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Research

Evaluation of Phytoconstituents and Hepatoprotective Activity (*In-Vitro*) of Flower Extract of *Hippaestrum Vittatum*

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Abstract:
The present study investigates the phytochemical profile and in-vitro hepatoprotective potential of the flower extract of *Hippeastrum vittatum*. Fresh flowers were collected, authenticated, shade-dried, powdered, and subjected to ethanolic extraction, yielding 5.8% w/w of crude extract. Pharmacognostical evaluation, including anatomical, organoleptic, ash value, and extractive value analysis, confirmed the purity and morphological identity of the plant material. Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, phenols, carbohydrates, and mucilage, indicating a rich composition of bioactive constituents. Quantitative estimation demonstrated high total phenolic content (94 µg/mg) and total flavonoid content (145 µg/mg), suggesting strong antioxidant potential. Antioxidant assays further supported this: the ethanolic extract showed significant free radical scavenging activity with an IC_{50} of 44.5 µg/ml in the DPPH assay, strong reducing power (absorbance 0.799 at 800 µg/ml), nitric oxide scavenging activity (IC_{50} = 83.53 µg/ml), and a total antioxidant capacity of 134 µg α-tocopherol equivalents/mg.Column chromatographic separation yielded two major compounds. Compound-I was identified as chlorogenic acid based on UV, IR, NMR, and mass spectrometric analysis. Compound-II was confirmed as quercetin, a well-known flavonoid with potent antioxidant and hepatoprotective properties. The presence of these compounds correlates with the strong antioxidant ability of the extract, indicating its potential role in mitigating oxidative stress–induced hepatic injury.

Keywords - Hippeastrum vittatum, phytoconstituents, antioxidant activity, chlorogenic acid, quercetin

Introduction

The liver is a vital organ that performs more than 500 essential physiological functions, including metabolism, detoxification, bile secretion, and regulation of nutrients and hormones. Its unique dual blood supply from the hepatic artery and portal vein enables efficient clearance of xenobiotics and metabolic by-products, making it central to maintaining systemic homeostasis. Hepatocytes, Kupffer cells, and Ito cells collectively contribute to immune defense, energy storage, and regeneration, underscoring the liver's indispensable role in human health. Despite its remarkable regenerative capacity, the liver is highly vulnerable to damage from toxins, drugs, infections, and metabolic disorders. Chronic liver diseases such as cirrhosis and hepatotoxicity remain major global health concerns, with rising incidence due to alcohol abuse, viral hepatitis, and non-alcoholic fatty liver disease. These conditions often progress silently until advanced stages, leading to complications such as jaundice, ascites, encephalopathy, and ultimately liver failure. The

burden of liver disease is particularly severe in developing countries, where limited access to healthcare exacerbates morbidity and mortality. Current therapeutic options rely heavily on synthetic drugs, which, although effective, are often associated with adverse effects, limited efficacy in advanced disease, and high treatment costs. This has prompted growing interest in herbal hepatoprotectives, which offer multi-targeted mechanisms of action, better safety profiles, and accessibility. Plants rich in phenolics, flavonoids, and alkaloids have demonstrated significant antioxidant and hepatoprotective properties, making them promising candidates for drug discovery.

Hippeastrum vittatum, a member of the Amaryllidaceae family, is widely cultivated as an ornamental plant but has also attracted attention for its medicinal potential. Taxonomically, it belongs to the genus *Hippeastrum*, comprising over 70 species natives to South America. Traditional uses of *H. vittatum* include applications in neurological disorders, diabetes, and inflammatory conditions. Phytochemical investigations have revealed the presence of diverse alkaloids, flavonoids, and phenolic compounds, many of which are known to exert antioxidant and cytoprotective effects. These bioactive constituents suggest that *H. vittatum* may serve as a valuable source of hepatoprotective agents.

The present study was undertaken to investigate the phytoconstituents of *H. vittatum* flower extract and evaluate its in-vitro hepatoprotective potential. By combining phytochemical profiling, antioxidant assays, and compound characterization, this work aims to establish a scientific basis for the therapeutic relevance of *H. vittatum* in mitigating oxidative stress–induced liver injury.

