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# Antioxidant activity of an ethanolic extract of entire plant of *Cissus quadrangularis* from their pharmacognostical and pharmacological studies a review

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**Abstract**
*Cissus quadrangularis* (Vitaceae), commonly known as Hadjod or Asthisamharaka, is a perennial succulent climber widely distributed across tropical and subtropical regions, especially in India. Traditionally revered in Ayurveda, Siddha, and Unani systems of medicine, the plant is primarily used for its bone-healing properties. Phytochemical investigations reveal a rich profile of bioactive compounds including flavonoids (quercetin, kaempferol), triterpenoids (α- and β-amyrins), stilbenes (resveratrol, quadrangularins), phytosterols, ketosteroids, and essential minerals like calcium and phosphorus. These constituents contribute to its diverse pharmacological activities such as fracture healing, anti-inflammatory, antioxidant, antimicrobial, anti-diabetic, and gastroprotective effects. The stem is the most therapeutically active part, often used in decoctions, powders, and topical applications. This review consolidates current knowledge on the phytochemistry, traditional uses, and pharmacological potential of *Cissus quadrangularis*, highlighting its relevance in modern phytomedicine and suggesting avenues for future research.

**Keywords:** Cissus quadrangularis, phytochemistry, bone healing, pharmacological activities, traditional medicine

## Introduction
*Cissus quadrangularis* (Vitaceae) is a perennial succulent climber that thrives across tropical and subtropical regions, including India, Pakistan, Bangladesh, and parts of Africa. It features stout, succulent, quadrangular stems, simple ovate-reniform leaves, and globose red berries, and is commonly found in lowland forests, scrub jungles, and wastelands [1, 2].

In India, C. quadrangularis is popularly known as Hadjod and Asthisamharaka, a nomenclature that reflects both its cultural prominence and its bone-mimicking stem morphology. Traditionally, nearly all aerial and underground parts have been harnessed in Ayurveda and Unani to support fracture healing, alleviate joint pain and swelling, manage hemorrhoids, scurvy, and various hemorrhagic disorders; stem paste is applied topically for burns and wounds, while decoctions and powders are consumed to treat digestive disturbances, respiratory ailments, and menstrual irregularities [1-3].

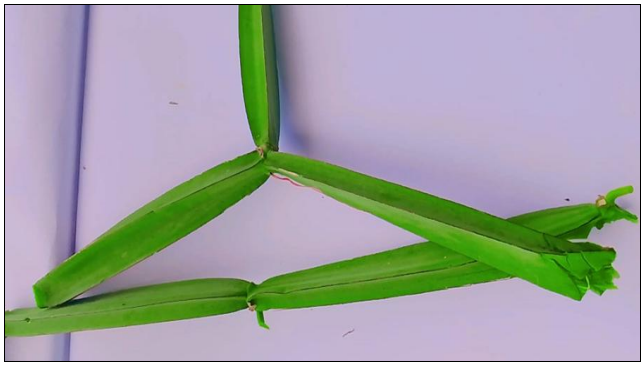
Comprehensive phytochemical analyses have demonstrated that C. quadrangularis is rich in tannins, flavonoids, triterpenoids, phytosterols, glycosides, saponins, alkaloids, and vitamin C, and serves as a significant dietary source of calcium. Comparative solvent screening reveals that methanolic extracts yield heightened levels of alkaloids, flavonoids, terpenoids, and saponins, whereas aqueous extracts are notably higher in tannins and steroids [1, 4].

Modern pharmacological investigations underline the plant's multifaceted bioactivity. Preclinical studies indicate it accelerates bone healing through glucocorticoid receptor antagonism and by stimulating osteoblastic proliferation, while additional research confirms its antimicrobial, antioxidant, anti-inflammatory, anti-ulcer, anti-diabetic, anti-obesity, hepatoprotective, and cardioprotective effects, positioning C. quadrangularis as a versatile candidate for integrative therapeutic applications [1].

Given its extensive traditional use and mounting scientific validation, there is a pressing need for systematic exploration of its molecular mechanisms, the standardization of extraction and formulation processes, and rigorously controlled clinical trials; these efforts will be crucial to substantiate efficacy and safety, and to develop novel phytopharmaceutical formulations targeting bone disorders, metabolic syndromes, and beyond.

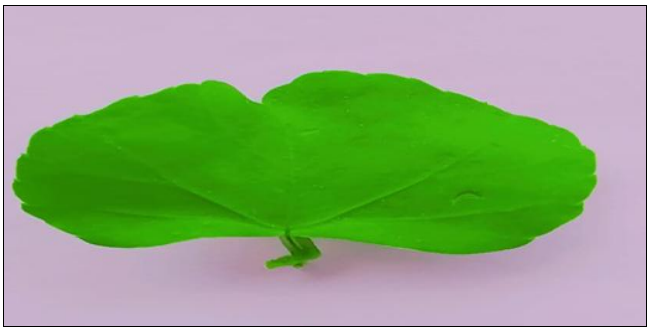
**Components**

**Stem**



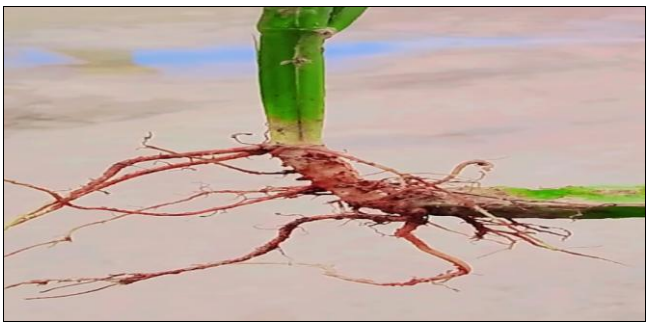
The stem part of *Cissus quadrangularis* have Antibacterial and Antimicrobial, Anticancer, Anti-inflammatory, Antiosteoporotic, Antidiabetic, Anti-obesity, Anti-convulsant, Analgesic, Antiulcer and Antiviral Properties.

**Leaves**



The leave part of *Cissus quadrangularis* have Analgesic and Anti-oxidant Activities.

**Root**



The root part of *Cissus quadrangularis* have Anticonvulsant property only.

**Whole plant / aerial part**

The whole plant / Aerial part of *Cissus quadrangularis* have Analgesic, Anti-inflammatory, Antipyretic, Anti-oxidant, Antigout, Anti-arthritic, Antiulcer, Estrogenic, Free radical scavenging, Anthelmintic, Anti-plasmodial, Anticancer, Antihistaminic, Anti-hyperlipidemic, Antifertility, Anti-haemorrhoid, Immunomodulatory, Anticonvulsant, Wound healing, Anti-osteoporotic properties.

