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# DEPRESSION-RELEVANT OXIDATIVE STRESS MODULATION BY *CLITORIA TERNATEA* FLOWER ETHANOLIC EXTRACT: ANTIOXIDANT CAPACITY AND $H_2O_2$-INDUCED NEURONAL INJURY RESCUE IN SH-SY5Y CELLS

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**ABSTRACT:**
Major depressive disorder is increasingly understood as a systems-level disorder in which oxidative stress, mitochondrial dysfunction, neuroinflammation, and impaired neuroplasticity interact to drive neuronal vulnerability. In this depression-relevant framework, we evaluated whether Clitoria ternatea flower ethanolic extract shows chemistry-supported antioxidant capacity and functional rescue against oxidative neuronal injury. The extract was prepared by 70% ethanolic maceration and characterized by physicochemical parameters (pH, specific gravity, alcoholic content, TDS), UV–Vis spectral profiling (λmax 312 nm), TLC fingerprinting (Rf 0.71, 0.59, 0.39, 0.32), qualitative phytochemical screening, GC–MS metabolite annotation, and FTIR functional group assignment. Antioxidant capacity was quantified using DPPH and ABTS radical scavenging assays and FRAP reducing power, using ascorbic acid as a reference. Neuronal protection was functionally validated in SH-SY5Y cells exposed to an $H_2O_2$ injury condition ($CTC_{50}$), followed by post-injury treatment with graded extract concentrations and MTT-based viability quantification. GC–MS highlighted phytosterols (β-sitosterol, stigmasterol) and fatty acids (palmitic acid, linoleic acid), while FTIR supported polyphenol- and lipid-associated functional groups. The extract showed strong DPPH activity, moderate ABTS activity, high reducing power in FRAP, and a protective low-dose window that improved viability under oxidative challenge, supporting oxidative stress modulation relevant to depression biology.

**KEYWORDS:** Oxidative stress; SH-SY5Y neuroblastoma; Clitoria ternatea

## INTRODUCTION

Depression is a complex neuropsychiatric disorder, in which classical neurotransmitter theories only partially explain the mechanisms underlying onset, heterogeneity, and treatment resistance. Accumulating evidence converges in support to a systems-biology framework of depression involving oxidative stress, mitochondrial dysfunction, inflammation and impaired neuroplasticity as intertwined pathogenic pathways9. Some of these elements, oxidative stress is implicated in a common mechanism that may cause cellular injury, amplify inflammatory signalling and disturb neuronal function. This mechanism is particularly relevant, since oxidative imbalance impairs synaptic and neuronal resilience leading to an aggravation of the cognitive and affective disturbances related to depression3.

Oxidative stress presents a discrepancy between generation of ROS and the efficacy of endogenous antioxidant defence. In neuronal networks, increased ROS generates lipid peroxidation, protein oxidation and nucleic acid degradation among mitochondria which also act both as a generator and a recipient of redox unbalance. Long-term oxidative stress might impair ATP generation, transmembrane potential and activate apoptotic signal pathways. Conversely, redox dysregulation can enhance pro-inflammatory signalling pathways including ROS-mediated transcriptional activation and inhibit neurotrophin-dependent synaptic plasticity5. Taken together, our results offer a biologically plausible reason to test redox-based interventions as potential neuroprotective strategies for depressive disorders that validate in models of neuronal oxidative stress.

Plant extracts are commonly comprised of polyphenols and flavonoids bearing electron-donor moieties, terpenoids that can interact with membranes, as well as phytosterols stabilizing lipid bilayers and inertia inflammatory pathways. From a biomanufacturing perspective, claims are not enough for establishing pharmacological viability; the product requires chemical fingerprinting to confirm consistent composition and functional assays to show measurable activity11. A unified protocol combining phytochemical screening, spectroscopic profiling, chromatographic assignment of metabolites, and biological validation offers a feasible methodology to define "chemistry-to-function" link in early neuroprotective studies.

