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ANTIOXIDANT AND ANTIPARKINSONIAN ACTIVITY OF ETHANOLIC EXTRACT OF *CLITORIA TERNATEA.*

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Abstract

This study investigates the antioxidant and antiparkinsonian potential of the leaf extract of Clitoria ternatea. Oxidative stress is a key contributor to the pathogenesis of Parkinson's disease, and natural antioxidants derived from medicinal plants offer promising neuroprotective strategies, the ethanolic extract demonstrated significant free radical scavenging activity. Total phenolic and flavonoid contents were quantified and in vitro antioxidant activity of extracts was determined through DPPH scavenging, catalase assay, ferric reducing antioxidant power assay. This study comprehensively evaluated the phytochemical composition, antioxidant potential, and neuroprotective properties of Clitoria terntea leaf extracts. Phytochemical screening and Soxhlet extraction revealed substantial total phenolic content (46.78 mg GAE/g) and moderate flavonoid levels (15.79 mg QE/g). Antioxidant activity was assessed using DPPH assay and hydrogen peroxide radical scavenging assays, ferric chloride antioxidant power assay, ABTS+ scavenging assay which demonstrated notable free radical scavenging capacity. Molecular docking experiments further supported the potential anti-parkinsonian effects of Clitoria Ternatea bioactive constituents, aligning with observed neuroprotective outcomes in experimental models. These findings provide a scientific basis for the traditional use of Clitoria Ternatea in herbal medicine and underscore its promise as a natural source of antioxidants for managing neurological disorders, particularly those linked to oxidative stress. The results advocate for further investigation into the therapeutic applications and mechanisms of Clitoria Ternatea in the context of neurodegenerative disease management.

Keywords: *Flavonoid, Clitoria Ternatea, antioxidant, DPPH and hydrogen peroxide radical scavenging.*

INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by motor dysfunction, caused mainly by the degeneration of dopaminergic neurons in the substantia nigra. One of the major factors involved in PD pathogenesis is oxidative stress (1). *Clitoria ternatea*, commonly known as butterfly pea, a perennial leguminous vine in the *Fabaceae* family, is traditionally used for its medicinal properties. Phytochemical analysis reveals the presence of *pentacyclic triterpenoids (taraxerol, taraxerone), proteins, carbohydrates, alkaloids, flavonoids, saponins, tannins, and ternatins,* supporting its diverse pharmacological activities. As the plant is rich in flavonoids and anthocyanins compounds known for antioxidant activity. This study evaluates the potential of its leaf extract to counter oxidative stress and Parkinsonian symptoms in animal models (2).

Materials And Methods

Fresh *Clitoria Ternatea* leaves from Kuzhalmannam, Palakkad were collected, washed, shade-dried, and ground into coarse powder for storage and analysis. Botanical identification was confirmed morphologically.

Physicochemical And Phytochemical Analysis

Hydroalcoholic leaf extracts underwent standard phytochemical screening to detect secondary metabolites using colorimetric assays. Key physicochemical parameters included:

- **Loss on Drying:** Determined by drying 10 g of powder at 105°C for one hour to assess moisture content.
- **Ash Value:** Total mineral content was measured by incinerating 1 g of dried sample at 100°C.
- **Acid-Insoluble Ash:** Ash treated with 2M HCl and heated at 500–600°C to quantify siliceous impurities.
- **Water-Soluble Ash:** Ash mixed with water, filtered, and residue dried at 400–500°C to estimate inorganic salts.
- **Alcohol-Soluble Extractive:** Five grams of sample macerated in alcohol for 18 hours, filtered, and extractive value calculated to evaluate alcohol-soluble constituents.

The extractive value provides insights into the solubility and potential bioactive compounds present in the sample. This information is crucial for understanding the chemical properties and possible applications of the material in various fields, including pharmaceuticals and food science (2).

Hydroalcoholic Extraction

Approximately 25 g of powdered sample was extracted in a Soxhlet apparatus with hydroalcoholic solvent at 40–60°C for seven hours (3). The process was complete when the siphon tube solvent cleared. Extracts and filter paper were oven-dried for three hours before weighing.

These methods provide a reliable assessment of the physicochemical quality and phytochemical profile of *Clitoria Ternatea* leaves, supporting further pharmacological research and quality control

Preliminary Phytochemical Analysis

Qualitative phytochemical screening of *Clitoria Ternatea* leaf extracts confirmed the presence of key secondary metabolites, including alkaloids, carbohydrates, glycosides, proteins, amino acids, flavonoids, phenolic compounds, tannins, saponins, lignins, quinones, anthraquinones, coumarins, and resins. Identification was based on characteristic color changes or precipitate formation in standard assays, indicating a diverse phytochemical profile.

Estimation of Phenolic and Flavonoid Content

1. **Total Phenolic Content:** The Folin-Ciocalteu method was used, with absorbance measured at 765 nm. Results were expressed as milligrams of gallic acid equivalents (mg GAE/g) per gram of extract, reflecting the overall phenolic concentration.
2. **Total Flavonoid Content:** Quantification was performed using an aluminum chloride colorimetric assay, with absorbance read at 415 nm. Values were reported as milligrams of quercetin equivalents (mg QE/g) per gram of dry extract.(3)

These methods provide a reliable assessment of the phenolic and flavonoid content, which are important indicators of the antioxidant capacity and potential health benefits of the extracts. Further analysis may also explore the relationship between these compounds and their biological activities.

