VI	0.165	1.875	19.25
4	EA-VI	0.163	1.852	19.02
5	AC-VI	0.148	1.682	17.27
Benkara malabarica				
1	AQ-BM	0.095	1.080	11.08
2	PE-BM	0.098	1.114	11.43
3	ET-BM	0.170	1.932	19.83
4	EA-BM	0.130	1.477	15.17
5	AC-BM	0.104	1.182	12.13

ET-VI showed the highest TAC value of **0.165**, corresponding to **1.875**-fold increase over control and **19.25%** activity compared to ascorbic acid; **ET-BM** showed the highest TAC value of **0.170**, corresponding to **1.932**-fold increase over control and **19.83%** activity compared to ascorbic acid

DPPH ASSAY (2,2-Diphenyl-1-Picrylhydrazyl Assay)

The antioxidant activity of *Vicoa indica and Benkara malabarica* extract was evaluated using the DPPH free-radical scavenging assay. The extract exhibited concentration-dependent quenching of the DPPH radical, indicating its ability to donate hydrogen atoms or electrons to neutralize free radicals. The characteristic purple colour of the DPPH solution gradually decreased upon incubation with *Vicoa indica* extract, reflecting its radical scavenging potential. This behaviour demonstrates the presence of phytoconstituents capable of contributing to the overall antioxidant capacity of the plant.

Table 8: Absorbance Values of *Vicoa indica* Extracts and Standard (Ascorbic Acid) at Various Concentrations

Conc. (µg/mL)	STD	BLANK	AQ-VI	PE-VI	ET-VI	EA-VI	AC-VI
1.00	3.966	0.6632	1.1159	2.4579	0.7915	0.9876	2.8482
0.50	3.7955	0.5720	1.0908	2.1668	0.6626	0.9463	2.0085

0.25	3.5848	0.5644	1.0542	2.1465	0.5069	0.9355	1.9901
0.125	3.5195	0.4264	0.9458	2.0139	0.4595	0.8971	1.9626
0.0625	2.6538	0.4104	0.6952	1.7013	0.5424	0.8684	1.4308
0.03125	2.5058	0.3660	0.5826	1.5032	0.3805	0.7624	1.4074
0.0156	2.4677	0.2820	0.5377	1.4834	0.2437	0.4935	1.4012
0.0078	2.4030	0.2729	0.4652	1.4154	0.2427	0.3876	1.2650

Table 9: Average Absorbance and Percentage Inhibition of *Vicoa indica* Extracts in the DPPH Radical Scavenging Assay

Sample	Average Absorbance	% Inhibition
AQ-VI	0.8110±0.021	55.92 ± 2.4^{c}
PE-VI	1.8610±0.045	12.64 ± 1.1^{e}
ET-VI	0.5062±0,018	88.60 ± 3.2^{a}
EA-VI	0.7877±0.025	57.68 ± 2.8^{e}
AC-VI	1.9152±0.039	10.22 ± 0.9^{e}
Standard	3.4870± 0.235	87.26±2.9*

Inhibitory effect of various solvent extracts of *Vicoa indica* at a concentration of 1.00 µg/mL . Data are expressed as Mean ± SEM (n=3). Statistical significance was evaluated using a One-Way ANOVA followed by Tukey's post-hoc test. Values in the '% Inhibition' column sharing different superscript letters are significantly different from each other at $p < 0.05$. The standard positive control was evaluated separately and is denoted by an asterisk (*).

ET-VI showed the highest antioxidant activity (88.60%), very close to the standard ascorbic acid (87.26%).

Table 10: Absorbance Values of *Benkara malabarica* Extracts and Standard (Ascorbic Acid) at Various Concentrations

Conc. (µg/mL)	STD	BLANK	AQ-BM	PE-BM	ET-BM	EA-BM	AC-BM
1.00	2.976	2.800	2.410	2.150	1.950	2.610	2.120
0.50	2.702	2.650	2.220	2.010	1.780	2.320	1.950
0.25	2.521	2.490	2.140	1.940	1.600	2.270	2.180
0.125	2.432	2.360	1.980	1.830	1.520	2.130	1.860
0.0625	2.006	1.950	1.750	1.650	1.400	1.920	1.720
0.03125	1.821	1.790	1.540	1.420	1.300	1.740	1.530
0.0156	1.775	1.740	1.390	1.320	1.210	1.580	1.410
0.0078	1.703	1.700	1.260	1.210	1.150	1.420	1.360

Table 11: Average Absorbance and Percentage Inhibition of *Benkara malabarica* Extracts in the DPPH Radical Scavenging Assay

Sample	Average Absorbance	% Inhibition
AQ-BM	1.836±0.041	20.5 ± 1.2^{c}
PE-BM	1.704±0.038	26.2 ± 1.5^{b}
ET-BM	1.489±0.029	35.6 ± 2.1^{a}
EA-BM	2.123±0.055	8.1 ± 0.8^{e}
AC-BM	1.891±0.047	18.2 ± 1.1^{d}
STD	2.447±0.169	72.8±2.9*

Inhibitory effect of various solvent extracts of *Benkara malabarica* at a concentration of 1.00 µg/mL . Data are expressed as Mean ± SEM (n=3). Statistical significance was evaluated using a One-Way ANOVA followed by Tukey's post-hoc test. Values in the '% Inhibition' column sharing different superscript letters are significantly different from each other at $p < 0.05$. The standard positive control was evaluated separately and is denoted by an asterisk (*).

ET-BM exhibited the highest DPPH scavenging activity among the BM samples (35.6%), suggesting potent antioxidant potential.

AChE Inhibition Assay

Table 12: % Ache Inhibition of *Vicoa Indica* Extracts At Different Concentrations and Their IC_{50} Value

SAMPLE-1 (*Vicoa indica*)		**AChE inhibitory assay**				
Concentration (µg/ml)	**(**standard) **Donepezil**	**AQ-VI**	**PE-VI**	**ET-VI**	**EA-VI**	**AC-VI**
	% Inhibition	% Inhibition	% Inhibition	% Inhibition	% Inhibition	% Inhibition
10	15	5	13	8	3	12
20	21	14	17	20	13	24
40	36	26	35	31	27	44
60	40	38	47	45	42	56
80	56	50	59	64	53	62
100	73	65	75	76	67	70
IC_{50} values	**IC_{50} values = 67.67 (µg/ml)**	**IC_{50} values = 78.04 (µg/ml)**	**IC_{50} values =64.70 (µg/ml)**	**IC_{50} values =64.19 (µg/ml)**	**IC_{50} values =74.39 (µg/ml)**	**IC_{50} values =60.13 (µg/ml)**