Clitoria ternatea L.(Fabaceae) (butterfly pea) is well known in traditional medicine and has been recently scientifically evaluated for its claimed antioxidant, neuroactive activities on different plant parts and extraction procedures. Flower extracts, in particular, contain secondary metabolites that are potentially redox-relevant being amongst the most diverse

of the plant organs6. However, most studies have been limited to chemical profiling or antioxidant assays of isolated substances and the association between composition and neuronal protection under controlled oxidative conditions remain unclear. Filling this gap not only requires extensive characterization of metabolites, but also a functional injury-rescue paradigm in a neuronal cell model.

Human SH-SY5Y neuroblastoma cells are a neuronal-like model system that allows the evaluation of oxidative stress-induced cytotoxicity and protection. Hydrogen peroxide (H2O2) is widely used as an oxidative stressor because it increases intracellular ROS and dose-dependently reduces cell viability, which makes it possible to reproducibly set an injury threshold (e.g., a 50% viability state) and screen for agents that have a potentially protective role12. With the inclusion of standard antioxidant tests including DPPH radical scavenging, ABTS radical scavenging and ferric reducing antioxidant power (FRAP), SH-SY5Y $H_2O_2$ model allows to study the antioxidative activity not only based upon chemical reactivity but also by the ability to maintain neuronal viability under oxidative challenge.

In this context, we perform the current study on the ethanol extract of flowers of Clitoria ternatea using an integrated biotechnological approach. The latter combines qualitative phytochemical screening, Fourier transform infrared (FTIR) functional group fingerprinting and gas chromatography–mass spectrometry (GC–MS) metabolite profiling with in vitro antioxidant activities ((DPPH, ABTS, FRAP), and functional validation in an H2O2 challenged SH-SY5Y injury-rescue model using MTT-based viability quantification. The principal aim is to determine whether the extract has an antioxidant profile supported by chemistry, and if so, to what extent this profile corresponds with a reduction in oxidative neuronal injury in vitro using a depression-relevant cellular stress model. This platform provides the basis for follow-up pathway-resolution studies (such as intracellular ROS determination, mitochondrial membrane potential analysis, apoptosis markers profiling and examination of redox-regulated gene expression) that may enlighten mechanisms by which oxidative stress is modulated in depression.

## MATERIALS AND METHODS

### Chemicals and reagents

Dimethyl sulfoxide (DMSO), phosphate-buffered saline (PBS), fetal bovine serum (FBS), Dulbecco's Modified Eagle Medium (DMEM), MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide), ethanol, hydrogen peroxide ($H_2O_2$), methanol, potassium persulfate, acetate buffer, ascorbic acid, n-butanol, acetic acid, ferric chloride, ammonium hydroxide, sulfuric acid, hydrochloric acid, acetic anhydride, sodium hydroxide, chloroform, vanillin-sulfuric acid reagent, and 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) were purchased from Sigma Aldrich (USA), SRL Chemicals (India), and Merck (Germany). Silica gel GF254 plates for thin-layer chromatography (TLC) were obtained from Merck.

### Instrumentation and equipment

A UV–Vis spectrophotometer (PerkinElmer Lambda 35, Germany) was used for absorbance measurements. A calibrated pH meter, specific gravity apparatus, and analytical balance were used for physicochemical assessments. Total dissolved solids (TDS) were measured using electrochemistry meters and probes. An oven was used for drying plant samples during moisture content estimation. GC–MS analysis was performed on an Agilent GC 7890A / MS 5975C system equipped with an Agilent DB5MS capillary column (30 m × 0.25 mm i.d. × 0.25 μm film thickness) and compound identification was carried out using the NIST library. FTIR spectra were recorded using a Thermo Fisher Nicolet iS5 FTIR spectrometer over 550–4000 $cm^{-1}$ for functional group identification.

### Plant material authentication

Clitoria ternatea specimens were collected from Vengampatti, Mallur, Salem, Tamil Nadu, India. The plant material was authenticated by Dr. P. Radha, Research Officer (Botany) Sci II & I/c, Siddha Medicinal Plants Garden (Central Council for Research in Siddha, Ministry of Ayush, Govt. of India), Mettur Dam, Salem District, Tamil Nadu (Authentication code: C060224117T).

### Cell line and culture conditions

SH-SY5Y human neuroblastoma cells were procured from the National Centre for Cell Science (NCCS), Pune, India. Cells were maintained in DMEM supplemented with 10% FBS, 1% penicillin-streptomycin antibiotic solution, and 1% non-essential amino acids in a humidified incubator at 37°C with 5% $CO_2$.