Herbal Extract's Antioxidant Activity

Radical Scavenging Activity DPPH

The DPPH assay is a method used to evaluate antioxidants' ability to scavenge free radicals in herbal extracts. It measures the presence of a persistent violet free radical, which is neutralized by an antioxidant.(4)

The DPPH assay uses 1,1-diphenyl-2-picryl as the principle to demonstrate free radical scavenging activity. The equation (DPPH) + (H-A), DPPH-H + (A), illustrates the reaction between DPPH and the antioxidant (H-A). The extent of discoloration determines the function of extracts or antioxidant compounds in scavenging hydrogen. Plant extracts are mixed with DMSO, DPPH solution is added, and absorbance is measured. The percentage of radical scavenging activity is calculated.(5)

Catalase Activity

Catalase is an antioxidant enzyme that breaks down hydrogen peroxide into water and oxygen. It is produced by some pathogens to defend against oxidative stress and hydrogen peroxide attacks, which are used by the host's immune system.

(6) A catalase-deficient mutant pathogen is more susceptible to oxidative stress and immune cell assaults. Catalase activity is determined by spectrophotometry, determining the hydrogen peroxide concentration in the solution at 240 nm.
The study used a phosphate buffer and 0.036% H2O2 to analyse enzyme activity. The absorbance ranged from 0.52 to 0.55 units. The enzyme activity was determined using spectrophotometry and a blank solution. The enzyme activity revealed a strong antioxidant system. The rate of reaction was determined using the initial linear section of the curve and the Extinction Coefficient.(7)

ABTS + Scavenging Activity

The study uses the radical cation 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+) test to evaluate the antioxidant activity of plant extracts. Through a reaction between ABTS and potassium persulfate, a blue/green ABTS chromophore is produced. Depending on the antioxidant activity, concentration, and reaction time, the radical cation is reduced to ABTS when antioxidants are added.(8)
Three distinct samples are used in the procedure to measure the concentrations of a diluted ABTS solution. (6) Ethanol-based Trolox standards should have a final concentration of 0–15 μM. The absorbance at 734 nm is determined with a UV-Vis spectrophotometer. By applying the Trolox standard curve equation Y=0.012x+2, the Trolox Equivalent Antioxidant Capacity (TEAC) is utilised to establish a connection between the Trolox concentration and 10% resistance. This relationship allows for the quantification of the antioxidant capacity of the ABTS solution in relation to the (9) Trolox standards. Consequently, the TEAC values obtained can provide insight into the antioxidant potential of various samples tested against the established Trolox curve.

Ferric Reducing Antioxidant Power (FRAP) Assay

The study's objective is to assess the plant extract's ability to reduce ferric acid by employing a violet-coloured solution synthesis approach. A combination of TPTZ, $FeCl_3$, and acetate buffer is the reagent employed, and it is incubated for 30 minutes at 37°C. "$FeSO_4$•7 H_2O" is the average.
The procedure uses reagents such as TPTZ, $FeCl_3 \cdot 6H_2O$, and acetate buffer to reduce a ferric 2, 4, 6-tripyridyl-s-triazine complex to its ferrous form. TPTZ, $FeCl_3 \cdot 6H_2O$, and acetate buffer are combined to generate the functioning FRAP reagent. The FRAP value is expressed as mmol/100 g, and the absorbance at 593 nm is measured at 4 minutes.(10)

Molecular Docking Studies

A common method in drug development is molecular docking, which involves locating ligands inside target binding sites. It calculates the free energy of receptor-ligand binding for every conceivable configuration. Using programmes like BIOVIA Discovery Studio Visualizer and Auto Dock Vina 4.2.6, small compounds are docked into the binding site of the receptor.
A C programme called AutoDock models the interactions between macromolecules and flexible ligands. (10) By offering molecular coordinates for docked ligand conformations using AutoDock Vina and a Lamarckian evolutionary method for energy estimations, it facilitates theoretical ligand design and research. Download 3D crystal structures, draw ligand structures, specify binding locations, extract ligands, save altered proteins, and determine binding affinity in order to use AutoDock.(11)

Result And Discussion

Preliminary Phytochemical Analysis

Result of Preliminary Phytochemical Analysis

CONSTITUENTS	SCREENED PLANTS	INFERENCE
Phenols	+ve	Phenols present
Flavanoids	+ve	Flavanoids present
Tannis	+ve	Tannis present
Glycosides	+ve	Glycosides present
resins	+ve	resins present

The colour of the solution and precipitate suggested the presence of large concentrations of tannins, flavonoids, phenols, glycosides, and resins, according to the phytochemical analysis of *Clitoria Ternatea* leaves.

Physicochemical Parameters Of Leaves *Clitoria Ternatea*

PHYSICOCHEMICAL PARAMETER	OBSERVATIONS
Loss on drying	6.40
Total ash	13.2 ± 3.49

Acid insoluble ash	4.8 ± 2.16
Alcohol soluble extractive value	18.4
Water soluble extractive value	25.2
pH	5.2.