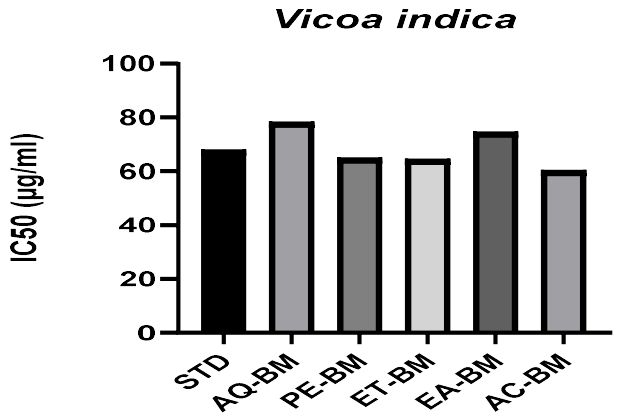

Figure 2: Graph Showing IC_{50} Values of Different Extracts of *Vicoa Indica* in the Acetylcholinesterase (Ache) Inhibition Assay

Table 13: % Ache Inhibition of *Benkara Malabarica* Extracts At Different Concentrations and Their IC_{50} Value

SAMPLE-2 (*Benkara Malabarica*)		**AChE Inhibitory Assay**				
Concentration (µg/ml)	**(**standard) **Donepezil**	**AQ-BM**	**PE-BM**	**ET-BM**	**EA-BM**	**AC-BM**
	% inhibition	% inhibition	% Inhibition	% inhibition	% inhibition	% inhibition
10	15	35	7	29	6	4
20	21	41	12	42	15	19
40	36	49	23	55	20	34
60	40	58	33	48	50	44
80	56	67	46	75	58	49
100	73	72	55	80	62	70
IC_{50} values	**IC_{50} values = 67.67 (µg/ml)**	**IC_{50} values =42.86 (µg/ml)**	**IC_{50} values =89.80 (µg/ml)**	**IC_{50} values =42.51 (µg/ml)**	**IC_{50} values =73.68 (µg/ml)**	**IC_{50} values =72.07 (µg/ml)**

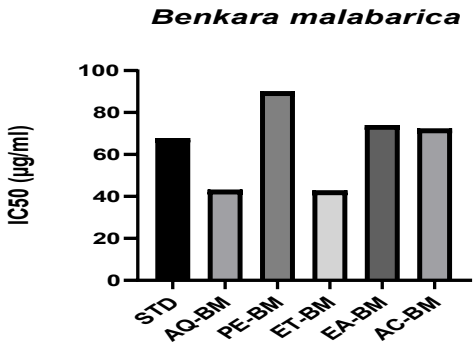

Figure 3: Graph Showing IC_{50} Values of Different Extracts of *Benkara Malabarica* in the Acetylcholinesterase (Ache) Inhibition Assay

Evaluation of ethanolic extracts of *Vicoa indica* (ET- VI) & *Benkara malabarica* (ET-BM) in amyloid-beta$_{1-42}$ (Aβ$_{1-42}$) intoxicated SH-SY5Y cell lines

Cell viability assessment – MTT assay [in presence of amyloid-beta$_{1-42}$ (Aβ$_{1-42}$)]

Cell viability was evaluated using the MTT assay to determine the protective or cytotoxic effects of the test samples against Aβ1–42–induced toxicity. Neuronal cells exposed to Aβ1–42 typically exhibit reduced metabolic activity due to impairment of mitochondrial function. In the present study, cells treated with Aβ1–42 alone served as the toxic control, while co-treatment with plant extracts/compounds was used to assess their ability to restore cell viability. The results depict the extent of metabolic recovery by quantifying the formation of formazan crystals, which reflects the percentage of viable cells. Aβ$_{1-42}$-induced neurotoxicity model to assess neuroprotective effects.

Table 14: Effect of ethanolic extracts of *Vicoa indica* (ET-VI) and *Benkara malabarica* (ET-BM) on the viability of Aβ1-42-challenged SH-SY5Y cells. Cell viability data were analysed using one-way ANOVA followed by Tukey's post hoc test in GraphPad Prism (version 10.0) and are presented as mean ± SEM. The superscript *** indicates p<0.001 versus the untreated control, while ###, ## and # denote p<0.001, p<0.01 and p<0.05, respectively, compared with the Aβ1-42-treated group (Aβ: amyloid beta).

S. No	Groups	% Viability of Cells
1	Control	99.87 ± 0.143
2	Aß$_{1-42}$ (10µM)	11.22 ± 0.473***
3	ET-VI	59.17 ± 4.671$^{###}$
4	ET-BM	39.82 ± 2.281$^{#}$

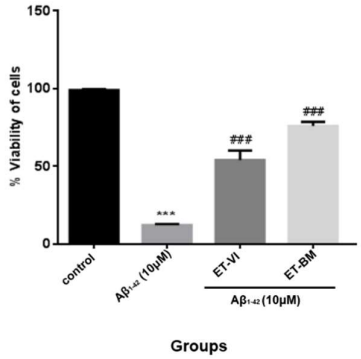

Figure 4: Effect of Ethanolic Extracts of *Vicoa Indica* (ET-VI) and *Benkara Malabarica* (ET-BM) on the Viability of Aβ1-42-Challenged SH-SY5Y Cells. Cell Viability Data Were Analysed Using One-Way ANOVA Followed by Tukey's Post Hoc Test in Graphpad Prism (Version 10.0) And Are Presented as Mean ± SEM. The Superscript *** Indicates P<0.001 Versus The Untreated Control, While ###, ## And # Denote P<0.001, P<0.01 And P<0.05, Respectively, Compared With The Aβ1-42-Treated Group (Aβ: Amyloid Beta).

Incubation of Aβ$_{1-42}$ in SH-SY5Y cell lines have shown significant (p<0.001) reduction in cell viability in comparison to control cells indicate the intensity of neurotoxicity exhibited by Aβ$_{1-42.}$ Post-

treatment with different extracts of ET-VI ($p<0.001$) and ET-BM ($p<0.001$) have shown notable increase in cell viability of SH-SY5Y cell lines evidence the protective effect exerted by extracts in $A\beta_{1-42}$ intoxicated condition.

Anti-Oxidant Evaluation – Spectroscopy Methods

Lipid Peroxide Assay (LPO)

Lipid peroxidation levels were quantified to assess the extent of oxidative damage induced under experimental conditions. The LPO assay measures malondialdehyde (MDA), a key end-product formed during the peroxidation of membrane lipids, which reflects cellular oxidative stress. In this study, MDA levels in control and treated groups were compared to evaluate the protective potential of the test samples against oxidative injury.

Table 15: Effect of Ethanolic Extracts of *Vicoa Indica* (ET-VI) and *Benkara Malabarica* (ET-BM) on the LPO Level in Aβ1-42-Challenged SH-SY5Y Cells. Cell Viability Data Were Analysed Using One-Way ANOVA Followed by Tukey's Post Hoc Test in Graphpad Prism (Version 10.0) And Are Presented as Mean ± SEM. The Superscript *** Indicates P<0.001 Versus The Untreated Control, While ###, ## And # Denote P<0.001, P<0.01 And P<0.05, Respectively, Compared With The Aβ1-42-Treated Group (Aβ: Amyloid Beta).