### Preparation of ethanolic extract

Fresh Clitoria ternatea flowers were washed thoroughly and shade-dried for one week. Dried flowers were powdered using a laboratory blender. The powdered material (100 g) was macerated with 70% ethanol (1:10 w/v) at room temperature for 72 h with intermittent shaking. The mixture was filtered through Whatman No. 1 filter paper and the filtrate was concentrated under reduced pressure at 40°C using a rotary evaporator. The concentrated extract was stored at 4°C until use.

### Physicochemical analysis

The physicochemical properties of the ethanolic extract were evaluated. For pH measurement, a 10 mL aliquot of the tincture was diluted in 100 mL distilled water and pH was recorded using a calibrated pH meter in triplicate. Specific gravity was assessed using a distillation-based approach: 25 mL sample was distilled with an equal volume of water, and the density of the distillate was measured using a pycnometer. Total dissolved solids (TDS) were determined by drying

samples to constant weight and weighing the residue, with additional validation using electrochemistry meters and probes where applicable.

**UV–Vis spectroscopy**
UV–Vis analysis was carried out using a UV–Vis spectrophotometer (PerkinElmer Lambda 35). The extract was scanned from 250–500 nm at defined intervals over 24 h to record absorbance features and assess spectral stability.Major absorbance peaks were documented.

**Thin-layer chromatography (TLC)**
TLC profiling was performed using silica gel GF254-coated plates as the stationary phase. Two mobile phase systems were used: (i) Toluene:Ethyl acetate:Formic acid (5:4.5:0.5) and (ii) n-Butanol:Acetic acid:Water (5:1:4). Samples were spotted and plates were developed in a saturated chamber. After development and drying, spots were visualized and Rf values were recorded along with spot colors.

**Qualitative phytochemical screening**
Preliminary phytochemical screening was performed using standard qualitative methods to identify major phytochemical classes. Alkaloids were assessed using Mayer's test; flavonoids using sulfuric acid-based reaction; steroids using Liebermann–Burchard test; terpenoids using Salkowski test; anthraquinones using hydrochloric acid and ammonia reaction; phenols using ferric chloride test; saponins by frothing test; tannins using ferric chloride; carbohydrates using Fehling's test; and oils/resins using filter paper transparency test. Results were recorded as present (+) or absent (−) based on characteristic reactions.

**GC–MS analysis**
GC–MS profiling of the ethanolic extract was performed using an Agilent GC 7890A system coupled to an MS 5975C detector. Separation was achieved using an Agilent DB5MS column (30 m × 0.25 mm × 0.25 µm). Helium was used as carrier gas at 1 mL/min. Injection volume was 1 µL in split mode (10:1). Oven temperature program: 60°C for 2 min; ramp 5°C/min to 280°C; hold 10 min. Mass spectra were acquired over 50–500 m/z under electron impact (EI) ionization at 70 eV. Samples were diluted appropriately and filtered through a 0.22 µm membrane filter before injection. Compound identification was based on NIST library matching; retention time, match score, and peak area percentage were recorded and used to report relative abundance. Peaks with low match quality were treated as tentative identifications.

**FTIR spectroscopy**
FTIR analysis was conducted using a Thermo Fisher Nicolet iS5 FTIR spectrometer. Spectra were recorded over 4000–500 $cm^{-1}$ at 4 $cm^{-1}$ resolution with 32 scans per sample. The dried extract was mixed with KBr and compressed into a pellet for analysis. Functional group assignments were performed by comparing observed peaks with standard IR absorption bands (for example O–H, C–H, C=O, N–H, C=C, C–O/C–O–C), emphasizing groups relevant to antioxidant and neuroprotective potential.