In order to determine the physicochemical analysis of *Clitoria ternatea,* the study used water-soluble extractive value, alcohol-soluble extractive value, pH, Total Ash value, and acid-insoluble ash. 5.2 was found to be the pH.

Determination Of Phenolic Content

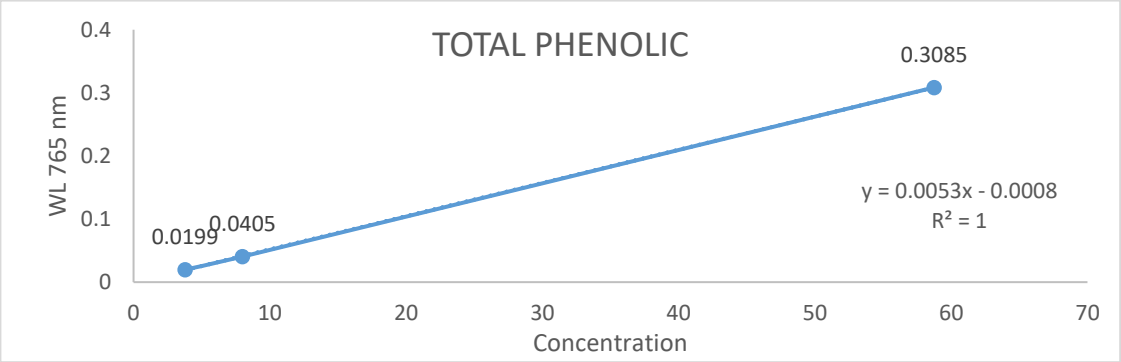
The graph shows *Clitoria ternatea* total phenolic content of 46.78 mg GAE/g, indicating a substantial amount of phenolic content in the sample.

STANDARD (CONC.)	CONCENTRATION	WL 765
standard 50	50	0.25813
standard 75	75	0.40109
standard 100	100	0.51921

Phenolic Standard Value

SAMPLE (CONC.)	CONCENTRATION	WL 765
sample 50	3.786	0.0199
sample 100	8	0.0405
sample 1000	58.7539	0.3085

Phenolic Sample Value



Phenolic Sample Value

Determination Of Flavanoid Content

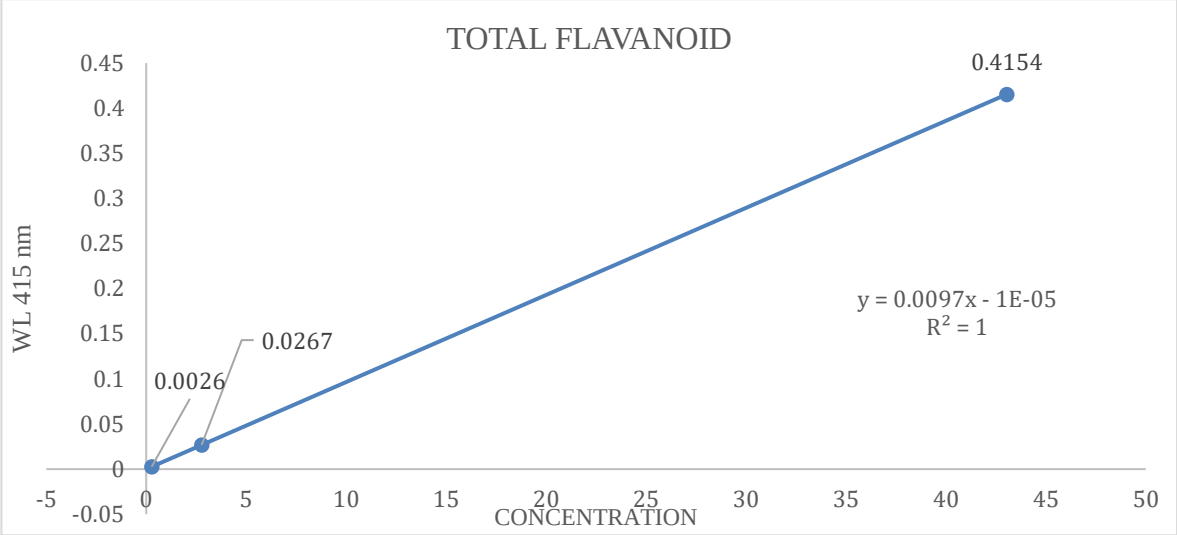
With a QE/g of 15.79, which indicates trace levels of flavonoids, the graph shows the total flavonoid content of *Clitoria ternatea*, expressed in mg of QE/g.