S. No	Groups	LPO nmoles/ml
1	Control	12.93 ± 1.74
2	$A\beta_{1-42}$ (10μM)	$95.30 \pm 3.38^{***}$
3	ET-VI	$62.15 \pm 1.72^{\#\#\#}$
4	ET-BM	$53.62 \pm 2.33^{\#\#\#}$

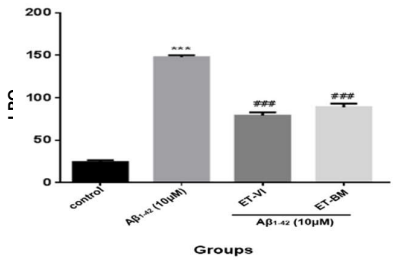

Figure 5: Effect of Ethanolic Extracts of *Vicoa Indica* (ET-VI) And *Benkara Malabarica* (ET-BM) on Lipid Peroxidation (LPO) Levels in $A\beta_{1-42}$-Challenged SH-SY5Y Cells. Data Are Expressed As Mean ± SEM (N = 6) And Were Analysed Using One-Way ANOVA Followed by Tukey's Post Hoc Test. ***P < 0.001 Vs. Control; ###P < 0.001, ##P < 0.01, and #P < 0.05 Vs. Aβ-Treated Group.

The LPO level was significantly ($p<0.001$) increased in $A\beta_{1-42}$ intoxicated SH-SY5Y cell lines in comparison to control cells indicate the induction of oxidative stress. Post-treatment with ET-VI ($p<0.001$) and ET-BM ($p<0.001$) have remarkably decreased the LPO level when compared to $A\beta_{1-42}$ treated cells represent the reduced lipid peroxidation level in $A\beta_{1-42}$ treated SH-SY5Y cell lines while treated with ET-VI and ET-BM extracts.

Nitric Oxide Assay (NO)

Nitric oxide levels were estimated to evaluate the extent of inflammatory and oxidative responses generated under the experimental conditions. The assay quantifies nitrite accumulation, a stable metabolite of NO, using the Griess reagent. In the present study, comparisons between control and treated groups were used to determine whether the test samples attenuated NO production, thereby indicating their potential anti-inflammatory and cytoprotective effects.

Table 16: Effect of Ethanolic Extracts of *Vicoa Indica* (ET-VI) and *Benkara Malabarica* (ET-BM) on the NO Level in Aβ1-42-Challenged SH-SY5Y Cells. The Data Were Analysed Using One-Way ANOVA Followed by Tukey's Post Hoc Test in Graphpad Prism (Version 10.0) And Are Presented As Mean ± SEM. The Superscript *** Indicates P<0.001 versus the Untreated Control, While ###, ## and # Denote P<0.001, P<0.01 and P<0.05, Respectively, Compared With the Aβ1-42-Treated Group (Aβ: Amyloid Beta).

S. No	Groups	NO nmoles/ml
1	Control	24.20 ± 2.10
2	$A\beta_{1-42}$ (10μM)	$147.63 \pm 2.39^{***}$
3	ET-VI	$78.94 \pm 3.76^{\#\#\#}$

4	ET-BM	$88.81 \pm 4.25^{\#\#\#}$

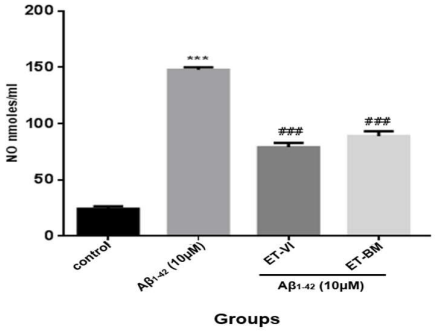

Figure 6: Effect of Ethanolic Extracts of *Vicoa Indica* (ET-VI) And *Benkara Malabarica* (ET-BM) on Nitric Oxide (NO) Levels in $A\beta_{1-42}$-Challenged SH-SY5Y Cells. Data Are Expressed As Mean ± SEM (N = 6) and Were Analysed Using One-Way ANOVA Followed by Tukey's Post Hoc Test. ***P < 0.001 Vs. Control; ###P < 0.001, ##P < 0.01, and #P < 0.05 Vs. Aβ-Treated Group.

A significant elevation in NO level ($p<0.001$) was observed in SH-SY5Y cell lines when treated with $A\beta_{1-42}$. Interestingly, the post-treatment with different extracts like of ET-VI ($p<0.001$) and ET-BM ($p<0.001$) have shown remarkable reduction in NO level in comparison to $A\beta_{1-42}$ toxicated cell lines denote the anti-oxidant potential of ET-VI and ET-BM in neurotoxicity circumstances.

Glutathione (GSH) assay

Reduced glutathione (GSH) levels were measured to assess the cellular antioxidant status under the experimental conditions. GSH is a major intracellular antioxidant, and its depletion reflects oxidative stress and impaired detoxification capacity. In this study, GSH concentrations were compared between control and treated groups to determine the ability of the test samples to restore or maintain antioxidant defense mechanisms.

Table 17: Effect of ethanolic extracts of *Vicoa indica* (ET-VI) and *Benkara malabarica* (ET-BM) on the GSH level in Aβ1-42-challenged SH-SY5Y cells. The data were analysed using one-way ANOVA followed by Tukey's post hoc test in GraphPad Prism (version 10.0) and are presented as mean ± SEM. The superscript *** indicates $p<0.001$ versus the untreated control, while ###, ## and # denote $p<0.001$, $p<0.01$ and $p<0.05$, respectively, compared with the Aβ1-42-treated group (Aβ: amyloid beta).

S. No	Groups	Cellular GSH (μM/mg protein)
1	Control	85.55 ± 4.33
2	$A\beta_{1-42}$ (10μM)	$11.23 \pm 1.44^{***}$
3	ET-VI	$59.92 \pm 2.96^{\#\#\#}$
4	ET-BM	$71.28 \pm 4.87^{\#\#\#}$

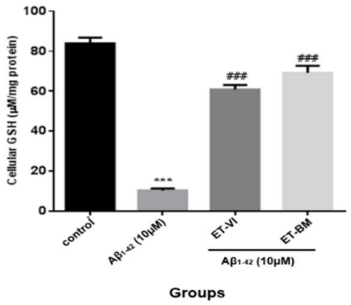

Figure 7: Effect of Ethanolic Extracts of *Vicoa Indica* (ET-VI) And *Benkara Malabarica* (ET-BM) on Reduced Glutathione (GSH) Levels in $A\beta_{1-42}$-Challenged SH-SY5Y Cells. Data Are Expressed As Mean ± SEM (N = 6) and Were Analysed Using One-Way ANOVA Followed by Tukey's Post Hoc Test. ***P < 0.001 Vs. Control; ###P < 0.001, ##P < 0.01, and #P < 0.05 Vs. Aβ-Treated Group.

$A\beta_{1-42}$ intoxicated SH-SY5Y cell lines have shown significant reduction of cellular GSH level ($p<0.001$) in comparison to control cells indicate the depletion of anti-oxidant enzyme level. Post-treatment with of ET-VI ($p<0.001$) and ET-BM ($p<0.001$) have shown notable elevation in anti-oxidant enzyme GSH level when compared to $A\beta_{1-42}$ incubated cell lines evidence the restoration of anti-oxidant system by above extracts in neurodegenerative conditions.