**Preparation of stock and working solutions**
A primary stock solution of Clitoria ternatea ethanolic extract (1 mg/mL) was prepared in dimethyl sulfoxide (DMSO) and used as the master stock for all in vitro experiments. Fresh working dilutions were prepared immediately before each assay. For the DPPH radical scavenging assay, the stock was diluted in methanol to obtain 40, 80, 120, 160, and 200 µg/mL. For the ABTS radical scavenging assay, dilutions were prepared in ethanol to achieve 25, 50, 75, 100, and 125 µg/mL. For the FRAP assay, the extract was diluted to 25, 50, 75, 100, and 125 µg/mL using phosphate buffer (pH 7.4) as per the working protocol (distilled water was used where buffer dilution was not applied). For the MTT-based cytotoxicity and oxidative injury rescue experiments, working concentrations of 50, 250, 500, 750, and 1000 µg/mL were prepared in complete DMEM supplemented with 10% fetal bovine serum (FBS). The final solvent concentration was kept constant across all treatment and control groups to avoid vehicle-driven variability.

**DPPH radical scavenging assay**
DPPH scavenging activity was measured following Brand-Williams et al. (1995) with minor modifications. A 500 µM DPPH solution was prepared in methanol. Reaction mixtures contained 100 µL DPPH solution and extract at 40–200 µg/mL; volume was adjusted to 1 mL with ethanol. Mixtures were prepared in triplicate and incubated for 30 min at room temperature in the dark. Absorbance was measured at 517 nm. Percent scavenging was calculated:

$$\text{Scavenging activity (\%)} = \left[\frac{\text{Absorbance (Control)} - \text{Absorbance(Sample)}}{\text{Absorbance (Control)}}\right] * 100$$

$IC_{50}$ values were estimated from nonlinear regression of the dose–response curve1.

**ABTS radical scavenging assay**

ABTS scavenging activity was assessed according to Re et al. (1999) with minor modifications. The ABTS radical cation ($ABTS^{\bullet+}$) was generated by reacting 7 mM ABTS with 2.45 mM potassium persulfate and incubating the mixture for 12–16 h in the dark at room temperature. The resulting $ABTS^{\bullet+}$ solution was diluted with ethanol to obtain an absorbance of 0.70 ± 0.02 at 734 nm. The reaction mixture consisted of 900 µL of $ABTS^{\bullet+}$ solution and the extract at 25, 50, 75, 100, and 125 µg/mL, with the final volume adjusted to 1 mL using ethanol. Samples were incubated at room temperature in the dark for 3 h, and absorbance was recorded at 734 nm. Percent scavenging and $IC_{50}$ values were calculated as described for the DPPH assay.

**FRAP reducing power assay**

FRAP assay was performed according to Benzie and Strain (1996). FRAP reagent was freshly prepared using 300 mM acetate buffer (pH 3.6), 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ in a 10:1:1 ratio and prewarmed to 37°C. Reaction mixtures contained 300 µL FRAP reagent and extract at 25–125 µg/mL, made up to 1 mL with distilled water. After incubation at 37°C for 15 min, absorbance was measured at 593 nm. Results were expressed as absorbance (reducing power) across concentrations (or $Fe^{2+}$ equivalents if a standard curve is included).

**MTT cytotoxicity assay and $H_2O_2$ injury optimization**

SH-SY5Y cells were seeded into 96-well plates at $1 \times 10^5$ cells/mL and allowed to adhere for 24 h. To determine cytotoxicity, cells were treated with extract (50, 250, 500, 750, 1000 µg/mL) or $H_2O_2$ (50–1000 µg/mL) for 24 h. After treatment, 20 µL of MTT (5 mg/mL in PBS) was added to each well and incubated for 4 h at 37°C. Medium was removed, and formazan crystals were dissolved in 150 µL DMSO. Absorbance was measured at 540 nm. Cell viability was calculated:

$$\text{Cell Viability } (\%) = \left[\frac{\text{Test OD}}{\text{Control OD}}\right] * 100$$

The $CTC_{50}$ (50% cytotoxic concentration) for $H_2O_2$ was determined by dose–response curve fitting and used as the oxidative injury concentration for the rescue experiment1.

**Neuroprotection (injury-mitigation) experiment**

To evaluate injury mitigation, cells were first treated with $H_2O_2$ at its $CTC_{50}$ concentration for 24 h to induce oxidative damage, followed by treatment with the extract (50, 250, 500, 750, 1000 µg/mL). Cell viability was then quantified using the MTT assay as described above. Protective effect was expressed as the percent increase in viability compared to the $H_2O_2$-only injury control.