**Chemical constituents and their activity:**

| Constituents | Source | Activty |
|---|---|---|
| ß-Carotene | Stem | Antioxidant, Free radical scavenging |
| Vitamin-C | Leaves | Antioxidant, Free radical scavenging |
| Quercetin | Aerial Part / Whole plant | Anti-inflammatory, Antioxidant |
| Daitzein | Leaves | Anti-inflammatory, Antioxidant |
| Calcium, Phosphorous | Aerial Part / Whole plant | Bone healing, Osteoprotection |
| ß-Sitosterol | Stem | Anti-inflammatory, Analgesic, Antihemorrhoidal |
| δ -Amyrin acetate, δ -Amyrone | Root / Stem | Anticonvulsant |
| Stilbenes(Resveratrol) | Leaves | Antioxidant, Antimicrobial, Analgesic |
| Saponins | Stem | Anti-inflammatory, Antimicrobial |
| n-Hexadecanoic acid | Stem | Antimicrobial, Cytotoxic |
| Kaempferol | Stem | Anti-inflammatory, Antimicrobial, Anticancer,Neuroprotective |
| Eicosyl Eicosanoate | Leaves | Analgesic, Antioxidant |
| Quadrangularin | Aerial Part / Whole plant | Anti-inflammatory, Antioxidant, Anticancer |
| Genistein | Aerial Part / Whole plant | Anti-inflammatory, Antioxidant |

**Introduction about the free radicals:** Free radicals are atoms or molecules containing one or more unpaired electrons, rendering them highly reactive. In biological systems, they primarily include reactive oxygen species (ROS) and reactive nitrogen species (RNS). At controlled levels, free radicals participate in signaling pathways and host defense. However, when their generation exceeds antioxidant defenses, oxidative stress ensues, damaging lipids, proteins, and nucleic acids.

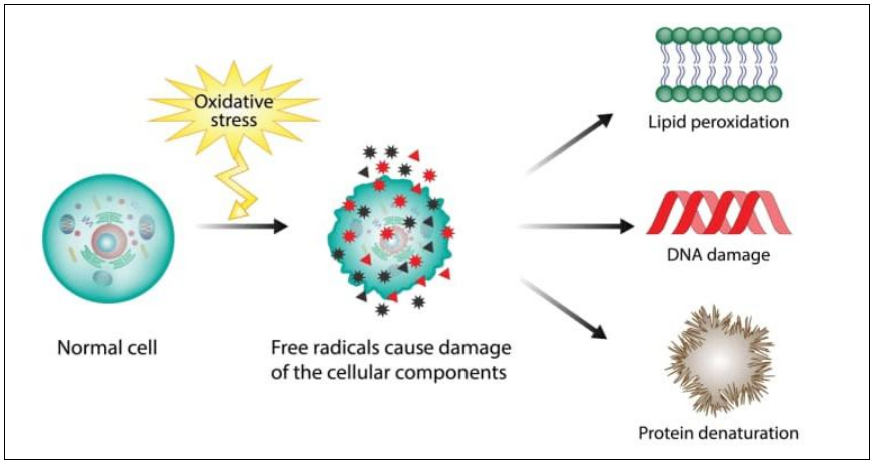
**Types of free radicals**

**Reactive Oxygen Species**

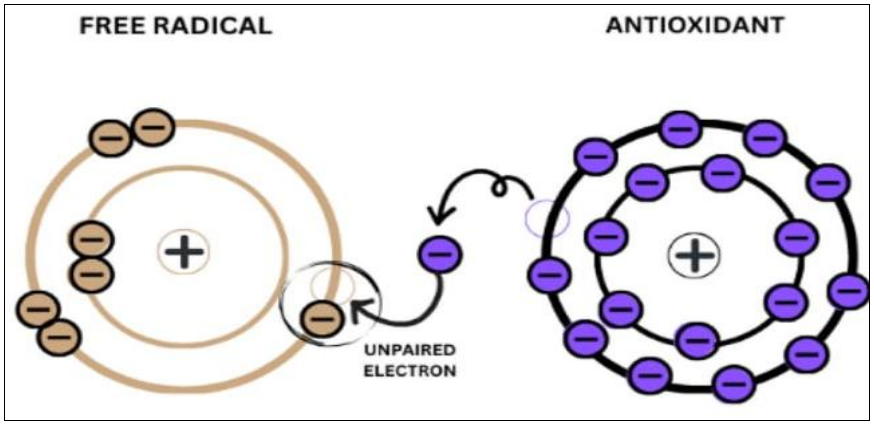
Superoxide ($O_2^{-}$•), Hydroxyl (OH•), Hydrogen peroxide ($H_2O_2$)

**Reactive Nitrogen Species**

Nitric oxide (NO•), Peroxynitrite ($ONOO^-$)




**Antioxidants:** Antioxidants are compounds that inhibit oxidation, a chemical reaction that produces free radicals capable of damaging biomolecules. They neutralize reactive oxygen and nitrogen species by donating electrons, thereby halting oxidative chain reactions without becoming reactive themselves. Antioxidants are molecules that can donate an electron to a free radical, stabilizing it and preventing it from causing further damage.




**Information about the *Cissus quadrangularis***

- Source of the plant material
- Collection of the plant material
- Pharmacognostical studies
- Chemical tests
- Invitro and in vivo studies

**Source of the plant material:** *Cissus quadrangularis*, commonly known as veldt grape, is a perennial plant primarily sourced from tropical and subtropical regions of Asia and Africa. It is widely found in India, Sri Lanka, Malaysia, Java, and West Africa. The plant is known for its medicinal properties and is used in traditional medicine for various ailments**.**

- **Synonym:** Vitis quadrangularis
- **Family:** Vitaceae

| Kingdom | Plantae |
|---|---|
| Subkingdom | Tracheobionta |
| Super division | Spermatophyta |
| Division | Magnoliophyta |
| Class | Magnoliopsida |
| Subclass | Rosidae |
| Order | Vitales |
| Family | Vitaceae |
| Genus | Cissus |
| SPECIES | C. quadrangularis |

**Collection of the plant material**

The various plant parts of *Cissus quadrangularis* were collected from the vallam village in Ramanathapuram District.

**Extraction**

**Preparation of extract:** Take the fresh stems of *Cissus quadrangularis* rinse with tape water first and after with distilled water cleaned stems of *Cissus quadrangularis* is shade dried at 40-50°C for 2 days. The dried material is powdered using a mechanical grinder. The solvent for extraction is Ethanol, Methanol, Aqueous. Weigh 20-50 g of powdered material Soak in 200-500 ml of solvent. Macerate for 24-72 hours of occasional shaking. Filter and Concentrate the extract using a Rotary Evaporater or Reduced under pressure. Stored the crude Extract at 4°C.