Superoxide Dismutase

Superoxide dismutase (SOD) activity was evaluated to determine the status of enzymatic antioxidant defense under the experimental conditions. SOD plays a crucial role in detoxifying superoxide radicals, and reduced activity indicates elevated oxidative stress. In the present study, SOD levels in control and treated groups were compared to assess the potential of the test samples to enhance endogenous antioxidant capacity.

Table 18: Effect of Ethanolic Extracts of *Vicoa Indica* (ET-VI) and *Benkara Malabarica* (ET-BM) On the SOD Level in Aβ1-42-Challenged SH-SY5Y Cells. Cell Viability Data Were Analysed Using One-Way ANOVA Followed by Tukey's Post Hoc Test in Graphpad Prism (Version 10.0) And Are Presented as Mean ± SEM. The Superscript *** Indicates P<0.001 versus the Untreated Control, While ###, ## And # Denote P<0.001, P<0.01 and P<0.05, Respectively, Compared With the Aβ1-42-Treated Group (Aβ: Amyloid Beta).

S. No	Groups	SOD (U/mg protein)
1	Control	25.17 ± 2.28
2	$A\beta_{1-42}$ (10μM)	$3.54 \pm 0.87^{***}$
3	ET-VI	$12.87 \pm 0.85^{\#\#}$
4	ET-BM	$17.58 \pm 0.84^{\#\#\#}$

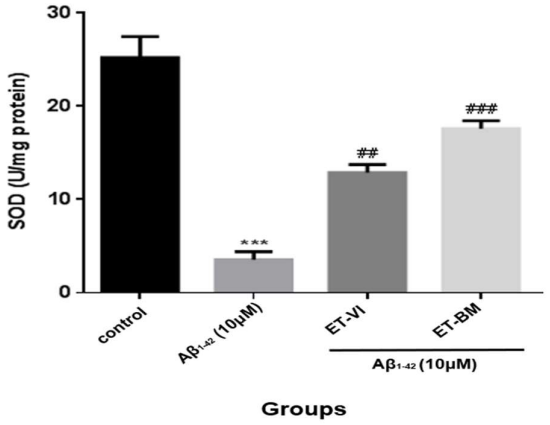

Figure 8: Effect of Ethanolic Extracts of *Vicoa Indica* (ET-VI) and *Benkara Malabarica* (ET-BM) on Superoxide Dismutase (SOD) Activity in $A\beta_{1-42}$-Challenged SH-SY5Y Cells. Data Are Expressed As Mean ± SEM (N = 6) And Were Analysed Using One-Way ANOVA Followed by Tukey's Post Hoc Test. ***P < 0.001 Vs. Control; ###P < 0.001, ##P < 0.01, and #P < 0.05 Vs. Aβ-Treated Group.

The SOD level ($p<0.001$) was significantly reduced in $A\beta_{1-42}$ intoxicated SH-SY5Y cell lines in comparison to control cells. Post-treatment with of ET-VI ($p<0.01$) and ET-BM ($p<0.001$) have remarkably elevated the SOD level in comparison to Aβ group indicate the free-radicals scavenging capacity of above extracts in neurotoxicity conditions.

Catalase

Catalase activity was measured to evaluate the enzymatic antioxidant response against oxidative stress generated under the experimental conditions. Catalase catalyzes the decomposition of hydrogen peroxide into water and oxygen, and a reduction in its activity reflects impaired antioxidant defense. In this study, CAT activity in control and treated groups was compared to determine the ability of the test samples to enhance or restore hydrogen peroxide detoxification.

Table 19: Effect of Ethanolic Extracts of *Vicoa Indica* (ET-VI) and *Benkara Malabarica* (ET-BM) on the CAT Activity in Aβ1-42-Challenged SH-SY5Y Cells. The Data Were Analysed Using One-Way ANOVA Followed by Tukey's Post Hoc Test in Graphpad Prism (Version 10.0) And Are Presented As Mean ± SEM. The Superscript *** Indicates P<0.001 versus the Untreated Control, While ###, ## and # Denote P<0.001, P<0.01 and P<0.05, Respectively, Compared With the Aβ1-42-Treated Group (Aβ: Amyloid Beta).

S. No	Groups	CAT (U/mg protein)
1	Control	0.907 ± 0.090
2	Aß$_{1-42}$ (10µM)	0.172 ± 0.035***
3	ET-VI	0.518 ± 0.029$^{\#\#}$
4	ET-BM	0.723 ± 0.017$^{\#\#\#}$

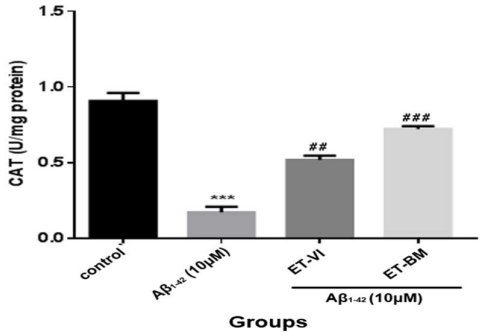

Figure 9: Effect of Ethanolic Extracts of *Vicoa Indica* (ET-VI) And *Benkara Malabarica* (ET-BM) on Catalase (CAT) Activity (U/Mg Protein) In Aβ$_{1-42}$-Challenged SH-SY5Y Cells. Data Are Expressed As Mean ± SEM (N = 6) and Were Analysed Using One-Way ANOVA Followed by Tukey's Post Hoc Test. ***P < 0.001 Vs. Control; ###P < 0.001, ##P < 0.01, and #P < 0.05 Vs. Aβ-Treated Group.

Incubation of Aβ$_{1-42}$ in SH-SY5Y cell lines has significantly reduced the CAT level ($p<0.001$) in comparison to control cells. Post-treatment with of ET-VI ($p<0.01$) and ET-BM ($p<0.001$) have increased the CAT level in Aβ toxicated cell lines indicate the anti-oxidant potential of above extracts in neurodegenerative conditions.

Anti-Inflammatory Assessments – ELISA

Pro-inflammatory cytokine levels were quantified using ELISA to evaluate the extent of inflammation induced under experimental conditions and to determine the modulatory effects of the test samples. Specific cytokines such as TNF-α, IL-1β were measured, as their elevation reflects activation of inflammatory pathways. In this study, cytokine concentrations in control and treated groups were compared to assess the potential anti-inflammatory efficacy of the samples.

IL-1β

Table 20: Effect of Ethanolic Extracts of *Vicoa Indica* (ET-VI) and *Benkara Malabarica* (ET-BM) On the IL-1β Level in Aβ1-42-Challenged SH-SY5Y Cells. The Data Were Analysed Using One-Way ANOVA Followed by Tukey's Post Hoc Test in Graphpad Prism (Version 10.0) And Are Presented As Mean ± SEM. The Superscript *** Indicates P<0.001 versus the Untreated Control, While ###, ## And # Denote P<0.001, P<0.01 and P<0.05, Respectively, Compared With the Aβ1-42-Treated Group (Aβ: Amyloid Beta).