**Statistical analysis**

All experiments were performed in triplicate and results are presented as mean ± SD. Statistical analyses were performed using GraphPad Prism (version 9.0). Group comparisons were conducted using one-way ANOVA followed by Tukey's post hoc test for multiple comparisons; $p < 0.05$ was considered statistically significant. $IC_{50}$ values for DPPH and ABTS were calculated using nonlinear regression.

**Results and Discussion**

**Physicochemical characterization**

The physicochemical analysis of Clitoria ternatea ethanolic extract revealed that the pH was 5.44 ± 0.19, indicating a mildly acidic nature. The specific gravity was 0.89 ± 0.06, which is consistent with typical ethanolic extracts. The alcoholic content was 6.11 ± 0.06%, and the total dissolved solids (TDS) were 4.94 ± 0.07% (w/v), indicating moderate solubility of the extract components. These baseline parameters support extract reproducibility and help contextualize spectroscopic stability and assay performance.

**UV–Vis spectral signature**

The UV–Vis absorption spectrum of the Clitoria ternatea ethanolic extract showed a prominent peak at 312 nm, indicating the presence of UV-active phytochemicals likely contributing to redox activity. The spectrum is presented as Figure 1.

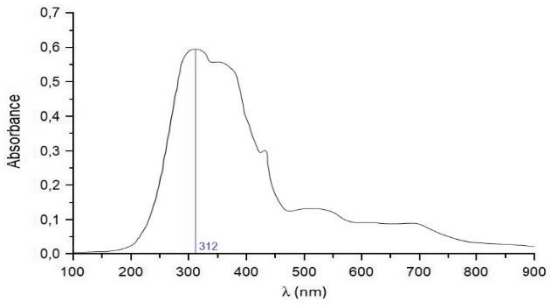


**Figure 1: UV–Vis spectrum of extract (peak at 312 nm)**

**TLC chemical fingerprint**

Thin-layer chromatography (TLC) of Clitoria ternatea ethanolic extract was performed under normal and UV light using the mobile phase n-butanol: acetic acid: water (5:1:4). Four bands were resolved with Rf values of 0.71, 0.59, 0.39, and 0.32, indicating multiple phytochemical constituents and confirming extract chemical diversity. This fingerprint supports the multi-component nature of the extract, which is relevant when interpreting concentration-dependent bioactivity patterns13.

**Qualitative phytochemical screening**

Phytochemical screening revealed the presence of flavonoids, steroids, terpenoids, phenols, carbohydrates, and oils & resins, while alkaloids, anthraquinones, saponins, and tannins were absent. The presence of flavonoids and phenolic compounds is consistent with antioxidant potential, while steroidal/terpenoid and lipid-associated fractions support plausibility for membrane-associated cytoprotection during oxidative injury2.

**GC–MS metabolite fingerprinting**

GC–MS analysis enabled tentative annotation of volatile and semi-volatile constituents and provided a metabolite-level fingerprint for the Clitoria ternatea ethanolic extract. Major peaks corresponded predominantly to phytosterols (stigmasterol and β-sitosterol) and fatty acids (n-hexadecenoic acid/palmitic acid and 9,12-octadecadienoic acid/linoleic acid), along with a lipophilic hydrocarbon fraction (E,Z-1,3,12-nonadecatriene)8. These constituents are frequently reported in plant extracts with antioxidant and cytoprotective properties and support biological plausibility for redox-linked neuronal protection. The high-confidence GC–MS identifications (retention time, peak area %, library hit, and match score/quality) are summarized in Table 1, and representative 2D chemical structures of key annotated constituents are presented in Figure 2. For analytical rigor, peaks with moderate or low library match can reflect co-elution, background contaminants, or common analytical species; therefore, such annotations should be considered tentative unless validated using solvent blanks, replicate runs, and, where feasible, authentic standards. Mechanistically, phytosterols are relevant because they are membrane-active metabolites widely discussed for anti-inflammatory and neuroprotective potential, while fatty acids contribute to lipid-phase redox biology and membrane dynamics, supporting a lipophilic extract fraction that may influence cellular stress responses7. When interpreted alongside the UV/TLC/FTIR fingerprints, the GC–MS profile provides chemical context consistent with the radical scavenging (DPPH/ABTS) and reducing power (FRAP) patterns observed in this study.