Materials and Methods

1. Plant Material Collection and Authentication

Fresh flowers of *Hippeastrum vittatum* were collected during the flowering season (March–April) from cultivated sources in Uttar Pradesh, India. The plant was identified and authenticated by a taxonomist at the Institute of Pharmaceutical Sciences & Research, Unnao. A voucher specimen (No. HV-2025) was deposited in the departmental herbarium for reference. Flowers were washed with distilled water to remove dust, shade-dried at room temperature (25–28 °C) for 10–12 days, and pulverized into coarse powder using a mechanical grinder. The powdered material was stored in airtight containers under desiccated conditions until extraction.

2. Preparation of Extract

Approximately 250 g of dried flower powder was subjected to Soxhlet extraction with 95% ethanol for 48 hours. The extract was concentrated under reduced pressure using a rotary evaporator at 40 °C to yield a dark brown residue. The percentage yield was calculated (5.8% w/w). The dried extract was stored in a refrigerator at 4 °C in amber-colored glass bottles to prevent photodegradation.

3. Pharmacognostical Evaluation

To establish identity and purity, standard pharmacognostical parameters were assessed:

1. **Organoleptic properties**: color, odor, taste, and texture were recorded.
2. **Microscopic examination**: transverse sections of petals and floral tissues were prepared, stained with safranin, and observed under a compound microscope for diagnostic features.
3. **Ash values**: total ash, acid-insoluble ash, and water-soluble ash were determined following WHO guidelines.
4. **Extractive values**: ethanol and water extractive values were calculated to assess solubility and purity.

4. Preliminary Phytochemical Screening

Qualitative phytochemical tests were performed on the ethanolic extract using standard protocols:

1. **Alkaloids**: Dragendorff's and Mayer's reagents.
2. **Flavonoids**: Shinoda test.
3. **Phenols and tannins**: Ferric chloride test.
4. **Carbohydrates**: Molisch's and Benedict's tests.
5. **Mucilage**: Ruthenium red staining.

5. Quantitative Estimation of Phytoconstituents

1. **Total Phenolic Content (TPC):** Determined by Folin–Ciocalteu method. Absorbance was measured at 765 nm using a UV–Vis spectrophotometer. Results expressed as µg gallic acid equivalents (GAE)/mg extract.
2. **Total Flavonoid Content (TFC):** Estimated by aluminum chloride colorimetric method. Absorbance measured at 415 nm. Results expressed as µg quercetin equivalents (QE)/mg extract.

6. Antioxidant Assays

The antioxidant potential of the extract was evaluated using multiple in-vitro assays:

1. **DPPH radical scavenging assay:** 2,2-diphenyl-1-picrylhydrazyl solution (0.1 mM) prepared in methanol. Different concentrations of extract (25–800 µg/ml) were incubated for 30 min in dark. Absorbance measured at 517 nm. IC_{50} values calculated.
2. **Reducing power assay:** Extract concentrations (100–800 µg/ml) incubated with phosphate buffer and potassium ferricyanide, followed by trichloroacetic acid and ferric chloride. Absorbance measured at 700 nm.
3. **Nitric oxide scavenging assay:** Sodium nitroprusside solution incubated with extract at physiological pH. Griess reagent added, absorbance measured at 546 nm. IC_{50} values calculated.
4. **Phosphomolybdate assay (Total Antioxidant Capacity):** Extract incubated with ammonium molybdate reagent at 95 °C for 90 min. Absorbance measured at 695 nm. Results expressed as µg α-tocopherol equivalents/mg extract.

7. Compound Isolation and Characterization

The ethanolic extract was subjected to column chromatography using silica gel (60–120 mesh) as stationary phase. Elution was carried out with gradient solvents (chloroform: methanol). Fractions were monitored by thin-layer chromatography (TLC) using appropriate solvent systems and visualized under UV light (254 nm, 366 nm). Fractions with similar Rf values were pooled and further purified.

Isolated compounds were characterized by:

1. **UV–Visible spectroscopy**: λmax recorded.
2. **FTIR spectroscopy**: functional groups identified.
3. **^{1}H and ^{13}C NMR spectroscopy**: chemical shifts analyzed for structural elucidation.
4. **Mass spectrometry (MS):** molecular ion peaks confirmed molecular weights.