S. No	Groups	IL-1β (pg/ml)
1	Control	105.23 ± 1.22
2	Aß$_{1-42}$ (10µM)	889.87 ± 17.89***
3	ET-VI	613.87 ± 32.65$^{\#\#\#}$
4	ET-BM	535.69 ± 9.54$^{\#\#\#}$

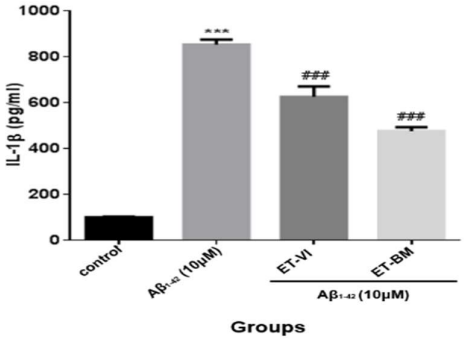

Figure 10: Effect of Ethanolic Extracts of *Vicoa Indica* (ET-VI) And *Benkara Malabarica* (ET-BM) on IL-1β Levels in $A\beta_{1-42}$-Challenged SH-SY5Y Cells. Data Are Expressed As Mean ± SEM (N = 6) And Were Analysed Using One-Way ANOVA Followed by Tukey's Post Hoc Test. ***P < 0.001 Vs. Control; ###P < 0.001, ##P < 0.01, and #P < 0.05 Vs. Aβ-Treated Group.

The IL-1β level was significantly ($p<0.001$) increased in $A\beta_{1-42}$ intoxicated SH-SY5Y cell lines in comparison to control cells indicate the induction of neuroinflammation. Post-treatment with of ET-VI ($p<0.001$) and ET-BM ($p<0.001$) have remarkably decreased the IL-1β level when compared to $A\beta_{1-42}$ treated cells represent the reduced inflammatory response by above extracts in $A\beta_{1-42}$ treated SH-SY5Y cell lines.

TNF-α

Table 21: Effect Of Ethanolic Extracts Of *Vicoa Indica* (ET-VI) And *Benkara Malabarica* (ET-BM) on the TNF-A Level In Aβ1-42-Challenged SH-SY5Y Cells. The Data Were Analysed Using One-Way ANOVA Followed by Tukey's Post Hoc Test in Graphpad Prism (Version 10.0) And Are Presented As Mean ± SEM. The Superscript *** Indicates P<0.001 versus the Untreated Control, While ###, ## and # Denote P<0.001, P<0.01 and P<0.05, Respectively, Compared With the Aβ1-42-Treated Group (Aβ: Amyloid Beta).

S. No	Groups	TNF-α (pg/ml)
1	Control	133.71 ± 5.37
2	$A\beta_{1-42}$ (10μM)	$977.84 \pm 36.52^{***}$
3	ET-VI	$665.24 \pm 18.14^{\#\#\#}$
4	ET-BM	$473.83 \pm 20.30^{\#\#\#}$

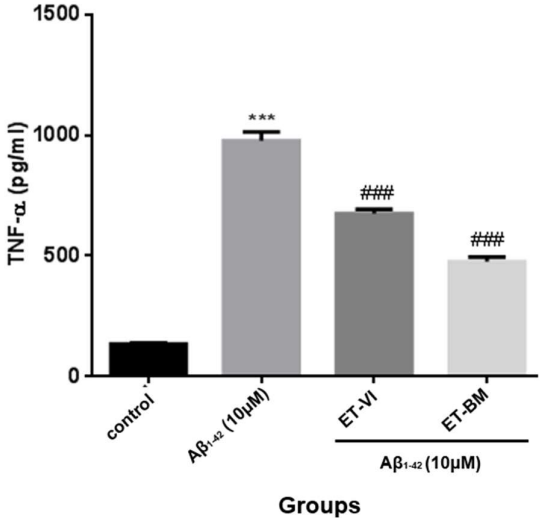

Figure 11: Effect of Ethanolic Extracts of *Vicoa Indica* (Et-Vi) And *Benkara Malabarica* (Et-Bm) on Tnf-A Levels in $A\beta_{1-42}$-Challenged Sh-Sy5y Cells. Data Are Expressed As Mean ± Sem (N = 6) and Were Analysed Using One-Way Anova Followed by Tukey's Post Hoc Test. ***P < 0.001 Vs. Control; ###P < 0.001, ##P < 0.01, And #P < 0.05 Vs. Aβ-Treated Group.

Remarkable elevation in TNF-α level ($p<0.001$) was observed in SH-SY5Y cell lines when treated with $A\beta_{1-42}$ intoxication. Fascinatingly, the post-treatment with different extracts like of ET-VI ($p<0.001$) and ET-BM ($p<0.001$) have shown significant reduction in TNF-α level in comparison to $A\beta_{1-42}$ toxicated cell lines denote the anti-neuroinflammatory capacity of above extracts in neurotoxicity conditions.

DISCUSSION

The findings of this study clearly demonstrate that the ethanolic extracts of *Benkara malabarica* (ET-BM) and *Vicoa indica* (ET-VI) exert a broad spectrum of protective effects that are highly relevant to the multifactorial pathology of Alzheimer's disease (AD). The strong antioxidant responses exhibited by both ET-BM and ET-VI reflected in their potent DPPH radical-scavenging activity and elevated total antioxidant capacity indicate the presence of bioactive phytochemicals capable of efficiently neutralizing ROS. These results align with previous findings on *Benkara malabarica*, where the leaf extracts demonstrated notable antioxidant and cytotoxic activities[40]. This observation aligns closely with extensive previous literature reporting that medicinal plants rich in polyphenols, flavonoids, and other redox-active compounds display robust free-radical-quenching properties. In particular, the comparatively higher antioxidant efficacy of ET-VI is in agreement with earlier studies showing that flavonoid-dominant extracts display stronger electron-donating potential and sustained antioxidant stability compared to alkaloid-rich fractions[41]. Such findings strengthen the hypothesis that the phytochemical composition of ET-VI may be especially suited for interrupting ROS-driven neurodegenerative cascades.

The neuroprotective effects of *Benkara malabarica* and *Vicoa indica* may be attributed to the combined action of flavonoids, phenolics, and alkaloids. Flavonoids are known to disrupt amyloid-β (Aβ) aggregation by interacting with β-sheet structures, while polyphenols suppress Aβ-induced oxidative stress through metal chelation and free-radical scavenging, consistent with reduced lipid peroxidation and nitric oxide levels. Alkaloids and phenolics may further attenuate tau hyperphosphorylation by modulating stress-activated kinases. Restoration of SOD, CAT, and GSH suggests preservation of mitochondrial function, while reduced nitric oxide indicates suppression of NF-κB–mediated neuroinflammation. Together, these effects support a multitarget mechanism relevant to Alzheimer's disease.