**Table 1. GC–MS metabolite fingerprint of Clitoria ternatea flower ethanolic extract (high-confidence hits only)**

| RT (min) | Peak area (%) | Identified compound (NIST) | Match quality |
|---|---|---|---|
| 22.762 | 7.17 | β-Sitosterol | 95 |
| 21.973 | 3.38 | Stigmasterol | 99 |
| 13.108 | 2.56 | n-Hexadecanoic acid (Palmitic acid) | 98 |
| 14.252 | 1.42 | 9,12-Octadecadienoic acid (Linoleic acid) | 98 |
| 17.330 | 1.62 | E,Z-1,3,12-Nonadecatriene | 95 |

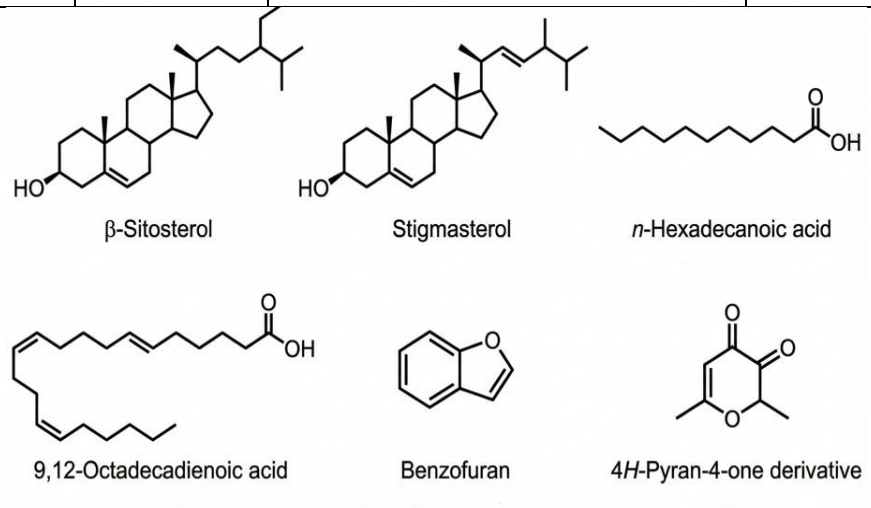


**Figure 2: 2D structures of key GC–MS-annotated constituents**

**FTIR functional group profiling**

FTIR spectroscopy provided a rapid functional group fingerprint of the extract and supported the presence of chemical features associated with antioxidant and cytoprotective phytochemistry. The spectrum showed broad bands attributable to hydroxyl groups (O–H stretching) consistent with polyphenols/flavonoids, aliphatic C–H stretching consistent with lipid/terpenoid fractions, carbonyl-related bands (C=O region) consistent with conjugated systems or ester/ketone functionalities, and C–O/C–O–C stretching bands often associated with glycosidic linkages and phenolic ethers. Together, these bands support the qualitative phytochemical results and complement GC–MS annotations by providing class-level confirmation across polar and semi-polar constituents10. Key FTIR peaks and assignments are summarized in Table 2.

**Table 2. FTIR peak assignments of Clitoria ternatea flower ethanolic extract**

| Wavenumber ($cm^{-1}$) | Functional group assignment | Likely contributing class |
|---|---|---|
| 3328.53 | O–H stretching (broad, H-bonded) | Phenols, flavonoids |
| 2973.70 | Aliphatic C–H stretching | Lipids, terpenoids, sterols |
| 2884.99 | Aliphatic C–H stretching | Lipids/terpenoids |
| 1654.63 | C=O / C=C region (conjugated/aromatic) | Polyphenols, conjugated systems |
| 1380.78 | C–H bending / phenolic O–H bending | Phenolics/flavonoids |
| 1272.79 | C–O stretching (phenols/esters) | Phenolics, glycosides |
| 1087.66 | C–O / C–O–C stretching | Carbohydrates, glycosides |
| 1045.23 | Strong C–O / C–O–C stretching | Carbohydrates/glycosides |
| 879.38 | Aromatic C–H out-of-plane / glycosidic region | Aromatics/glycosides |
| 802.24 | Aromatic C–H out-of-plane | Aromatic rings (phenolics) |