STANDARD ID	CONCENTRATION	WL 415
standard 30	30	0.26958
standard 40	40	0.35846
standard 50	50	0.53606

Flavonoid Standard Value

SAMPLE ID	CONCENTRATION	WL 415
sample 100	0.2703	0.0026
sample 500	2.7682	0.0267
sample 1000	43.043	0.4154

Flavonoid Sample Value



Flavonoid Sample Value

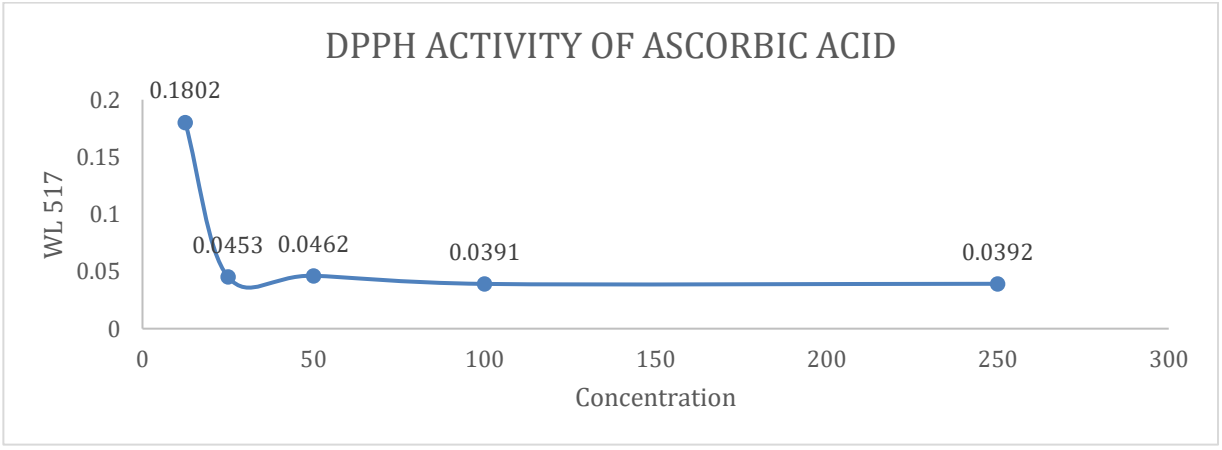
Antioxidant Activity

DPPH Radical Scavenging Activity

The study evaluates the antioxidant activity of *Clitoria Ternatea* hydro-alcoholic extract against DPPH radicals. Results indicate that the extract's scavenging activity increases in a concentration-dependent manner. The IC50 value, or the concentration needed to block 50% of DPPH radicals, indicates the antioxidant strength of the extract. The primary antioxidant component is phenol, and a rise in antioxidant activity is strongly correlated with its overall concentration.

CONCENTRATION	WL 517
12.5	0.1802
25	0.0453
50	0.0462
100	0.0391
250	0.0392

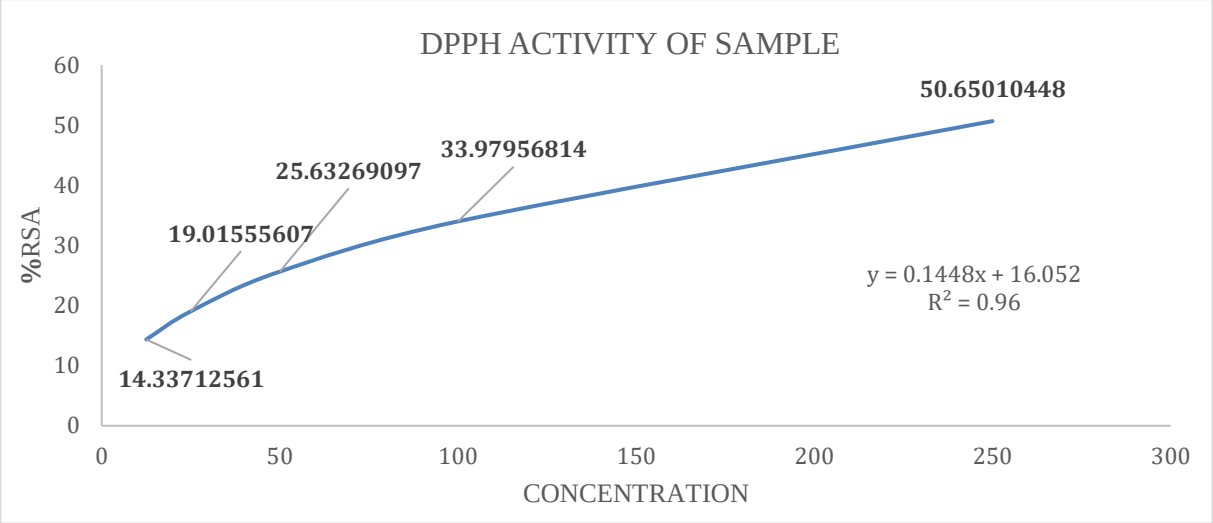
Standard Ascorbic Acid Value



Ascorbic Acid DPPH Activity

CONCENTRATION (µG/ML)	OD AT 517	%INHIBITION
12.5	0.7379	14.33712561
25	0.6976	19.01555607
50	0.6406	25.63269097
100	0.5687	33.97956814
250	0.4251	50.65010448

