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Research Article

Hydroalcoholic Extraction of *Cissus quadrangularis* and *In Vitro* Evaluation of Antifungal Properties

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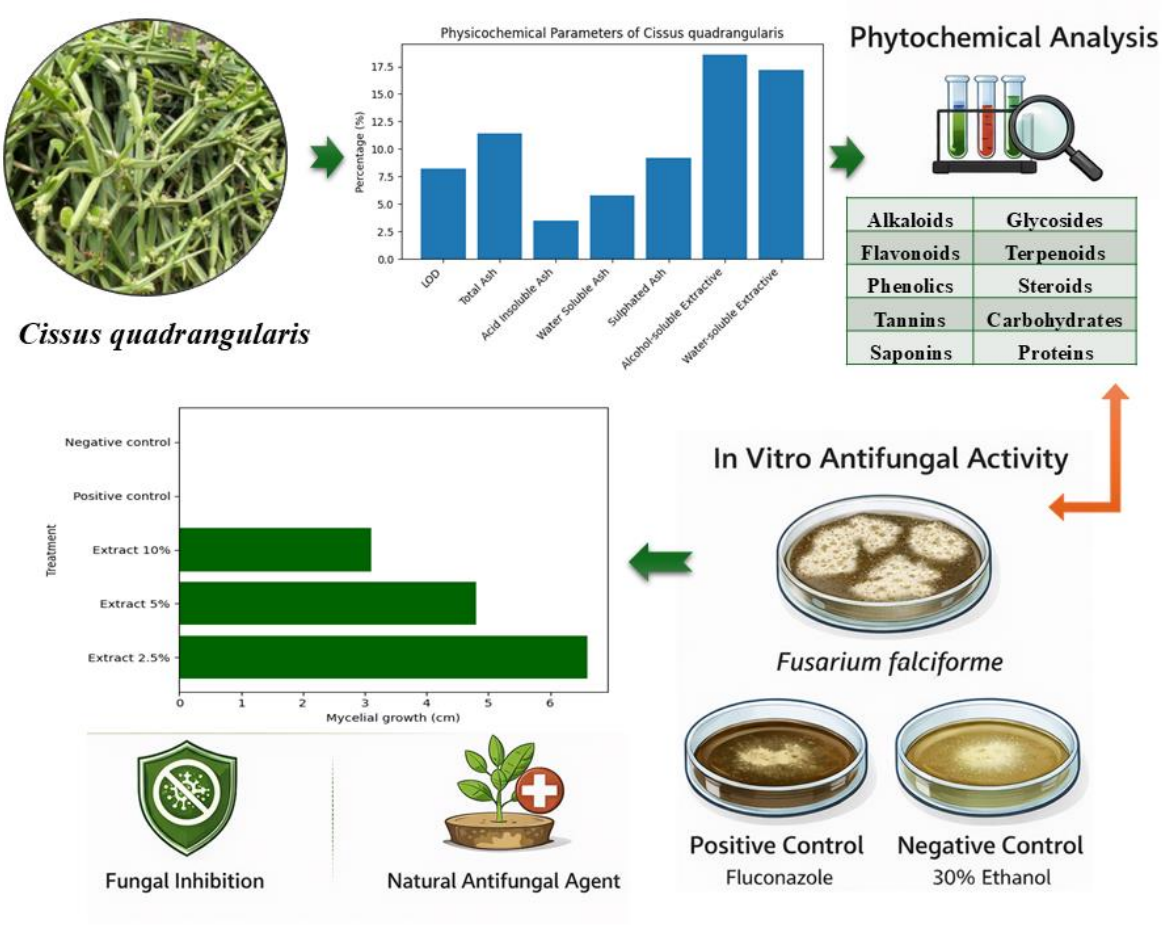
Abstract:
Cissus quadrangularis is a medicinal plant traditionally recognized for its therapeutic potential, yet its antifungal properties remain underexplored. The present study aimed to investigate the physicochemical characteristics, phytochemical composition, and in vitro antifungal activity of the hydroalcoholic extract of *Cissus quadrangularis.* Plant material was subjected to Soxhlet extraction using a hydroalcoholic solvent system (70% ethanol:30% water). Physicochemical evaluation demonstrated that the extract met acceptable quality standards, with slightly acidic pH, low moisture content, and minimal inorganic and siliceous impurities. The absence of foreign organic matter, heavy metals, and pesticide residues confirmed the purity and safety of the plant material. High alcohol- and water-soluble extractive values indicated efficient extraction of phytoconstituents. Preliminary phytochemical screening revealed flavonoids, phenolic compounds, tannins, saponins, glycosides, terpenoids, steroids, alkaloids, and carbohydrates, while proteins were absent, indicating enrichment of low-molecular-weight bioactive compounds. The antifungal potential of the extract was evaluated against *Fusarium falciforme* using the food poison method. The results demonstrated a concentration-dependent reduction in radial mycelial growth, with partial inhibition observed in extract-treated plates and the highest inhibition recorded at 10% extract concentration. Radial growth measured 6.6 cm (2.5%), 4.8 cm (5%), and 3.1 cm (10%), corresponding to approximately 17.5%, 40.0%, and 53.1% inhibition, respectively, compared with the control. Complete growth inhibition was observed only in the positive control (fluconazole), whereas the solvent control showed no antifungal effect. Overall, the study highlights the phytochemical richness and notable antifungal potential of *Cissus quadrangularis* as a promising natural source of antifungal agents.