**Pharmacognostical studies**
- Macroscopical studies
- Microscopical studies

**Macroscopical studies**
The stem pieces are sub-quadrangular, flattened, and winged, with joints and constricted nodes, featuring spindle shaped internodes. They appear smooth, shiny, and range in color from dull green to greyish-brown as they age. Branches exhibit a dichotomous pattern. Leaves, positioned alternately along the stem, are cauline and cordate-ovate in shape, lacking stipules. They are soft, thick, shiny, and have short petioles. Tendrils are brittle, long, slender, and twisted, emerging opposite the leaves at nodes.

**Stem**
- **Shape:** Typically Quadrangular in Cross Section
- **Surface and Texture:** Smooth, shiny, and fleshy Surface
- **Colour:** Dull green
- **Taste:** Bitter
- **Odour:** Distinctive
- **Leaves:**
- **Shape:** Ovate
- **Size:** 2.5-5 cm long and ± 5 cm wide
- **Margin:** denticulate (finely toothed), serrulate dentate, or crenate-serrate
- **Apex:** Rounded or Obtuse
- **Surface:** Typically glabrous

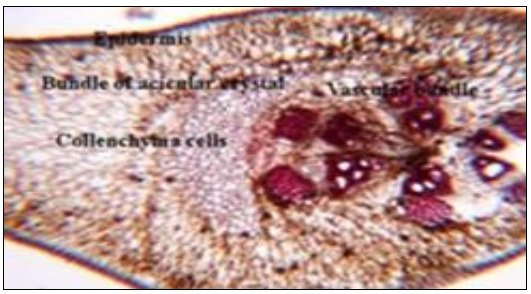
**Microscopic studies**
- **Epidermis**
- Single layer of small, rectangular cells covered by a continuous cuticle**.**
- Occasional tangentially divided cells forming a periderm-like zone in older stems.

**Cortex**
- Outer zone of 3-5 layers of angular collenchyma beneath each wing.
- Ground parenchyma richly interspersed with oval to circular secretory cavities.

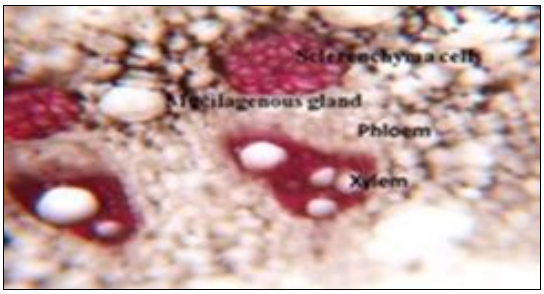
**Vascular Bundles**
- Four collateral bundles arranged in an arc within each stem wing.
- **Xylem:** 3-5 rows of wide vessels with scalariform and bordered pits.
- **Phloem:** 2-3 layers of sieve elements and companion cells, capped externally by a sclerenchymatous sheath.

**Pith**
- Central parenchymatous region of thin-walled, isodiametric cells.
- Scattered druses and raphides of calcium oxalate throughout.

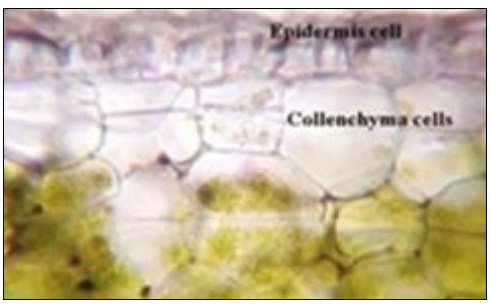
| Region | Cell type | Approximat Per Radial File (1mm Section) |
|---|---|---|
| Epidermis | Epidermal cell | 100 |
| Hypodermis | Collenchyma cell | 30 - 40 |
| Cortex | Parenchyma cell | 50 - 60 |
| Endodermis | Endodermal cell | 12 - 15 |
| Pericycle | Parenchyma / Fibre cell | 10 - 12 |
| Schlerenchyma Sheath | Schlerenchyma Fibre | 20 - 25 |
| Vascular Bundle (Xylem) | Vessel element | 5 - 8 |
| Vascular Bundle (Phloem) | Sieve tube + Campanion | 3 - 5 |
| Pith | Parenchyma cell | 20 - 30 |