DPPH Activity of sample

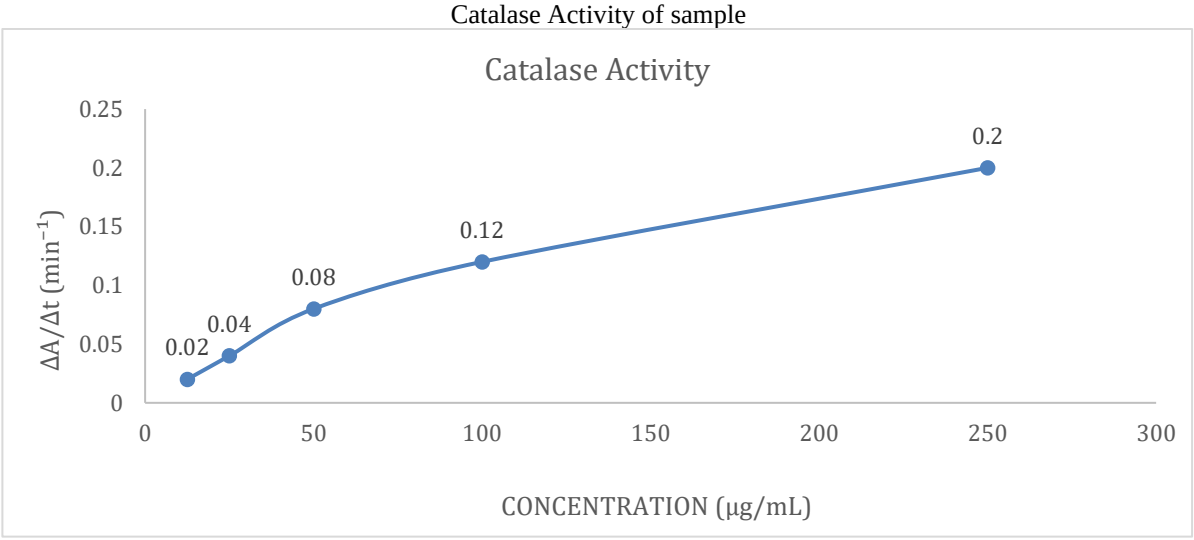


Catalase Activity

Clitoria Ternatea extracts exhibit catalase antioxidant activity, which increases with concentration, indicating a dose-dependent relationship. (12) The enzyme effectively decomposes hydrogen peroxide into water and oxygen, demonstrating its potent antioxidant properties. This enzyme is crucial in reducing hydrogen peroxide and protecting cells from oxidative damage.

CONCENTRATION (µg/ml)	ΔA/ΔT (min^{-1})	CATALASE ACTIVITY (u/ml)
12.5	0.02	0.0138
25	0.04	0.0275
50	0.08	0.0550
100	0.12	0.0825
250	0.20	0.1375

Catalase Activity of sample



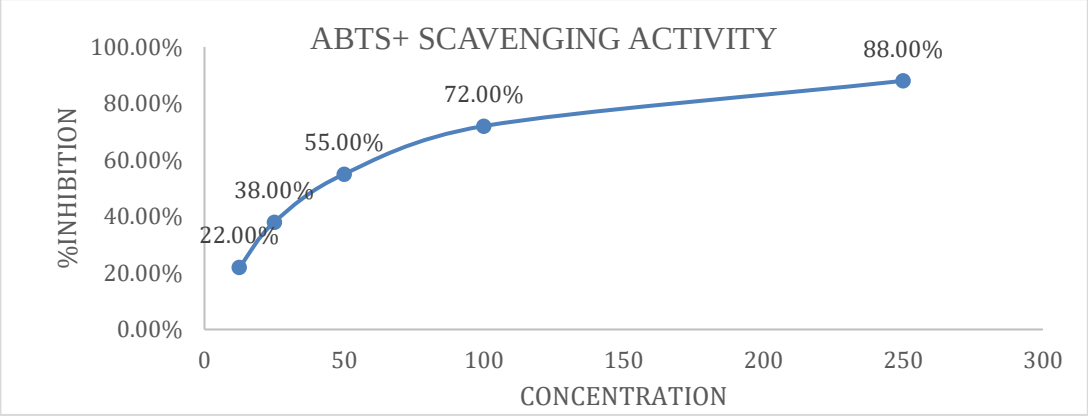
Catalase Activity of sample

ABTS + Scavenging Activity

The ABTS+ scavenging assay showed a concentration-dependent increase in antioxidant activity, with higher concentrations resulting in greater ABTS+ radical inhibition. The TEAC values showed strong antioxidant properties, with significant % inhibition at higher concentrations, suggesting it can neutralise free radicals and reduce oxidative stress. (13)

CONCENTRATION (µg/ml)	% INHIBITION	TEAC (µM Trolox equivalents)
12.5	22.0%	1666.67 µM
25	38.0%	3000.00 µM
50	55.0%	4416.67 µM
100	72.0%	5833.33 µM
250	88.0%	7166.67 µM