**Antioxidant capacity**

The extract demonstrated concentration-dependent antioxidant activity across three complementary assays, providing convergent evidence of redox activity. In the DPPH radical scavenging assay, Clitoria ternatea showed a dose-dependent increase in inhibition, with a maximum inhibition of 93.4% at 200 µg/mL, comparable to ascorbic acid (93%) at the same concentration, indicating strong free-radical neutralization capacity. In the ABTS assay, the extract exhibited moderate radical scavenging, reaching 69.29% inhibition at 125 µg/mL, while ascorbic acid reached 92.7%, indicating higher activity of the reference standard in this system. In the FRAP assay, the extract demonstrated strong ferric reducing power with maximum absorbance of 0.819 at 125 µg/mL, slightly higher than ascorbic acid (0.795), suggesting comparatively stronger reducing capacity under the assay conditions. All three antioxidant plots will be presented as a single composite figure: Figure 3a (DPPH), Figure 3b (ABTS), and Figure 3c (FRAP). From a depression-relevant mechanistic perspective, these assays matter because oxidative stress is a consistently implicated biochemical axis in depression-associated neuronal vulnerability3. The agreement between radical scavenging (DPPH/ABTS) and reducing power (FRAP) supports that the extract contains multiple redox-active constituents, consistent with its phenolic/flavonoid signatures and GC–MS metabolite profile.

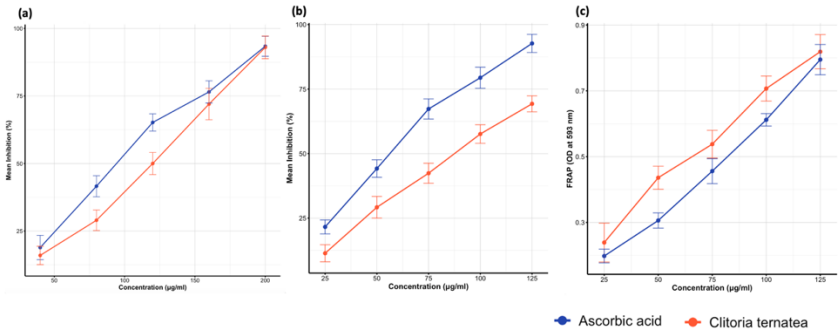


**Figure 3:Antioxidant activity of Clitoria ternatea ethanolic extract compared to ascorbic acid. (a) DPPH inhibition assay, (b) ABTS inhibition assay, (c) FRAP assay. Data are presented as mean ± SD. Clitoria ternatea shows comparable DPPH inhibition, moderate ABTS activity, and stronger FRAP activity at higher concentrations.**

**SH-SY5Y oxidative injury rescue**

A neuronal oxidative stress paradigm is relevant because depression biology frequently intersects with oxidative damage, mitochondrial stress, and impaired stress adaptation in neuronal systems. SH-SY5Y cells provide a human-derived neuronal-like platform that is experimentally tractable for modelling oxidative injury and quantifying cytoprotection. $H_2O_2$ challenge is a standardized oxidative insult that increases intracellular ROS burden and produces dose-dependent loss of viability, allowing functional testing of redox-active interventions in a controlled injury-rescue design4.

**Viability outcomes and interpretation**

The MTT assay showed that the Clitoria ternatea ethanolic extract improved SH-SY5Y viability at lower concentrations under $H_2O_2$-induced oxidative stress. At 50 µg/mL, viability increased to 70.14% relative to the $H_2O_2$ injury control (50%), indicating a protective effect within a lower-dose window. At higher concentrations (750 and 1000 µg/mL), viability decreased, suggesting concentration-associated cytotoxicity at the upper range. This dose pattern is consistent with a protective window followed by reduced viability at high exposure, a common feature of complex plant extracts where protective redox effects can be outweighed by non-specific cytotoxicity beyond an optimal concentration range. The MTT dose-response (control vs $H_2O_2$ vs extract concentrations) will be shown as Figure 4, and representative SH-SY5Y morphology images (control, $H_2O_2$-injured, and protective dose condition) will be presented as Figure 5. Taken together, these results provide functional validation that the extract can mitigate oxidative neuronal injury in a depression-relevant cellular stress paradigm, supported by chemical fingerprinting (GC–MS, FTIR) and convergent antioxidant capacity (DPPH/ABTS/FRAP).