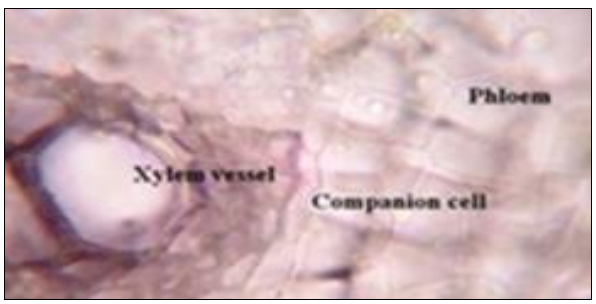

TS of stem passing through angle



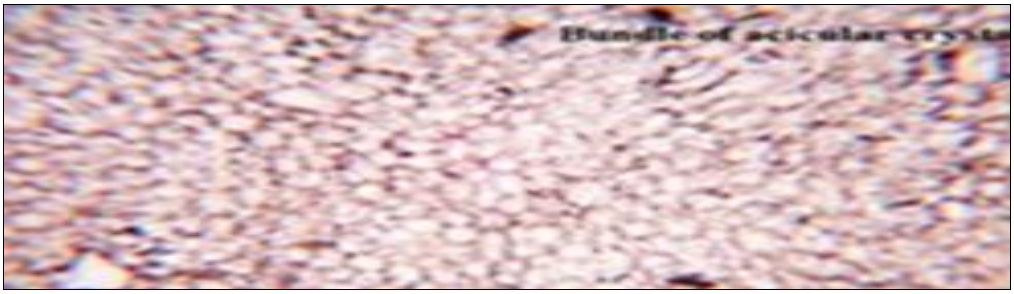

Stele region in enlarge view



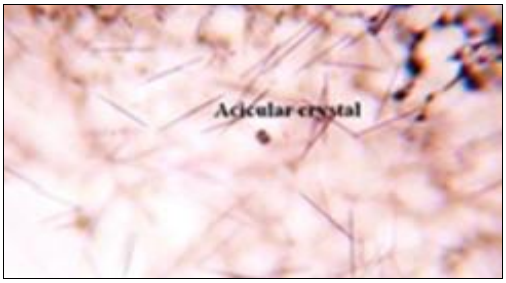

Epidermis and hypodermis



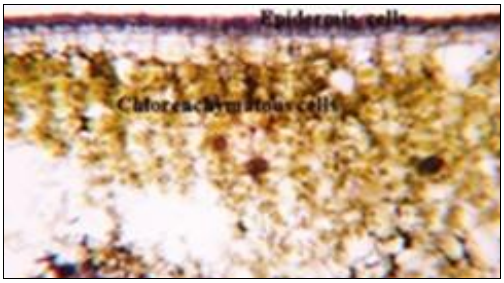

Cortical region with vascular bundle



**Pith consisting bundles of acicular crystals of calcium oxalate**




Crystals in scattered form




TS with overall arrangement of tissues


Cortex in enlarge view shows cluster and rosette crystals of calcium oxalate

**Chemical Tests**

**Qualitative phytochemical screening**

As per the usual procedures, through phytochemical Analyses of both extracts were performed. All *Cissus quadrangularis* extracts were subjected to qualitative phytochemical analysis to identify the presence of various phytochemicals, including Flavonoids, triterpenoids, phytosterols, Stilbenes, iridoids, glycosides, alkaloids, saponins, tannins and phenolic acids, minerals and vitamins.

**Test for flavonoids**

**1. Alkaline Reagent Test**

Add a few drops of 10% sodium hydroxide to the plant extract. The appearance of a yellow color indicates the presence of flavonoids. If the yellow color disappears upon the addition of dilute hydrochloric acid, it further confirms the presence of flavonoids.

**2. Lead Acetate Test**

Add a few drops of 10% lead acetate solution to the plant extract. The formation of a yellow precipitate suggests the presence of flavonoids

**Test for triterpenoids**

**1. Liebermann-Burchard Test**

Add a few drops of acetic anhydride to the plant extract. Then add concentrated sulfuric acid carefully along the side of the test tube. Formation of a green, blue, or violet ring at the interface indicates the presence of triterpenoids.

**2. Salkowski Test**

Mix the extract with chloroform, then add concentrated sulfuric acid. A red or reddish-brown coloration in the chloroform layer indicates the presence of triterpenoids.

**Test for stilbenes**

**Qualitative Tests**

- **Ferric Chloride Test**
  This test involves adding a few drops of ferric chloride solution to a sample of the *Cissus quadrangularis* extract. A positive result, indicated by a color change (often blue, green, or violet), suggests the presence of phenolic compounds, including stilbenes.
- **Lead Acetate Test**
  A bulky white precipitate formed upon addition of lead acetate solution to the extract indicates the presence of phenolic compounds.

**Test for glycosides**

**Keller-Kiliani Test**

Mix 5ml of the extract with 2ml of glacial acetic acid containing one drop of ferric chloride solution, then add 1ml of concentrated sulfuric acid. A brown ring at the interface between the layers indicates the presence of cardiac glycosides.

**Test for alkaloids**

**1. Mayer's Test:** Few drops of Mayer's reagent (potassium mercuric iodide solution) are added to the plant extract. The

formation of a yellowish or white precipitate indicates the presence of alkaloids.

**2. Wagner's Test:** A Wagner's reagent (solution of iodine and potassium iodide) is added to the plant extract.The formation of a reddish-brown precipitate suggests the presence of alkaloids.

**3. Dragendorff's Test**
Dragendorff's reagent (potassium bismuth iodide solution) is added to the plant extract. The formation of a reddish-orange precipitate indicates the presence of alkaloids.

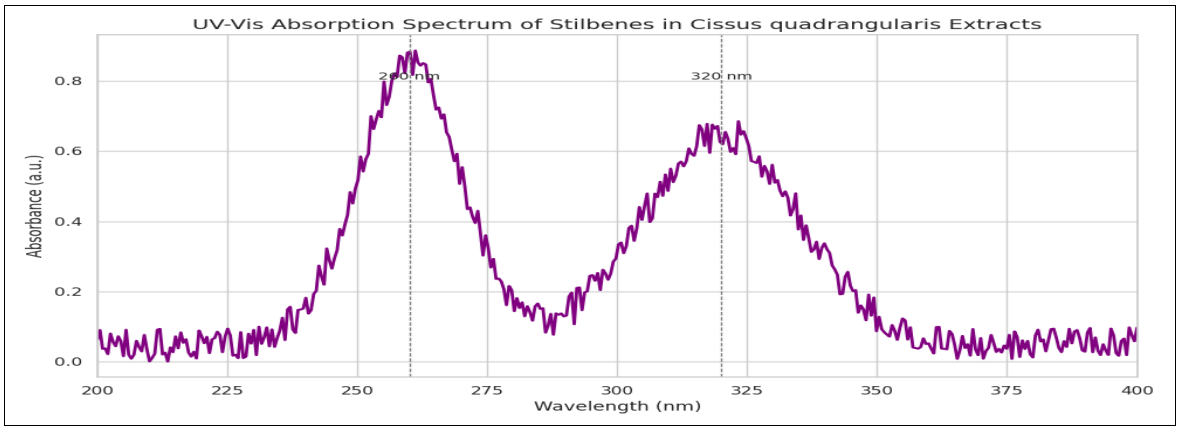
**Test for tannins**
**Gelatin Test:** Add 1% gelatin solution (containing sodium chloride) to the plant extract. Observe for the formation of a white precipitate. The presence of a precipitate indicates tannins.

**Braymer's Test (Ferric Chloride Test)**
Add 10% alcoholic ferric chloride solution to the plant extract. Observe for a blue or greenish-blue color change. This color change is due to the reaction of ferric ions with tannins, forming a complex.