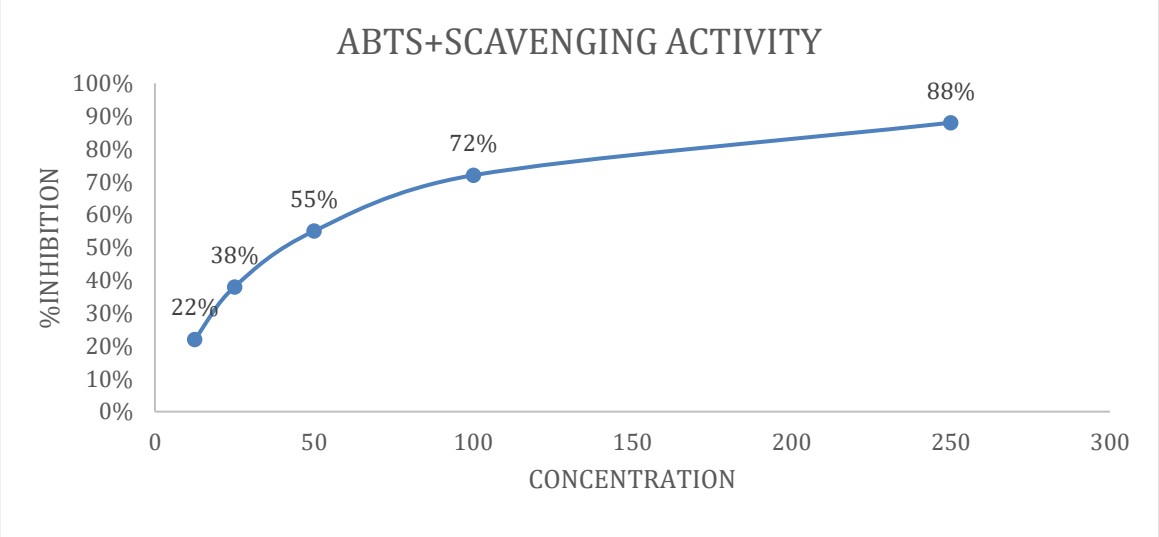
ABTS+ Scavenging Activity of TEAC



ABTS+ Scavenging Activity of TEAC

CONCENTRATION (µg/ml)	ABSORBANCE (sample)	% INHIBITION
12.5	0.624	22%
25	0.496	38%
50	0.360	55%
100	0.224	72%
250	0.096	88%

ABTS+ Scavenging Activity of Sample



ABTS+ Scavenging Activity of Sample

Ferric Reducing Antioxidant Power (FRAP) Assay

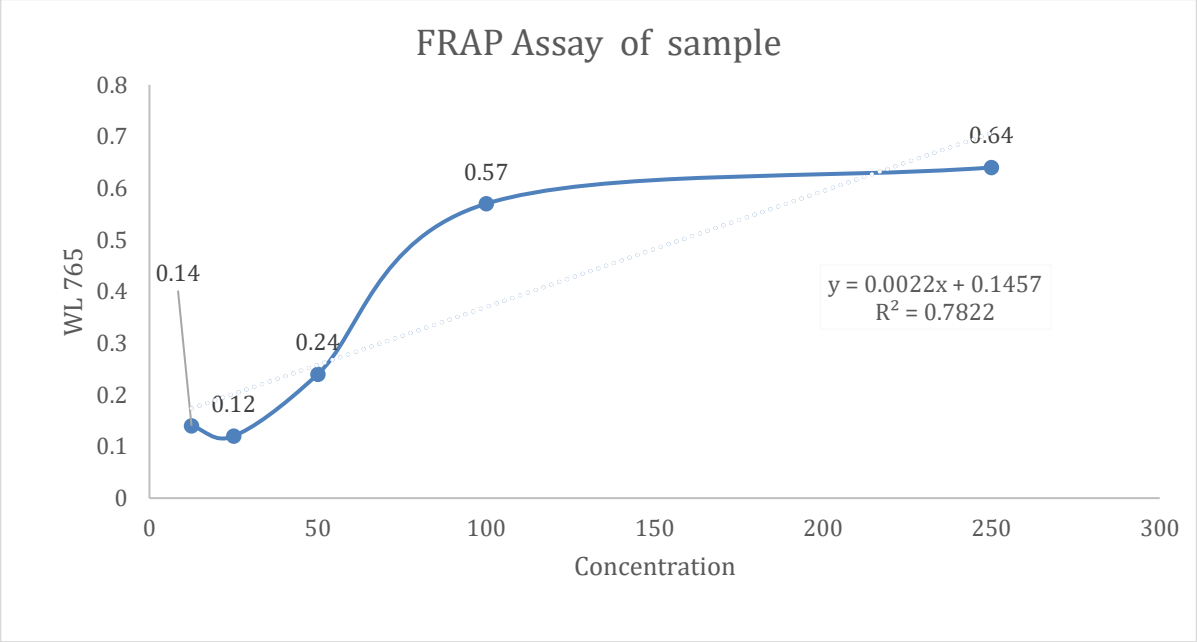
The study found that *Clitoria Ternatea* extract, a natural antioxidant, showed a dose-dependent increase in absorbance with increasing concentration, indicating higher ferric reducing antioxidant power. However, compared to vitamin C, the extract showed slightly lower absorbance values, suggesting its potential as a natural antioxidant source.