A direct comparison showed that ET-VI consistently outperformed ET-BM across antioxidant and neuroprotection assays, including DPPH scavenging, total antioxidant capacity, $A\beta_{1–42}$–induced cytoprotection, and suppression of oxidative and inflammatory markers. Although both extracts were neuroprotective, ET-VI more effectively restored cell viability and reduced lipid peroxidation and nitric oxide levels. This enhanced efficacy is likely due to ET-VI's higher flavonoid and phenolic content, compounds strongly linked to redox regulation and amyloid-β modulation, whereas ET-BM contained a broader but less redox-potent metabolite profile. These findings suggest flavonoid enrichment as a key driver of anti-Alzheimer's activity. The membrane-stabilizing activity observed in the RBC hemolysis assay provides further support for the cytoprotective capabilities of both extracts[40]. Stabilization of the erythrocyte membrane is widely recognized as a preliminary indicator of a compound's ability to protect cellular structures from peroxidative damage[42]. The membrane-stabilizing and neuroprotective effects of the ethanolic extracts of *Benkara malabarica* (ET-BM) and *Vicoa indica* (ET-VI) are consistent with those reported for polyphenol- and flavonoid-rich botanicals used in neurodegenerative research. Phenolic compounds enhance membrane integrity by limiting lipid peroxidation and protecting membrane-associated proteins under oxidative stress. The marked reduction in erythrocyte hemolysis, $A\beta_{1–42}$-induced cytotoxicity, lipid peroxidation, and nitric oxide levels in SH-SY5Y cells indicates that ET-BM and ET-VI act primarily through antioxidant-mediated membrane stabilization and modulation of redox-sensitive inflammatory pathways. These mechanisms are highly relevant to Alzheimer's disease, where oxidative membrane damage, mitochondrial dysfunction, and neuroinflammation are closely linked..The membrane-stabilizing effects of ET-BM and ET-VI likely arise from reinforcement of the lipid bilayer, suppression of lipid peroxidation, and modulation of membrane-associated enzymes mechanisms frequently reported for polyphenol-rich extracts. Similar biophysical insights are described in studies using model lipid membranes, where small bioactive molecules enhance bilayer packing and reduce destabilization by thermal or oxidative stress. These parallels suggest that ET-BM and ET-VI act through mechanisms consistent with those observed in controlled membrane models, supporting their role as effective natural membrane-protective agents[43].

The neuroprotective effect against $A\beta_{1–42}$-induced cytotoxicity in SH-SY5Y cells further underscores the therapeutic potential of these extracts. The neuroprotective actions of ET-BM and ET-VI against $A\beta_{1–42}$-induced cytotoxicity are consistent with previous evidence that plant-derived antioxidants safeguard neurons by stabilizing mitochondrial membrane potential, reducing ROS

overproduction, and preventing intrinsic apoptosis. Comparable mechanisms are highlighted in the potential of xanthotoxin in the treatment of cognitive disorders, where xanthotoxin is shown to alleviate oxidative stress, preserve mitochondrial integrity, and limit neuronal degeneration[44]. The parallel between these findings suggests that ET-BM and ET-VI may act through conserved mitochondrial-protective and ROS-modulatory pathways similar to xanthotoxin. Given that mitochondrial dysfunction is an early hallmark of AD, the convergence of these mechanistic insights reinforces the therapeutic relevance of such antioxidant-based interventions.

The significant reduction in oxidative and nitrosative stress markers, including malondialdehyde (MDA) and nitric oxide (NO), provides biochemical confirmation of these protective effects. Similar decreases in MDA and NO levels following treatment with polyphenolic plant extracts have been reported in several experimental models of Aβ-induced toxicity, reinforcing the interpretation that ET-BM and ET-VI exert potent antioxidative and anti-inflammatory effects[45,46]. The reduction in NO levels is especially relevant, as excessive nitrosative stress is closely linked to microglial activation and neuroinflammation two pathological processes strongly implicated in the progression of AD[47,48]. Thus, the ability of these extracts to mitigate NO production suggests that their bioactive constituents may modulate microglial reactivity or inhibit inducible nitric oxide synthase (iNOS), aligning with patterns seen in other anti-inflammatory botanicals[49].

Taken together, the biological activities demonstrated by ET-BM and ET-VI reflect a multitarget therapeutic profile that is consistent with the behaviour of numerous plant-derived neuroprotective agents previously studied in AD research. Their ability to simultaneously modulate oxidative stress, preserve membrane integrity, reduce inflammation, and protect neuronal cells against Aβ-induced toxicity supports the concept that these extracts influence several interconnected pathological pathways. This is particularly relevant in the context of AD, where therapeutic strategies targeting single pathways have shown limited efficacy, and multidimensional approAChEs are increasingly being prioritized.

Despite the encouraging findings, the present study has certain limitations. The results are based solely on *in vitro* models, which may not fully reflect the complexity of Alzheimer's disease pathology *in vivo*. In addition, crude ethanolic extracts were used, preventing definitive identification of the individual bioactive compounds responsible for the observed neuroprotective effects and their possible synergistic interactions. Furthermore, the blood–brain barrier permeability and pharmacokinetic properties of the active constituents remain unknown. Future studies should therefore focus on bioactivity-guided isolation of active compounds, assessment of BBB penetration, and validation in relevant in vivo Alzheimer's disease models to establish translational potential.

Overall, the study suggests that *Benkara malabarica* and *Vicoa indica* possess pharmacological characteristics comparable to many well-studied medicinal plants recognized for their neuroprotective potential. Their demonstrated antioxidant, anti-inflammatory, cytoprotective, and anti-amyloidogenic activities not only validate their traditional medicinal use but also highlight their promise as multi-mechanistic candidates for further preclinical development in Alzheimer's disease therapeutics. Continued investigation into their bioactive constituents, mechanisms of action, and *in vivo* efficacy could provide valuable insights into their potential integration into future AD treatment strategies.

CONCLUSION

The findings of the present investigation strongly support the therapeutic relevance of *Benkara malabarica* and *Vicoa indica* ethanolic extracts in mitigating pathological processes associated with Alzheimer's disease. Both ET-BM and ET-VI demonstrated high antioxidant potency, effective membrane-stabilising capability, and marked protection against $A\beta_{1-42}$-induced neuronal toxicity. Their ability to suppress oxidative and nitrosative stress further reflects modulation of disease-driving events such as ROS generation, lipid peroxidation, microglial activation, and downstream inflammatory pathways.

Among the two extracts, ET-VI exhibited superior efficacy across multiple assays, suggesting enrichment of highly active flavonoids or polyphenolic constituents with strong neuroprotective potential. The multi-target profile shown by these extracts positions them as compelling candidates for early-stage AD intervention, addressing several molecular hallmarks simultaneously rather than a single pathological trigger. Future investigations should focus on bioassay-guided fractionation to isolate and structurally characterize the active phytoconstituents responsible for the observed neuroprotective effects. Validation of these findings in well-established animal models of Alzheimer's disease will be essential to confirm in vivo efficacy and safety. In parallel, detailed studies on molecular mechanisms, blood–brain barrier permeability, and pharmacokinetic behavior of the active compounds are warranted to assess their translational potential.

Overall, these outcomes justify detailed future studies focusing on phytochemical isolation, structure activity relationship assessment, molecular pathway elucidation using omics-based

approAChEs, and preclinical evaluation in AD animal models. Such efforts will be instrumental in determining the translational feasibility of these extracts as novel plant-derived therapeutics for Alzheimer's disease.