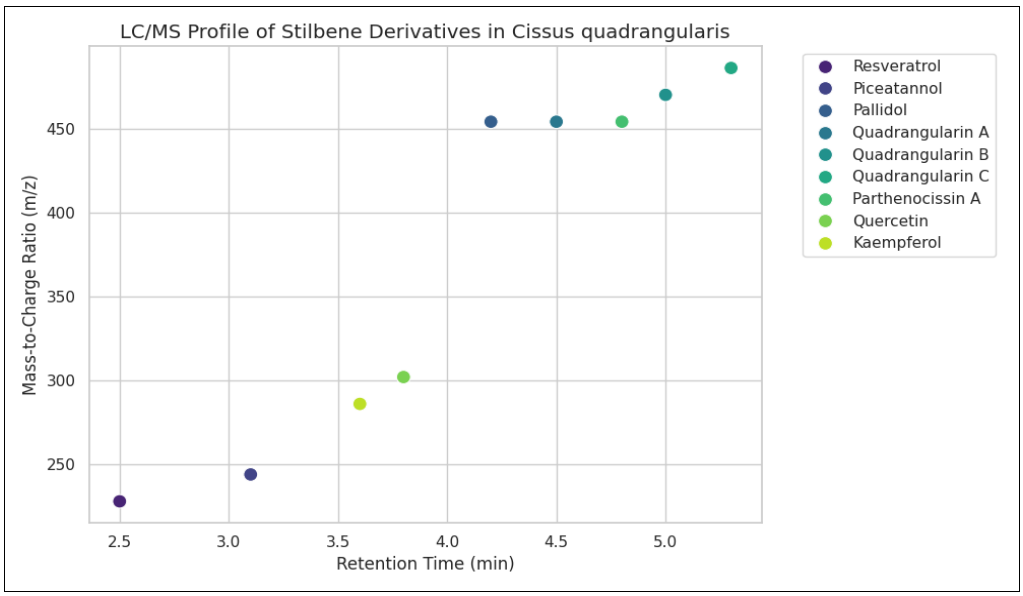
**Test for saponins**
**Foam Test (Froth Test)**
A small amount of the plant extract is added to distilled water and shaken vigorously. The formation of a persistent, honeycomb-like froth that remains stable for a period of time indicates the presence of saponins.

**Hemolytic Test**
A few drops of fresh blood are mixed with the plant extract on a glass slide or a TLC plate is immersed in a blood cell suspension. The appearance of a clear zone around the sample (on the slide) or a white spot against a pink background (on the TLC plate) indicates the presence of saponins.

**Analytical techniques**
**Spectrophotometric Analysis**
**UV-Vis Spectroscopy:** Stilbenes, being phenolic compounds, exhibit characteristic absorption peaks in the UV-Vis spectrum, particularly in the 250-350 nm range. By analyzing the absorption spectra of *Cissus quadrangularis* extracts, the presence of stilbenes based on these peaks.

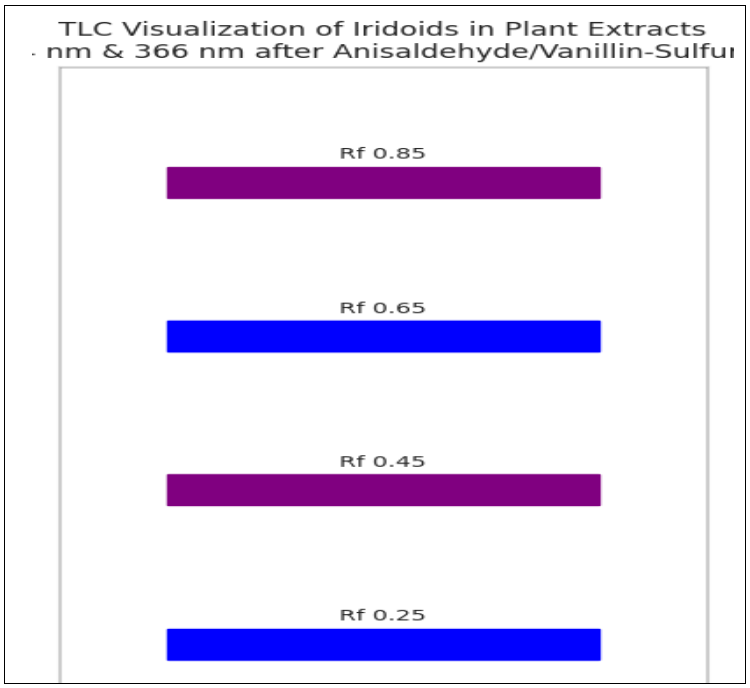



**LC/MS:** Liquid Chromatography-Mass Spectrometry (LC/MS) is a powerful technique for separating and identifying compounds in complex mixtures. It can be used to identify specific stilbene derivatives in *Cissus quadrangularis* extracts by analyzing their mass-to-charge ratios and retention times.



**Test for iridoids**

- **Thin Layer Chromatography (TLC)**
- **Procedure:** Extract the plant material using appropriate solvents like ethanol or aqueous alcohol. Apply the extract to a TLC plate coated with silica gel. Develop the plate using a solvent system tailored for iridoid separation, for instance, a mixture of hexane and ethyl acetate.
- **Detection:** Visualize the separated bands under UV light (254 nm and 366 nm) and spray the plate with a detection reagent like anisaldehyde-sulfuric acid or vanillin-sulfuric acid. Iridoids often produce characteristic colors, like blue or purple, upon spraying and heating.
- **Interpretation:** The presence of bands with specific retention factors (Rf values) and characteristic color reactions can indicate the presence of iridoids.