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Graphical Abstract



1. Introduction

Fungal infections represent a growing challenge in public health, agriculture, and food security owing to the emergence of resistant strains and the limited efficacy of existing antifungal agents (1,2). Opportunistic and phytopathogenic fungi, including Fusarium species, are of particular concern because they cause significant plant diseases and can also lead to infections in humans, especially in immunocompromised individuals (3–5). The increasing resistance to conventional antifungal drugs, coupled with their associated toxicity and high cost, has intensified the search for alternative, safer, and more cost-effective antifungal agents derived from natural sources.

Medicinal plants have long been recognized as valuable reservoirs of bioactive compounds with diverse pharmacological properties (6,7). Among them, *Cissus quadrangularis*, a perennial plant belonging to the family Vitaceae, has been extensively used in traditional medicine for the treatment of bone fractures, inflammation, pain, and various metabolic disorders (8). The therapeutic potential of this plant has been attributed to its rich phytochemical composition, including flavonoids, phenolic compounds, tannins, saponins, glycosides, terpenoids, and steroids (9,10). These secondary metabolites exhibit antimicrobial, antioxidant, and anti-inflammatory activities, suggesting that the plant may also possess antifungal potential.

Extraction plays a crucial role in isolating bioactive constituents from plant materials, and the choice of solvent significantly influences the quality and quantity of extracted phytochemicals. Hydroalcoholic extraction, which typically employs a combination of ethanol and water, is widely preferred because of its ability to effectively extract both polar and moderately polar compounds. Ethanol is considered a safe and

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environmentally acceptable solvent, and the addition of water enhances the extraction of hydrophilic constituents, making hydroalcoholic systems particularly suitable for comprehensive phytochemical recovery (11,12).

Despite the well-documented medicinal applications of *Cissus quadrangularis*, systematic investigations focusing on its antifungal activity using standardized in vitro methods remain limited. Evaluating the antifungal potential of plant extracts through reliable laboratory assays is essential to establish scientific evidence supporting their traditional use and explore their possible application as natural antifungal agents.

In this context, the present study was designed to prepare a hydroalcoholic extract of *Cissus quadrangularis* and to assess its in vitro antifungal activity using an established experimental approach. By integrating extraction methodology with biological evaluation, this study aims to contribute to the growing body of research on plant-based antifungal agents and to provide a scientific basis for the potential use of *Cissus quadrangularis* in antifungal applications. Therefore, the primary objective of the present study was to systematically prepare and characterize a hydroalcoholic extract of *Cissus quadrangularis* through physicochemical evaluation and preliminary phytochemical screening, and to explicitly assess its in vitro antifungal efficacy against *Fusarium falciforme* using the food poisoning method.

2. Experimental Method

2.1 Collection of Plant Material

Fresh and healthy plant material of *Cissus quadrangularis* was collected from the local region of Buldhana, Maharashtra, India. The collected plant material was thoroughly washed with running tap water to remove the adhering soil and debris, followed by rinsing with distilled water. The plant material was shade-dried at room temperature for an adequate period to prevent the degradation of bioactive constituents and then coarsely powdered using a mechanical grinder. The powdered material was stored in an airtight container until further use.