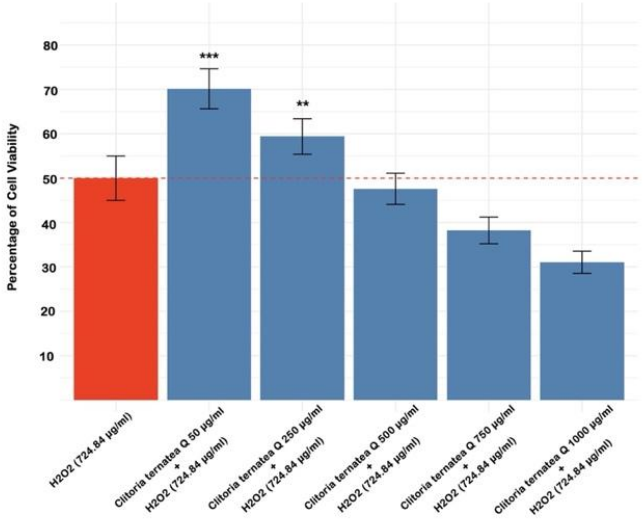


**Figure 4: MTT assay showing the effect of Clitoria ternatea ethanolic extract on $H_2O_2$-induced cytotoxicity in SH-SY5Y cells. Statistical significance vs $H_2O_2$ control was evaluated using one-way ANOVA followed by Tukey's post hoc test.**

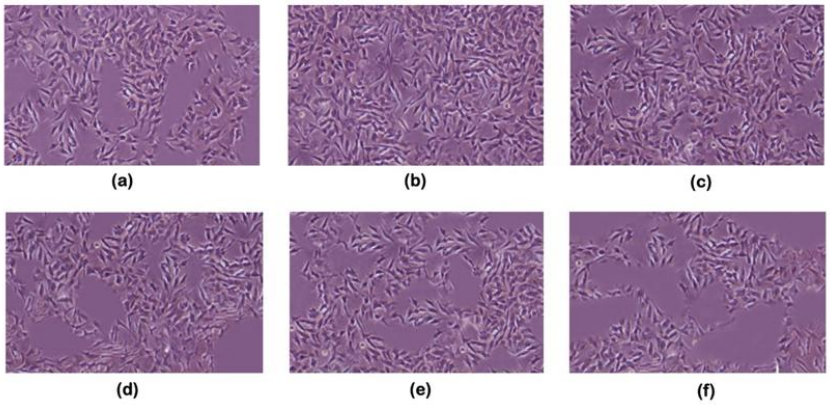


**Figure 5: Effect of Clitoria ternatea flower ethanolic extract on SH-SY5Y cells under $H_2O_2$-induced oxidative stress. Microscopic images: (a) $H_2O_2$ alone (reduced viability); (b) 50 µg/mL + $H_2O_2$ (protective); (c) 250 µg/mL + $H_2O_2$ (enhanced protection); (d) 500 µg/mL + $H_2O_2$; (e) 750 µg/mL + $H_2O_2$; (f) 1000 µg/mL + $H_2O_2$. Lower concentrations (b, c) show protection, while higher concentrations (e, f) show reduced viability.**

## CONCLUSION

This study establishes that Clitoria ternatea flower ethanolic extract possesses a chemically supported antioxidant profile and a measurable capacity to mitigate oxidative neuronal injury in a depression-relevant cellular stress model. Physicochemical characterization and UV–Vis/TLC fingerprinting supported extract consistency and multi-component composition, while GC–MS and FTIR profiling confirmed the presence of redox-active and membrane-associated phytochemical signatures, including phytosterols, fatty acids, and phenolic-like scaffolds. Functionally, the extract demonstrated strong radical scavenging and reducing power across DPPH, ABTS, and FRAP assays and produced a clear protective window in $H_2O_2$-challenged SH-SY5Y cells, improving viability at lower concentrations while showing

reduced viability at higher doses. Together, these integrated chemical and biological data support the extract as a promising candidate for oxidative stress modulation relevant to depression-associated neuronal vulnerability and justify further mechanistic validation using intracellular ROS, mitochondrial function, and apoptosis-related endpoints.

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