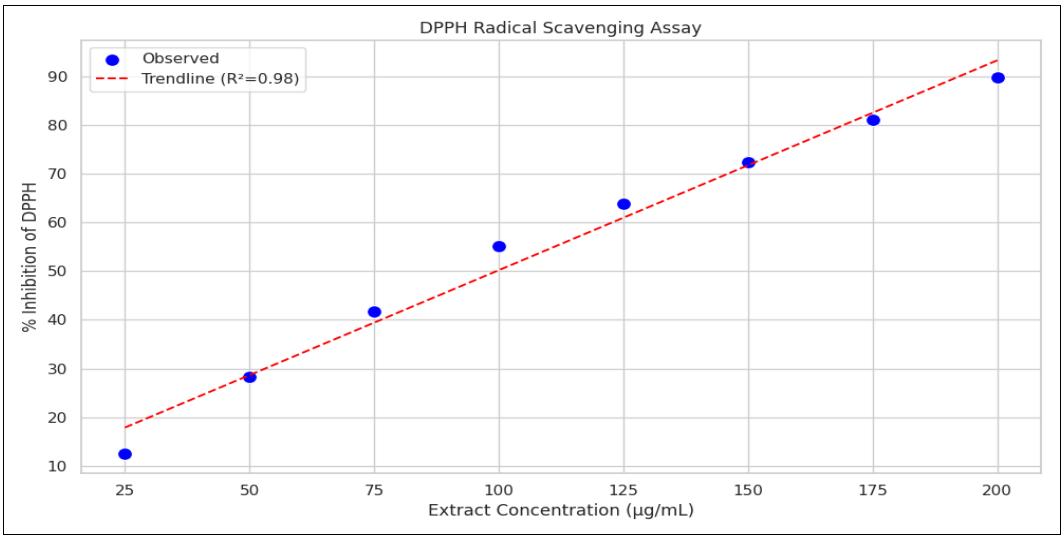



***In vitro* and invivo studies**

***In vitro* STUDIES**

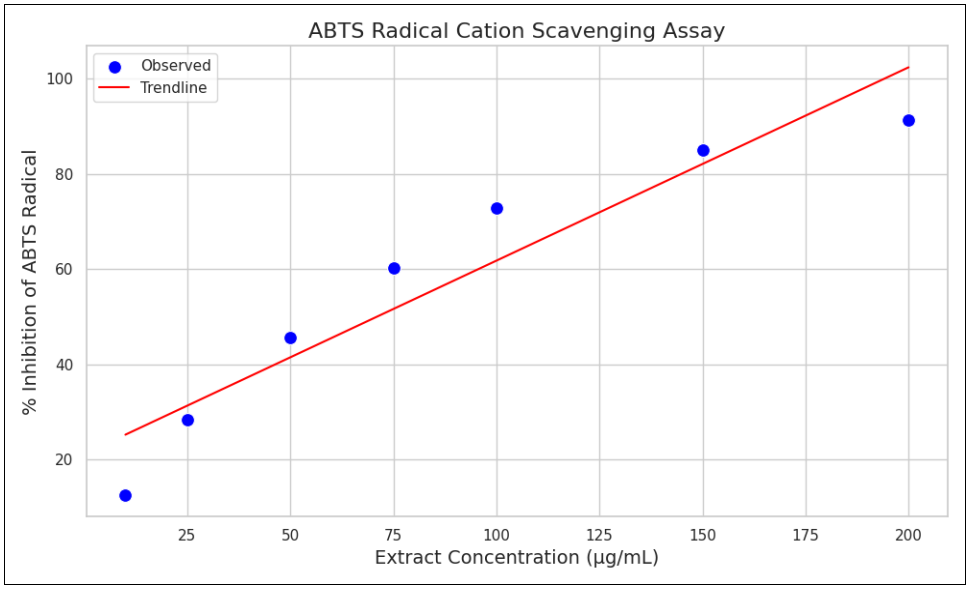
**1. DPPH Radical Scavenging Assay**

Prepare 0.1 mM DPPH in methanol. Mix 1 mL of extract at different concentrations (25-200µg/mL). Incubate in dark for 30 minutes and measure absorbance at 517 nm. DPPH (2,2-Diphenyl-1-Picrylhydrazyl) is purple colour turns to yellow colour when reduced by antioxidants. Calculate the % of inhibition = [A-control - A-sample)A-control]×100.



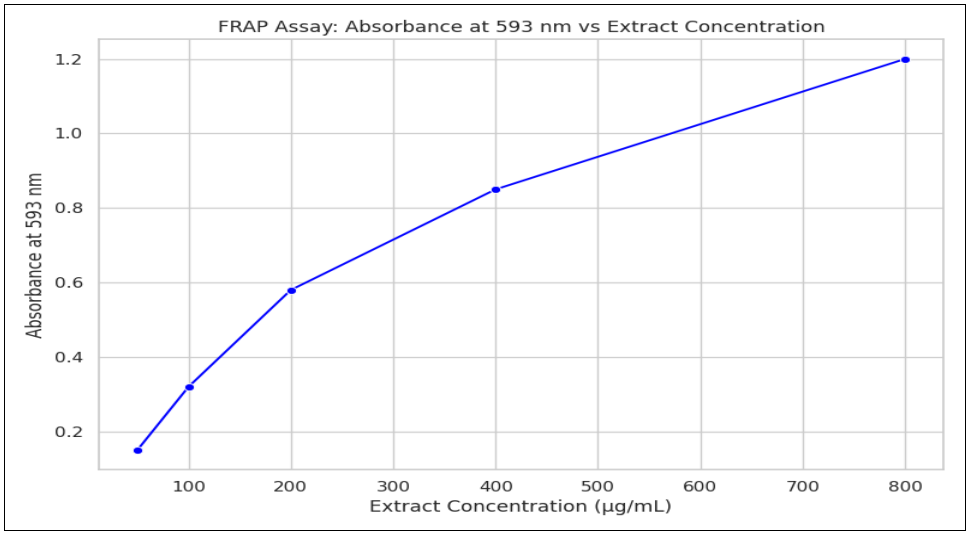

**2. ABTS Radical Cation Scavenging Assay**

Generate ABTS radical by mixing ABTS and Potassium persulfate. Add extract and incubate for 30 minutes. Measure absorbance at 734 nm. The ABTS 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) blue-green is decolourized by antioxidants. Calculate the % of inhibition indicates scavenging activity.



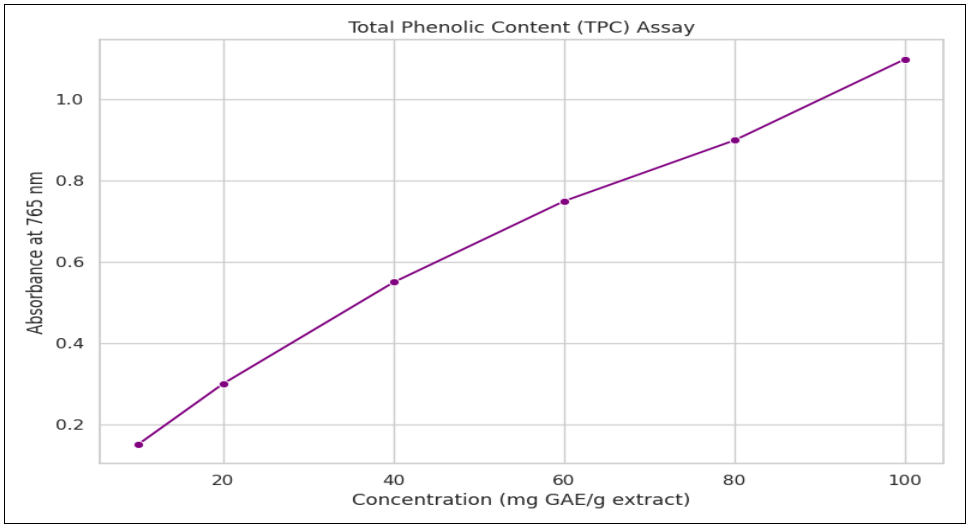

**3. Ferric Reducing Antioxidant Power (FRAP) Assay**

Mix the FRAP reagent with the extract and incubate at 37° C for 30 minutes and measure the absorbance at 593 nm. The antioxidant reduc es Fe3+ to Fe2+, forming a blue complex.