Two major compounds were identified: **chlorogenic acid** and **quercetin**, both known for potent antioxidant and hepatoprotective properties.

Result

Phytochemical Profile

Preliminary phytochemical screening of the ethanolic flower extract of *Hippeastrum vittatum* revealed the presence of diverse bioactive constituents. Alkaloids, flavonoids, phenols, carbohydrates, and mucilage were detected, confirming the rich phytochemical composition of the extract. These findings suggest that the plant material contains secondary metabolites of therapeutic relevance, particularly those associated with antioxidant and hepatoprotective activity.

Quantitative Estimation of Phytoconstituents

Quantitative analysis demonstrated a high concentration of phenolic and flavonoid compounds in the extract.

1. Total Phenolic Content (TPC): 94 µg gallic acid equivalents (GAE)/mg extract.
2. Total Flavonoid Content (TFC): 145 µg quercetin equivalents (QE)/mg extract.

The elevated levels of phenolics and flavonoids indicate strong antioxidant potential, as these compounds are known to scavenge free radicals and protect against oxidative stress.

Antioxidant Activity

The ethanolic extract exhibited significant antioxidant activity across multiple in-vitro assays:

1. DPPH radical scavenging assay: The extract showed potent free radical scavenging with an IC_{50} value of 44.5 µg/ml, indicating strong hydrogen-donating ability.
2. Nitric oxide scavenging assay: The extract inhibited nitric oxide radicals with an IC_{50} value of 83.53 µg/ml, suggesting its role in mitigating reactive nitrogen species.
3. Reducing power assay: The extract demonstrated concentration-dependent reducing activity, with maximum absorbance of 0.799 at 800 µg/ml, confirming its electron-donating capacity.
4. Phosphomolybdate assay (Total Antioxidant Capacity): The extract exhibited a total antioxidant capacity equivalent to 134 µg α-tocopherol/mg extract, further validating its strong antioxidant potential. Collectively, these results highlight the ability of *H. vittatum* extract to neutralize free radicals and reduce oxidative stress.

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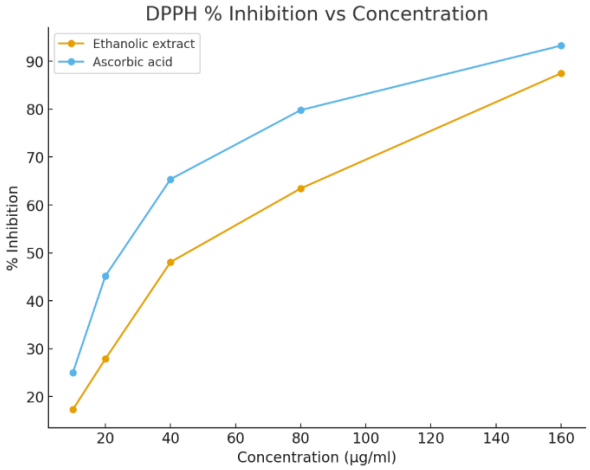