SAMPLE	CONCENTRATION(MG/ML)	ABSORBANCE 765 NM
Vitamin C (Std)	12.5	0.14
	25	0.16
	50	0.29
	100	0.62
	250	0.81

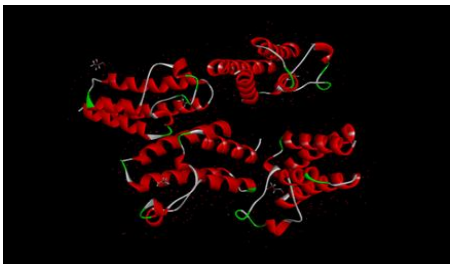
FRAP Assay of Vitamin C

SAMPLE	CONCENTRATION(MG/ML)	ABSORBANCE 765 NM
Clitoria Ternatea	12.5	0.14
	25	0.12
	50	0.24
	100	0.57
	250	0.64

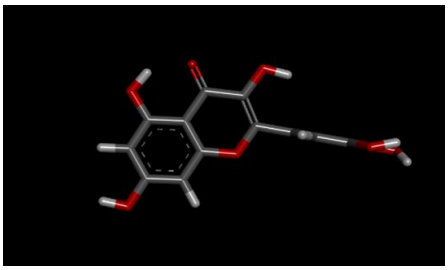
FRAP Assay of sample



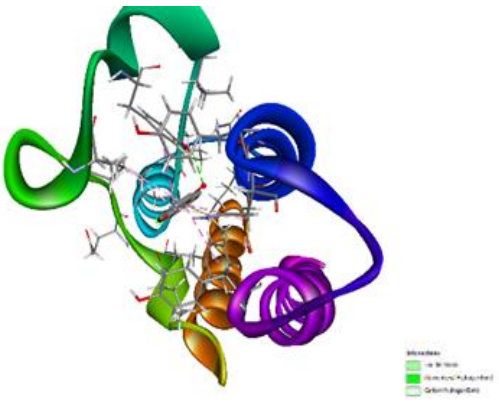
MOLECULAR DOCKING STUDIES - QUERCETIN – CAT



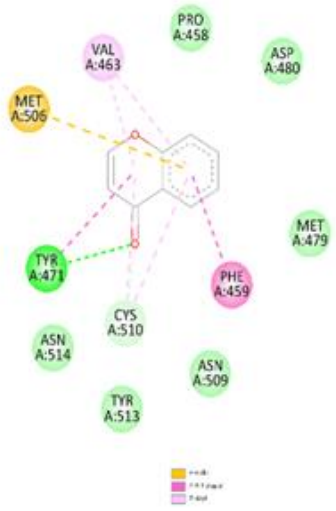
CAT – 8ru1



QUERCITIN



3D interaction

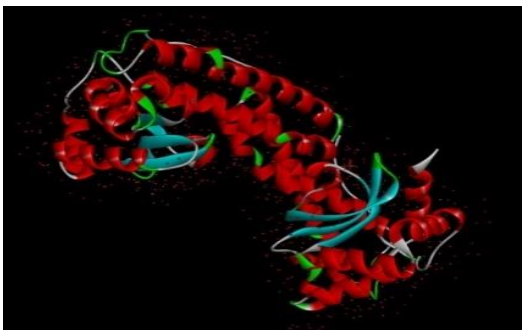


2D interaction

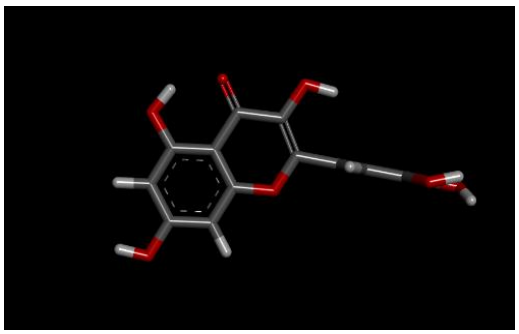
MODE	AFFINITY (kcal/mol)	DISTANCE FROM BEST MODE	
		RMSD L.B	RMSD U.B
1	-8.5	0	0
2	-8.3	1.843	2.841
3	-7.8	2.995	5.862
4	-7.8	2.942	6.929
5	-7.7	2.751	6.889
6	-7.5	3.204	7.121
7	-7.5	2.512	7.295
8	-7.4	3.741	6.817
9	-5.9	5.895	7.868