2.2 Hydroalcoholic Extraction Using Soxhlet Apparatus

The shade-dried plant material of *Cissus quadrangularis* was coarsely powdered and accurately weighed prior to extraction. The powdered samples were packed into a cellulose extraction thimble and placed in a Soxhlet extractor. Extraction was carried out using a hydroalcoholic solvent system consisting of 70% ethanol and 30% distilled water to ensure efficient recovery of both polar and moderately polar phytoconstituents (11,13). The extraction process was performed at a controlled temperature corresponding to the boiling point of the solvent mixture and was allowed to proceed for 6–8 h until the siphon tube solvent appeared nearly colorless, indicating exhaustive extraction. After completion, the extract was filtered and concentrated under reduced pressure using a rotary evaporator to remove the solvent without thermal degradation of the active constituents (14). The concentrated extract was further dried to a constant weight, and the percentage yield was calculated. The dried hydroalcoholic extract was stored in an airtight container until further analysis.

2.3 Physicochemical Analysis and Preliminary Phytochemical Screening

The hydroalcoholic extract of *Cissus quadrangularis* was subjected to physicochemical evaluation to determine quality and purity parameters, including loss on drying, total ash, acid-insoluble ash, and extractive values, following standard pharmacopoeial procedures. These parameters were assessed to ensure consistency in the plant material and to detect the presence of any extraneous matter (15,16).

Subsequently, preliminary phytochemical screening of the extract was performed using standard qualitative chemical tests to identify the major classes of phytoconstituents. The extract was analyzed for the presence of alkaloids, flavonoids, phenolic compounds, tannins, saponins, glycosides, terpenoids, steroids, carbohydrates, and proteins. The development of characteristic color changes or precipitates has been used as an indicator of positive reactions (17–19). This combined evaluation provided essential information regarding the physicochemical stability and phytochemical composition of the extract prior to the biological activity studies. Heavy metals were analyzed using Atomic Absorption Spectroscopy (AAS), and pesticide residues

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were screened by Gas Chromatography–Mass Spectrometry (GC–MS). None were detected in the sample, confirming the safety and suitability of the plant material for further phytochemical and biological investigations.

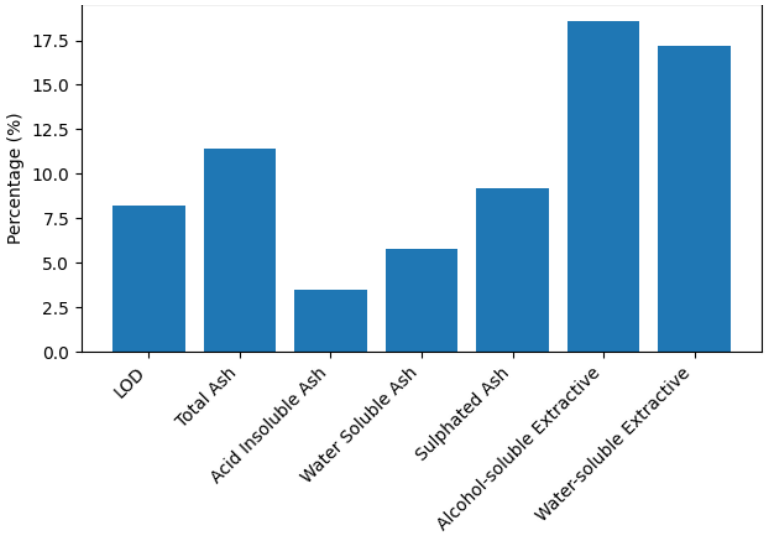
2.4 *In Vitro* Antifungal Activity

The in vitro antifungal activity of the hydroalcoholic extract of *Cissus quadrangularis* was evaluated against *Fusarium falciforme* using the food poisoning technique. The plant extracts were tested at three concentrations (2.5%, 5%, and 10% w/v), prepared using sterile 30% ethanol as the solvent. Sterile potato dextrose agar (PDA) medium was melted and cooled to 45–50 °C. The prepared extract solutions were aseptically incorporated into molten PDA to obtain the required final concentrations and mixed thoroughly. PDA plates incorporated with the plant extract served as test plates (20,21). PDA plates supplemented with fluconazole (100 mg/mL) were used as the positive control, whereas PDA plates containing 30% ethanol alone served as the negative control. For each concentration, three plates were prepared, corresponding to the plant extract, positive control, and negative control. After solidification, all plates were centrally inoculated with a 5 mm diameter mycelial disc taken from the actively growing margin of *Fusarium falciforme* culture. Inoculated plates were incubated under conditions suitable for fungal growth. After the incubation period, antifungal activity was evaluated by measuring radial mycelial growth in each plate using a standard scale (20–22). Mycelial growth was recorded in centimeters and used to assess the antifungal effect of the extract in comparison with the control plates.