Conflict of Interest

The authors have no conflicts of interest regarding this investigation.

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Anoop T.V: Literature review, Writing original draft; Umamaheswari D: Writing original draft, Conceptualization, Critical Evaluation; Antony Justin: Conceptualization; Gomathi Vengatachalam: Critical Evaluation Review and editing, Supervision, Evaluation, Visualization.

REFERENCE

1. Arora R, Malhotra P, Chawla R, Gupta D. Herbal Drugs for Oncology: Current Status and Future Directions in Cancer Chemoprevention. In: Herbal Medicine: A Cancer Chemopreventive and Therapeutic Perspective. Jaypee Brothers Medical Publishers (P) Ltd.; 2010. p. 1–1. doi:10.5005/jp/books/11166_1
2. Ng JY, Anant S, Parakh ND. Characteristics of the research literature on herbal medicines corresponding with herbal supplements yielding the highest total sales: A bibliometric analysis. Adv Integr Med. 2023 May;10(2):64–79. doi:10.1016/j.aimed.2023.05.004
3. Rehman MU, Wali AF, Ahmad A, Shakeel S, Rasool S, Ali R, et al. Neuroprotective Strategies for Neurological Disorders by Natural Products: An update. Curr Neuropharmacol. 2019 Feb 14;17(3):247–67. doi:10.2174/1570159X16666180911124605
4. Kamatham PT, Shukla R, Khatri DK, Vora LK. Pathogenesis, diagnostics, and therapeutics for Alzheimer's disease: Breaking the memory barrier. Ageing Res Rev. 2024 Nov;101:102481. doi:10.1016/j.arr.2024.102481
5. Alzheimer's Facts and Figures Report | Alzheimer's Association [Internet]. [cited 2025 Dec 6]. Available from: https://www.alz.org/alzheimers-dementia/facts-figures
6. Alzheimer's Disease Puzzle: Delving into Pathogenesis Hypotheses. Aging Dis. 2024. doi:10.14336/AD.2023.0608
7. Mathur S, Gawas C, Ahmad IZ, Wani M, Tabassum H. Neurodegenerative disorders: Assessing the impact of natural vs drug-induced treatment options. AGING MEDICINE. 2023 Mar 22;6(1):82–97. doi:10.1002/agm2.12243
8. Anoop TV, Vengatachalam G. Vicoa indica: unveiling the therapeutic properties and phytochemical profiling of a lesser-known medicinal plant. Biol Nyssana. 2025;16(1). doi:10.46793/BiolNyss.16.1.22A
9. Malík M, Tlustoš P. Nootropic Herbs, Shrubs, and Trees as Potential Cognitive Enhancers. Plants. 2023 Mar 18;12(6):1364. doi:10.3390/plants12061364
10. Kushwah S, Maurya NS, Kushwaha S, Scotti L, Chawade A, Mani A. Herbal Therapeutics for Alzheimer's Disease: Ancient Indian Medicine System from the Modern Viewpoint. Curr Neuropharmacol. 2023 Apr;21(4):764–76. doi:10.2174/1570159X21666230216094353
11. Tiwari VJ. Validity of Ethnopharmacological uses of *Vicoa indica* (L.) DC., Family-*Asteraceae*. Research Journal of Pharmacognosy and Phytochemistry. 2018;10(3):207. doi:10.5958/0975-4385.2018.00033.X
12. Bitwell C, Indra S Sen, Luke C, Kakoma MK. A review of modern and conventional extraction techniques and their applications for extracting phytochemicals from plants. Sci Afr. 2023 Mar;19:e01585. doi:10.1016/j.sciaf.2023.e01585
13. Jha DK, Panda L, Lavanya P, Ramaiah S, Anbarasu A. Detection and Confirmation of Alkaloids in Leaves of Justicia adhatoda and Bioinformatics Approach to Elicit Its Anti-tuberculosis Activity. Appl Biochem Biotechnol. 2012 Nov 17;168(5):980–90. doi:10.1007/s12010-012-9834-1
14. LAB 9 - TESTS FOR CARBOHYDRATES - Chemistry LibreTexts [Internet]. [cited 2026 Feb 3]. Available from: https://chem.libretexts.org/Courses/Triton_College/Elementary_Organic_Chemistry_(Lab_Manual)/09%3A_Lab_9-_Tests_for_Carbohydrates

15. Chemical Tests For Glycosides: General and specific - Gpatindia: Pharmacy Jobs, Admissions, Scholarships, Conference,Grants, Exam Alerts [Internet]. [cited 2026 Feb 3]. Available from: https://gpatindia.com/chemical-tests-for-glycosides-general-and-specific/#google_vignette
16. View of PRELIMINARY PHYTOCHEMICAL AND STANDARDIZATION PARAMETERS OF IPOMOEA QUAMOCLIT LINN WHOLE PLANT-AN ETHNOMEDICINALLY IMPORTANT PLANT | International Journal of Pharmacy and Pharmaceutical Sciences [Internet]. [cited 2026 Feb 3]. Available from: https://journals.innovareacademics.in/index.php/ijpps/article/view/1409/10382
17. SEN K, BAGCHI P. Studies on the Ultraviolet Absorption Spectra of Coumarins and Chromones. II. Hydroxy Derivatives [1]. J Org Chem. 1959 Mar 1;24(3):316–9. doi:10.1021/jo01085a008
18. Godlewska K, Pacyga P, Najda A, Michalak I. Investigation of Chemical Constituents and Antioxidant Activity of Biologically Active Plant-Derived Natural Products. Molecules. 2023 Jul 21;28(14):5572. doi:10.3390/molecules28145572
19. Rahimah SB, Djunaedi DD, Soeroto AY, Bisri T. The The Phytochemical Screening, Total Phenolic Contents and Antioxidant Activities in Vitro of White Oyster Mushroom (Pleurotus Ostreatus) Preparations. Open Access Maced J Med Sci. 2019 Jun 30;7(15):2404–12. doi:10.3889/oamjms.2019.741
20. Laboratory Test of Proteins: Step-by-Step Procedures & Tips [Internet]. [cited 2026 Feb 3]. Available from: https://www.vedantu.com/chemistry/laboratory-test-of-proteins
21. Devmurari *. Phytochemical screening study and antibacterial evaluation of Symplocos racemosa Roxb. Arch Appl Sci Res [Internet]. 2010 [cited 2026 Feb 3];2(1):354–9. Available from: www.scholarsresearchlibrary.com
22. Ivanova M, Hanganu A, Dumitriu R, Tociu M, Ivanov G, Stavarache C, et al. Saponification Value of Fats and Oils as Determined from 1H-NMR Data: The Case of Dairy Fats. Foods. 2022 May 18;11(10):1466. doi:10.3390/foods11101466
23. Singh PK, Singh J, Medhi T, Kumar A. Phytochemical Screening, Quantification, FT-IR Analysis, and *In Silico* Characterization of Potential Bio-active Compounds Identified in HR-LC/MS Analysis of the Polyherbal Formulation from Northeast India. ACS Omega. 2022 Sep 20;7(37):33067–78. doi:10.1021/acsomega.2c03117
24. Rao A, Kumari S, Laura JS, Dhania G. Qualitative Phytochemical Screening of Medicinal Plants Using Different Solvent Extracts. Oriental Journal Of Chemistry. 2023 Jun 30;39(3):621–6. doi:10.13005/ojc/390312
25. Kalusalingam M, Balakrishnan V. Estimation of Antioxidant and Cytotoxicity Activities of Extracts Obtained from the Leaves of Folk Medicinal Plant Benkara malabarica (Lam). Indian Journal of Pharmaceutical Education and Research. 2022 Jun 30;56(3):838–45. doi:10.5530/ijper.56.3.136
26. Evans BC, Nelson CE, Yu SS, Beavers KR, Kim AJ, Li H, et al. Ex Vivo Red Blood Cell Hemolysis Assay for the Evaluation of pH-responsive Endosomolytic Agents for Cytosolic Delivery of Biomacromolecular Drugs. Journal of Visualized Experiments. 2013 Mar 9;(73). doi:10.3791/50166
27. BLOIS MS. Antioxidant Determinations by the Use of a Stable Free Radical. Nature. 1958 Apr;181(4617):1199–200. doi:10.1038/1811199a0
28. Baliyan S, Mukherjee R, Priyadarshini A, Vibhuti A, Gupta A, Pandey RP, et al. Determination of Antioxidants by DPPH Radical Scavenging Activity and Quantitative Phytochemical Analysis of Ficus religiosa. Molecules. 2022 Feb 16;27(4):1326. doi:10.3390/molecules27041326
29. Riegerová P, Brejcha J, Bezděková D, Chum T, Mašínová E, Čermáková N, et al. Expression and Localization of AβPP in SH-SY5Y Cells Depends on Differentiation State. Journal of Alzheimer's Disease. 2021 Jul 21;82(2):485–91. doi:10.3233/JAD-201409
30. Kaya ZB, Santiago-Padilla V, Lim M, Boschen SL, Atilla P, McLean PJ. Optimizing SH-SY5Y cell culture: exploring the beneficial effects of an alternative media supplement on cell proliferation and viability. Sci Rep. 2024 Feb 27;14(1):4775. doi:10.1038/s41598-024-55516-5
31. Ahmad N, Feyes DK, Agarwal R, Mukhtar H. Photodynamic therapy results in induction of WAF1/CIP1/P21 leading to cell cycle arrest and apoptosis. Proceedings of the National Academy of Sciences. 1998 Jun 9;95(12):6977–82. doi:10.1073/pnas.95.12.6977

32. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979 Jun;95(2):351–8. doi:10.1016/0003-2697(79)90738-3
33. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. Anal Biochem. 1982 Oct;126(1):131–8. doi:10.1016/0003-2697(82)90118-X
34. Jollow DJ, Mitchell JR, Zampaglione N, Gillette JR. Bromobenzene-Induced Liver Necrosis. Protective Role of Glutathione and Evidence for 3,4-Bromobenzene Oxide as the Hepatotoxic Metabolite. Pharmacology. 1974;11(3):151–69. doi:10.1159/000136485
35. A modified spectrophotometric assay of superoxide dismutase - PubMed [Internet]. [cited 2025 Dec 8]. Available from: https://pubmed.ncbi.nlm.nih.gov/6490072/
36. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase - PubMed [Internet]. [cited 2025 Dec 8]. Available from: https://pubmed.ncbi.nlm.nih.gov/14938361/
37. Waterborg JH. The Lowry Method for Protein Quantitation. In. 2009. p. 7–10. doi:10.1007/978-1-59745-198-7_2
38. Giraldo-Osorno PM, Wirsig K, Asa'ad F, Omar O, Trobos M, Bernhardt A, et al. Macrophage-to-osteocyte communication: Impact in a 3D in vitro implant-associated infection model. Acta Biomater. 2024 Sep;186:141–55. doi:10.1016/j.actbio.2024.08.005
39. Prism - GraphPad [Internet]. [cited 2025 Dec 8]. Available from: https://www.graphpad.com/features
40. Kalusalingam M, Balakrishnan V. Estimation of Antioxidant and Cytotoxicity Activities of Extracts Obtained from the Leaves of Folk Medicinal Plant Benkara malabarica (Lam). Indian Journal of Pharmaceutical Education and Research. 2022 Jul 1;56(3):838–45. doi:10.5530/ijper.56.3.136
41. Zhang W, Chen H, Wang Z, Lan G, Zhang L. Comparative studies on antioxidant activities of extracts and fractions from the leaves and stem of Epimedium koreanum Nakai. J Food Sci Technol. 2013 Dec 16;50(6):1122–9. doi:10.1007/s13197-011-0447-4
42. Ydyrys A, Zhamanbayeva G, Zhaparkulova N, Aralbaeva A, Askerbay G, Kenzheyeva Z, et al. The Systematic Assessment of the Membrane-Stabilizing and Antioxidant Activities of Several Kazakhstani Plants in the Asteraceae Family. Plants. 2023 Dec 28;13(1):96. doi:10.3390/plants13010096
43. Peetla C, Stine A, Labhasetwar V. Biophysical Interactions with Model Lipid Membranes: Applications in Drug Discovery and Drug Delivery. Mol Pharm. 2009 Oct 5;6(5):1264–76. doi:10.1021/mp9000662
44. Agarwal U, Pannu A, Tonk RK, Jaiswal P, Jain K. The potential of xanthotoxin in the treatment of cognitive disorders: current insights and future perspectives. Futur J Pharm Sci. 2024 Oct 22;10(1):147. doi:10.1186/s43094-024-00717-y
45. Aguiar S, Borowski T. Neuropharmacological Review of the Nootropic Herb *Bacopa monnieri*. Rejuvenation Res. 2013 Aug;16(4):313–26. doi:10.1089/rej.2013.1431
46. Wu X, Zhou Y, Xi Y, Zhou H, Tang Z, Xiong L, et al. Polyphenols: Natural Food-Grade Biomolecules for the Treatment of Nervous System Diseases from a Multi-Target Perspective. Pharmaceuticals. 2024 Jun 13;17(6):775. doi:10.3390/ph17060775
47. Liy PM, Puzi NNA, Jose S, Vidyadaran S. Nitric oxide modulation in neuroinflammation and the role of mesenchymal stem cells. Exp Biol Med. 2021 Nov 9;246(22):2399–406. doi:10.1177/1535370221997052
48. Iova OM, Marin GE, Lazar I, Stanescu I, Dogaru G, Nicula CA, et al. Nitric Oxide/Nitric Oxide Synthase System in the Pathogenesis of Neurodegenerative Disorders—An Overview. Antioxidants. 2023 Mar 20;12(3):753. doi:10.3390/antiox12030753
49. Rodríguez-Yoldi MJ. Anti-Inflammatory and Antioxidant Properties of Plant Extracts. Antioxidants. 2021 Jun 7;10(6):921. doi:10.3390/antiox10060921