Molecular Docking of Quercetin - CAT

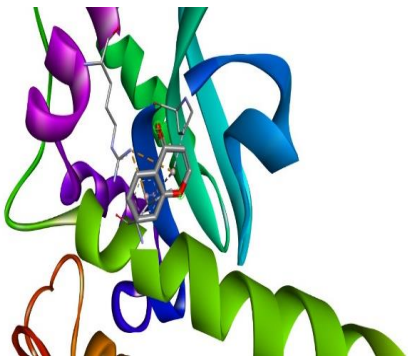
QUERCETIN - SOD



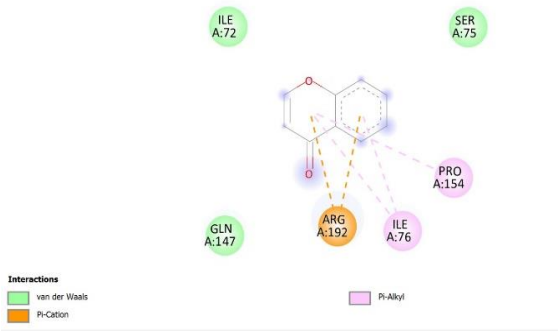
SOD – 8vj5



QUERCITIN



3D interaction

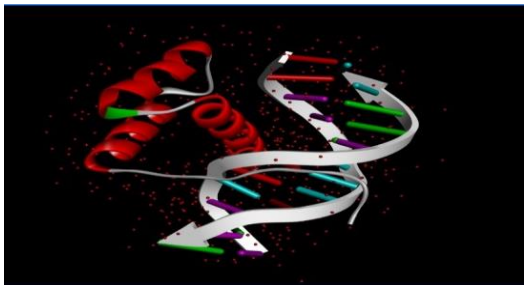


2D interaction

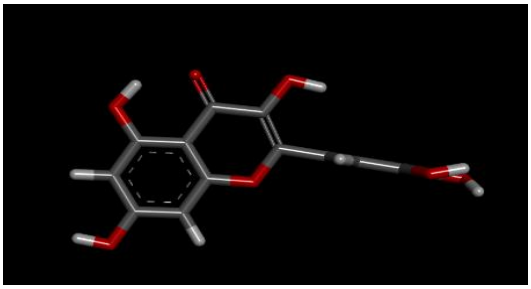
MODE	AFFINITY (kcal/mol)	DISTANCE FROM BEST MODE	
		RMSD L.B	RMSD U.B
1	-5.6	0	0
2	-4.9	1.219	2.952
3	-4.6	1.331	1.961
4	-4.5	2.107	6.874
5	-4.5	1.811	6.932
6	-4.2	2.16	3.514
7	-3.8	2.17	6.87
8	-3.5	2.503	8.144
9	-3.5	2.446	7.882

Molecular Docking of Quercetin - SOD

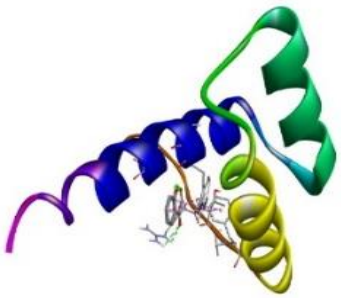
QUERCETIN – HO-1



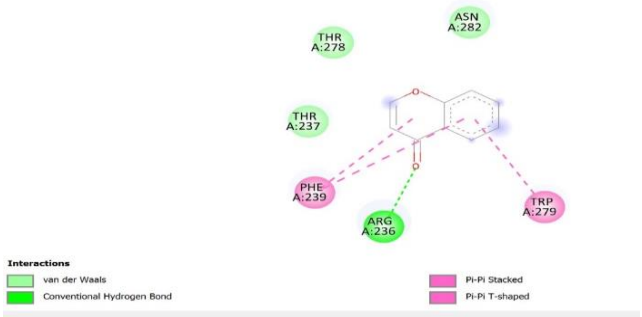
HO-1 – 8pmf



QUERCITIN



3D interaction



2D interaction

MODE	AFFINITY (kcal/mol)	DISTANCE FROM BEST MODE	
		RMSD L.B	RMSD U.B
1	-8.2	0	0
2	-7.9	2.174	3.624
3	-7.6	0.771	1.478
4	-7.4	2.444	6.875
5	-7.4	1.73	7.163
6	-7.4	1.67	7.179
7	-7.3	1.792	7.06
8	-7	1.877	2.138
9	-6.9	2.058	2.871

Molecular docking of quercetin HO-1

Discussion

The study highlights the antioxidant and antiparkinsonian properties of *Clitoria Ternatea* leaf extract, which has been found to be effective in managing neurodegenerative diseases like Parkinson's disease (PD)(14). The extract showed significant free radical scavenging activity, with robust DPPH radical neutralisation and dose-dependent increases in catalase activity. This is due to the high concentrations of phenolic compounds and flavonoids present in the leaves, which are known for their ability to mitigate oxidative stress, a key contributor to PD and other neurodegenerative disorders.(15) The extract's ferric reducing antioxidant power, slightly lower than vitamin C, further supports its role as a potent natural antioxidant.

The pronounced biological activities of *C. ternatea* are closely linked to its diverse phytochemical constituents, including tannins, flavonoids, phenols, glycosides, and resins (16). Molecular docking studies show that plant-derived flavonoids, such as quercetin, interact with key antioxidant enzymes, forming stable complexes that may enhance enzyme activity and protect against oxidative damage.

The findings underscore the therapeutic promise of *Clitoria Ternatea* in neurodegenerative disease management. Further research is needed to isolate and characterise the active compounds responsible for these effects and elucidate their molecular mechanisms of action.(17)

Conclusion

The exploration of *Clitoria Ternatea* leaf extract reveals its remarkable promise as a rich source of bioactive compounds, highlighted by its potent antioxidant and neuroprotective properties(18). The diverse phytochemical profile, particularly the presence of phenolic and flavonoids, underpins its various biological activities, suggesting a multifaceted approach to harnessing its health benefits.(20) Plant-derived flavonoids, including quercetin, interact with important antioxidant enzymes to produce stable complexes that may increase enzyme activity, according to molecular docking studies.

However, to fully unlock the therapeutic potential of this plant, further research is essential to isolate the active constituents and delve deeper into their molecular mechanisms. Such investigations could pave the way for innovative applications in neuroprotection and contribute significantly to the field of natural product pharmacology.

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