3. Results and Discussion

3.1 Physicochemical Analysis

Physicochemical evaluation of *Cissus quadrangularis* revealed acceptable quality parameters. The pH of the extract was slightly acidic (6.3 for the 1% solution and 5.9 for the 10% solutions. Foreign organic matter was absent, indicating proper collection and processing of the plant material. The loss of the drying value was 8.2%, suggesting low residual moisture and good stability of the dried sample. The total ash value was 11.4%, reflecting a moderate level of inorganic content, whereas the acid-insoluble ash value was 3.5%, indicating minimal siliceous impurities. The water-soluble ash value (5.8%) and sulfated ash value (9.2%) further support the purity and acceptable mineral composition of the plant material. The extractive values demonstrated the efficient extraction of phytoconstituents, with alcohol-soluble and water-soluble extractive values of 18.6% and 17.2%, respectively. Heavy metal and pesticide residues were not detected in the sample, confirming the safety and suitability of the plant material for further phytochemical and biological investigations. Physicochemical characterization of the hydroalcoholic extract of Cissus quadrangularis, showing pH, ash values, moisture content, and extractive values used to assess the quality and purity of the plant material are shown in Figure 1.



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Figure 1. Physicochemical characterization of the hydroalcoholic extract of *Cissus quadrangularis*, showing pH, ash values, moisture content, and extractive values used to assess quality and purity of the plant material.

3.2 Preliminary Phytochemical Screening

Qualitative phytochemical screening of the hydroalcoholic extract of *Cissus quadrangularis* revealed a rich and diverse phytochemical profile (Table 1). Positive reactions were observed for major secondary metabolites including flavonoids, phenolic compounds, tannins, saponins, glycosides, terpenoids, and steroids. Alkaloids and carbohydrates were also detected in the extract, indicating the presence of nitrogenous- and carbohydrate-based constituents. In contrast, no proteins were detected, suggesting that hydroalcoholic extraction favored the enrichment of low-molecular-weight secondary metabolites over macromolecular constituents. The overall phytochemical profile highlighted the chemical complexity of the extract and provided a foundational understanding of its composition for subsequent analytical and biological investigations.

Table 1. Preliminary phytochemical screening of hydroalcoholic extract of *Cissus quadrangularis*

Phytoconstituent	Result
Alkaloids	+
Flavonoids	+
Phenolics	+
Tannins	+
Saponins	+
Glycosides	+
Terpenoids	+
Steroids	+
Carbohydrates	+
Proteins	−

3.3 *In Vitro* Antifungal Activity

The antifungal activity of the hydroalcoholic extract of *Cissus quadrangularis* against *Fusarium falciforme* was assessed by measuring radial mycelial growth using the food poisoning method (Table 2). The results showed a concentration-dependent reduction in mycelial growth in plates treated with the plant extract. No mycelial growth was observed in either the positive control (fluconazole, 100 mg/mL) or negative control (30% ethanol), indicating complete inhibition under the experimental conditions. Among the tested concentrations, the lowest mycelial growth was recorded at 10% extract concentration, followed by 5% and 2.5%, respectively. The effects of different concentrations (2.5%, 5%, and 10% w/v) of the hydroalcoholic extract of *Cissus quadrangularis* on radial mycelial growth of *Fusarium falciforme,* illustrating concentration-dependent antifungal activity compared with control treatments, are shown in Figure 2.

Table 2. Effect of hydroalcoholic extract of *Cissus quadrangularis* on mycelial growth of *Fusarium falciforme*

Treatment	Concentration	Mycelial growth (cm)
***Cissus quadrangularis* (Plant extract)**	2.5%	6.6
	5%	4.8
	10%	3.1
Positive control (Fluconazole)	100 mg/mL	0
Negative control (30% Ethanol)	—	0