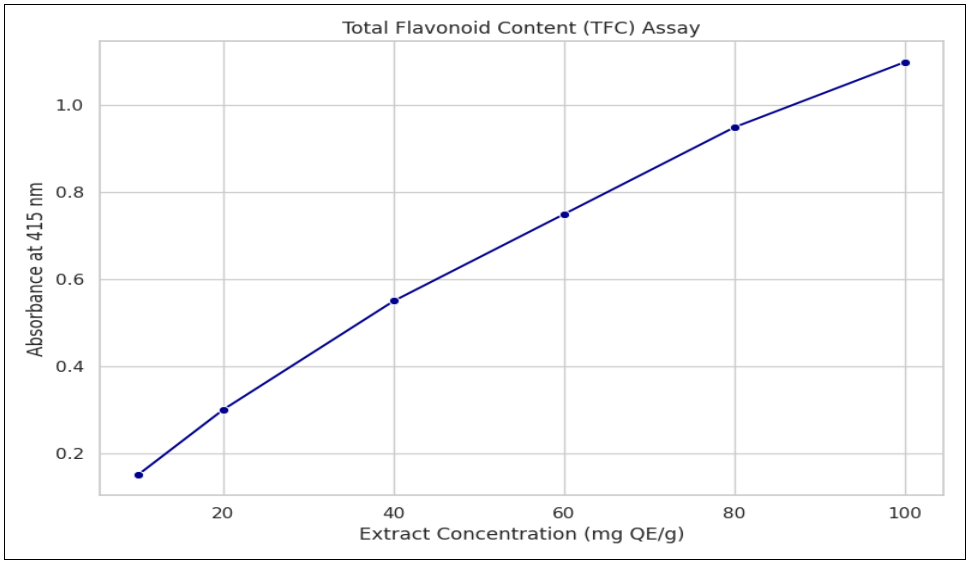

**4. Total Phenolic Content (TPC)**

Mix the extract with Folin-Ciocalteus reagent and Na2CO3 and incubate for 30 minutes and measures the absorbance at 765 nm. The result is expressed in mg gallic acid equivalent (GAE)/g extract.



**5. Total Flavonoid Content (TFC)**

Mix the extract with Aluminium chloride (Alcl3) reagent and incubate for 30 minutes and measures the absorbance at 415 nm. The results are expressed in mg quercetin equivalent (qe)/g extract.




| Assay | Purpose |
|---|---|
| DPPH assay | Measures Free radical Scavenging |
| ABTS assay | Measures radical Cation Scavenging |
| FRAP assay | Measures Reducing Power |
| Nitric oxide scavenging | No radical inhibition |
| Total Phenolic Content(TPC) | Measures phenolic content |
| Total Flavonoids Content (TFC) | Measures flavonoids content |

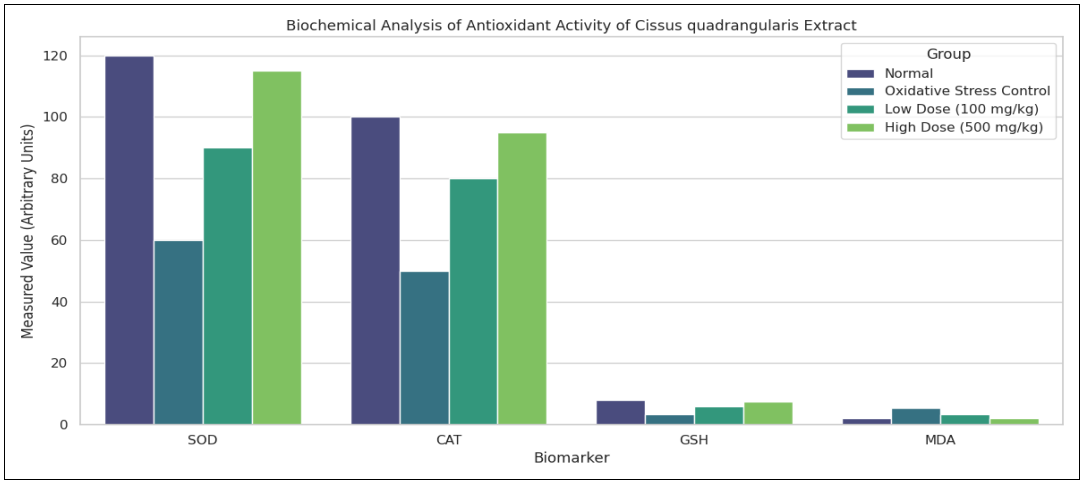
***In vivo* Studies**

**Induction of Oxidative Stress**

In invivo studies of antioxidants activity in *Cissus quadrangularis* is first select the animal of Wrister rats or albino mice are taken and divide into four groups.

- 1st group is Normal group
- 2nd group is Oxidative Stress Control (CCl4, STZ, HFD)
- 3rd group is *Cissus quadrangularis* treated - Low dose
- 4th group is *Cissus quadrangularis* treated - High dose

The Carbon Tetrachloride injected for Hepatotoxicity, Streptozotocin for Oxidative Stress and High fat diet for obesity induced oxidative stress and treat with dose of 100-500 mg/kg *Cissus quadrangularis* extract orally for 14 to 28 days. After Treatment, Sacrifice animal ethically. Collect blood and liver/ kidney tissue for biochemical analysis.




**5. Biochemical Estimation**

| Marker | Function |
|---|---|
| MDA (Malondialdehyde) | Lipid Peroxidation marker |
| SOD (Superoxide Dismutase) | Converts Superoxide radicals to hydrogen peroxide |
| CAT (Catalase) | Decomposes hydrogen peroxide |
| GSH (Glutathione) | Detoxifies reactive oxygen species |

## Results

### Extraction Yield

The 1:1 ratio of Ethanolic extraction of the *Cissus quadrangularis* yielded 10-14 %, Methanolic extraction of *Cissus quadrangularis* yielded 12-16 % and The Aqueous extraction of *Cissus quadrangularis* is yielded 6-9 %.