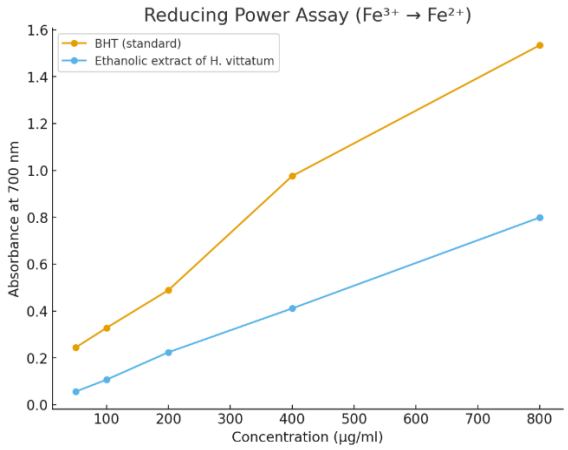

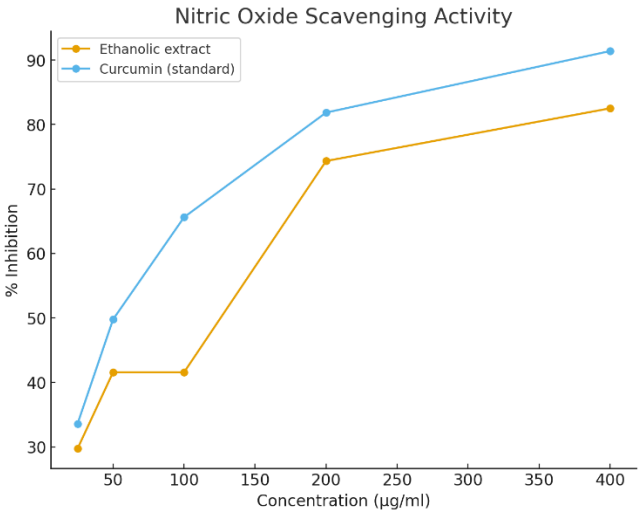

Compound Identification

Column chromatographic separation followed by spectroscopic characterization (UV, FTIR, NMR, and MS) led to the identification of two major compounds: Chlorogenic acid and Quercetin. Both compounds are well-documented for their antioxidant and hepatoprotective properties. Their presence in the extract correlates with the observed bioactivity, supporting the therapeutic relevance of *H. vittatum* in liver protection.

Discussion

The present study demonstrated that the ethanolic flower extract of *Hippeastrum vittatum* is rich in phytoconstituents, particularly alkaloids, flavonoids, and phenolic compounds. These classes of secondary metabolites are widely recognized for

their ability to neutralize reactive oxygen species (ROS) and protect cellular integrity. The high total phenolic (94 µg/mg) and flavonoid (145 µg/mg) content observed in the extract strongly correlates with its potent antioxidant activity across multiple assays, including DPPH, nitric oxide scavenging, reducing power, and total antioxidant capacity. This suggests that the hepatoprotective potential of *H. vittatum* is largely attributable to its phytochemical composition.

Among the isolated compounds, chlorogenic acid and quercetin were identified as major constituents. Chlorogenic acid is a polyphenolic compound known to inhibit lipid peroxidation, modulate glucose metabolism, and reduce oxidative stress in hepatocytes. Quercetin, a flavonoid, has been extensively studied for its hepatoprotective properties, including stabilization of cell membranes, inhibition of pro-inflammatory cytokines, and enhancement of endogenous antioxidant defenses such as glutathione. The presence of these compounds in *H. vittatum* provides a mechanistic basis for the observed antioxidant activity, as both are capable of scavenging free radicals and attenuating oxidative damage that underlies hepatic injury.

Comparison with standard hepatoprotective agents further validates the therapeutic relevance of *H. vittatum*. Silymarin, derived from *Silybum marianum*, is widely used as a reference compound in hepatoprotective st0udies. Like silymarin, the *H. vittatum* extract demonstrated strong radical scavenging and reducing power, suggesting comparable antioxidant mechanisms. While silymarin acts primarily through stabilization of hepatocyte membranes and modulation of nuclear transcription factors, chlorogenic acid and quercetin contribute through direct ROS neutralization and enhancement of endogenous antioxidant systems. This complementary activity profile positions *H. vittatum* as a promising alternative or adjunct to established hepatoprotective agents.

The implications of these findings extend to herbal drug development. The identification of chlorogenic acid and quercetin as bioactive markers supports the standardization of *H. vittatum* extracts for therapeutic use. Furthermore, the multi-targeted antioxidant and hepatoprotective mechanisms observed highlight the potential of this plant in managing oxidative stress–related liver disorders. Future studies should focus on in-vivo validation, pharmacokinetic profiling, and formulation development to translate these findings into clinically viable herbal therapeutics.

Conclusion

The ethanolic flower extract of *Hippeastrum vittatum* demonstrated strong antioxidant and hepatoprotective potential, as evidenced by its rich phytochemical profile and significant activity in multiple in-vitro assays. The high levels of phenolic and flavonoid compounds correlated directly with free radical scavenging, reducing power, and nitric oxide inhibition, underscoring the extract's ability to mitigate oxidative stress.

Spectroscopic characterization confirmed the presence of chlorogenic acid and quercetin, two well-established bioactive compounds with documented hepatoprotective properties. Their identification not only validates the therapeutic relevance of *H. vittatum* but also provides chemical markers for standardization of future formulations.

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