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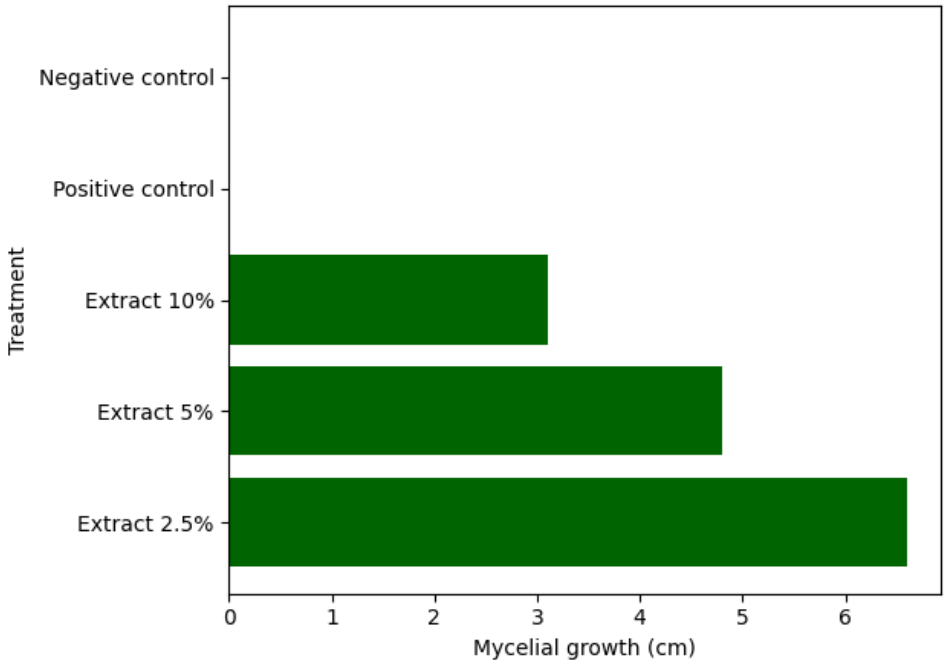


Figure 2. Effect of different concentrations (2.5%, 5%, and 10% w/v) of hydroalcoholic extract of Cissus quadrangularis on radial mycelial growth of Fusarium falciforme, illustrating concentration-dependent antifungal activity compared with control treatments.

The present study demonstrates that the hydroalcoholic extract of *Cissus quadrangularis* effectively inhibited the growth of *Fusarium falciforme* in a concentration-dependent manner, as evidenced by the progressive reduction in radial mycelial growth with increasing extract concentration. The strongest inhibitory effect was observed at a concentration of 10%, indicating enhanced antifungal efficacy at higher extract concentrations. The absence of mycelial growth in both the positive and negative control plates indicated complete growth suppression under experimental conditions. The lack of growth in the negative control confirmed that the solvent system did not support fungal proliferation, whereas the positive control validated the assay conditions.

The antifungal activity observed in the extract-treated plates may be attributed to the presence of bioactive phytoconstituents such as flavonoids, phenolic compounds, tannins, and saponins, which are known to disrupt fungal cellular structures and metabolic processes. These findings support the potential use of *Cissus quadrangularis* as a source of antifungal agents.

4. Conclusion

In conclusion, the present study demonstrated that the hydroalcoholic extract of *Cissus quadrangularis* possesses acceptable physicochemical qualities, safety, and phytochemical richness. The extract showed low moisture content, minimal inorganic and siliceous impurities, satisfactory extractive values, and the absence of heavy metals and pesticide residues, confirming its suitability for further investigation. Preliminary phytochemical screening revealed the presence of diverse bioactive secondary metabolites, including flavonoids, phenolics, tannins, saponins, glycosides, terpenoids, and steroids, while proteins were absent, indicating the enrichment of low-molecular-weight bioactive compounds. The in vitro antifungal study confirmed concentration-dependent inhibition of *Fusarium falciforme*, with maximum activity observed at 10% extract concentration, validating the antifungal potential of the plant extract. However, the study was limited to preliminary in vitro evaluation against a single fungal strain, and the exact bioactive compounds responsible for the observed antifungal activity were not isolated or structurally characterized. In addition, the absence of mechanistic studies and toxicity assessments represents a limitation in translating these findings into therapeutic applications. Overall, these findings highlight *Cissus quadrangularis* as a promising natural source of antifungal agents. Future research should focus on bioassay-guided fractionation, isolation, and

characterization of active constituents, detailed mechanistic investigations, evaluation against a broader spectrum of fungal pathogens, toxicity profiling, and in vivo validation to establish its clinical relevance and safety.

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None

Conflict of Interest

The authors declare no conflict of interest.

Authors' Contribution

Conceptualization; methodology; software; validation; formal analysis; investigation; resources; data curation; writing—original draft preparation; writing—review and editing; visualization; supervision; project administration by Qurratulayen Siddiqui and Yasmeen A. Shaikh. All authors have read and agreed to the published version of the manuscript.

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