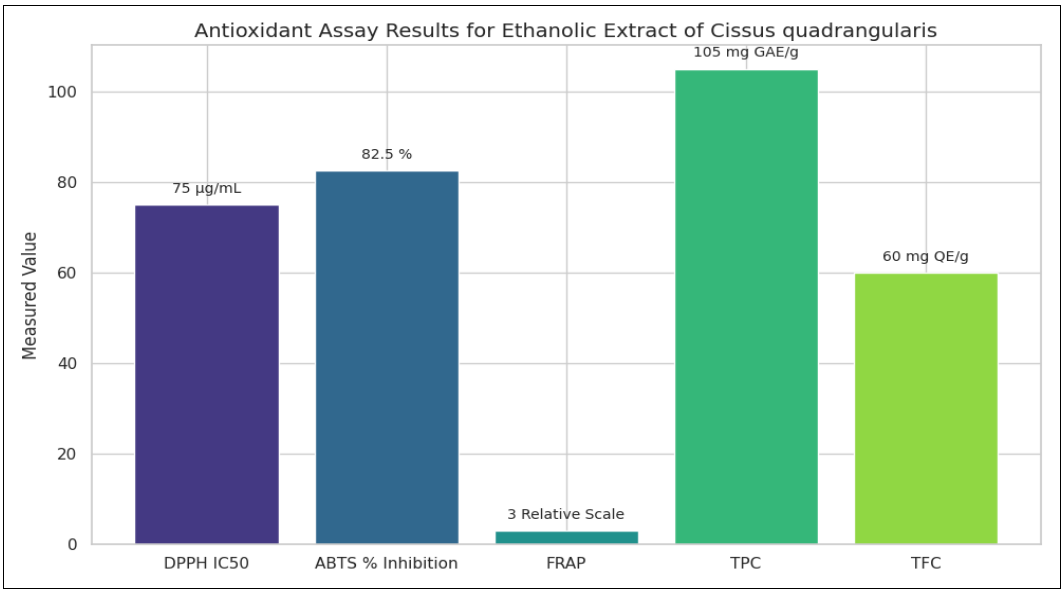
### Chemical Test

The result showed that Flavonoids, triterpenoids, phytosterols, Stilbenes, iridoids, glycosides, alkaloids, saponins, tannins and phenolic acids, minerals and vitamins were detected in all extracts of *Cissus quadrangularis*. The results of qualitative phytochemical analysis of *Cissus quadrangularis* extracts is summarized in table.

| Pytoconstituents | Test Names | PhCQ | ChCQ | EtCQ | WCQ |
|---|---|---|---|---|---|
| Alkaloids | Marquis Reagent | + | + | + | - |
| | Wagner's Reagent | + | + | + | + |
| | Mayers Test | + | + | + | + |
| Flavonoids | Ammonia Test | + | + | + | + |
| | Sodium hydroxide Test | + | + | + | + |
| Flavones | Sulphuric acid Test | + | + | - | + |
| Chalcones & Aurones | Sulphuric acid Test | - | - | + | - |
| Terpenoids | Salkowski Test | + | + | + | + |
| Phytosterols | Liberman burchard | + | - | + | - |
| Phenols | 5% aq.FeCl3 | - | - | + | - |
| Tannins | Alcoholic Ferric Chloride | - | - | + | - |
| Saponins | Foam Test | - | - | + | + |
| Glycosides | 1% aq. FeCl3 Test | + | + | + | + |
| | Sodium hydroxide Test | + | + | + | + |
| Cardiac Glycosides | Kellar Killiani Test | + | + | + | + |
| Anthraquinone Glycosides | Borntrager's Test | - | - | - | - |
| Protein | CuSO4 Test | - | + | + | - |

### *In vitro* Studies

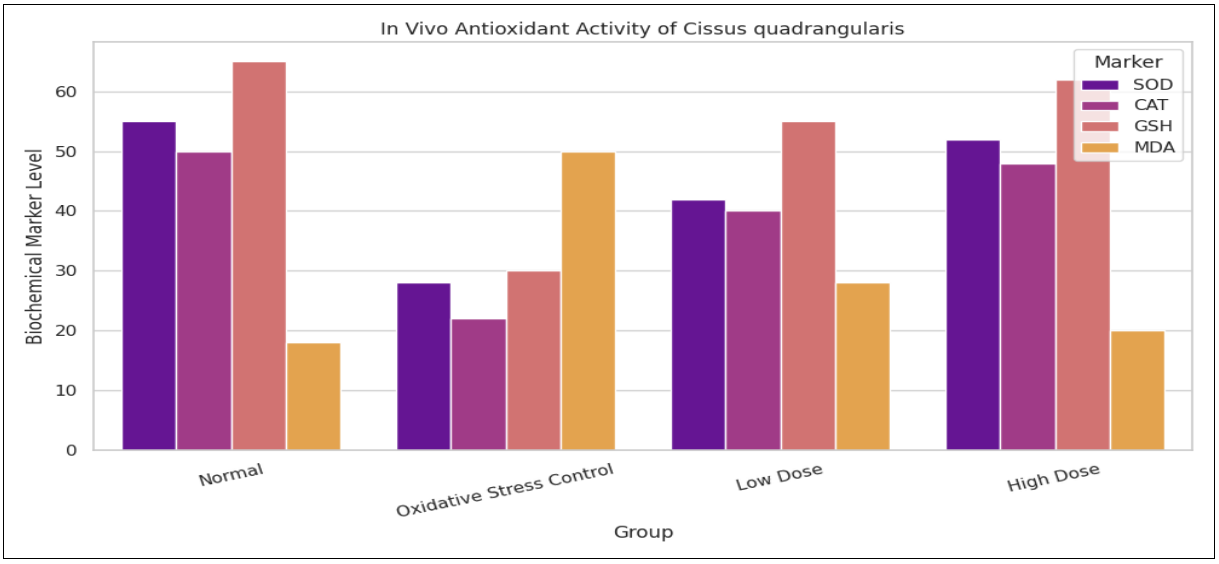
| Assay | Ethanolic extract |
|---|---|
| DPPH IC50 | 75µ / mL |
| ABTS % inhibition | 80-85 % |
| FRAP | Moderate to strong reducing power |
| TPC | 90-120 mg GAE /g |
| TFC | 50-70 mg qe /g |




The free radical scavenging ability and Antioxidant potential of *Cissus quadrangularis* extracts using chemical assays is shows good Antioxidant activity.

### *In vivo* Studies

| Parameter | Control (Oxidative Stress) | Treated with *Cissus quadrangularis* |
|---|---|---|
| MDA ↑ | High (Oxidative damage) | ↓ Reduced |
| SOD ↓ | Low | ↑ Restored |
| CAT ↓ | Low | ↑ Incresed |
| GSH ↓ | Depleted | ↑ Replenished |




*In vivo* studies of *Cissus quadrangularis* Produces protective effect against Oxidative Stress in Animal of rat so it shows good Antioxidant Activity.

**Discussion**

In our research for the biological activity with respect to Oxidative stress, inview of the Ethanolic extract of *Cissus quadrangularis*.

**Conclusion**

Results obtained from the pharmacognostical and pharmacological screening of the plant *Cissus quadrangularis* Ethonolic extract showed the presence of Flavonoids, triterpenoids, phytosterols, Stilbenes, iridoids, glycosides, alkaloids, saponins, tannins and phenolic acids, minerals and vitamins.

This study showed that the Ethanolic extract of *Cissus quadrangularis* had a promising Antioxidant Activity and Decreases the Oxidative Stress in Rats.

A further more studies formulate an Herbal Formulation of *Cissus quadrangularis* in our Studies.

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