



Phytochemical analysis and antimicrobial potential of *Cissus quadrangularis* extracts

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Abstract

Cissus quadrangularis, a succulent plant used in traditional medicine, was studied for its antimicrobial and phytochemical properties. Extracts were prepared using various solvents (methanol, ethanol, chloroform, ethyl acetate) and tested against bacterial and fungal strains using agar well diffusion. The phytochemical screening revealed the presence of steroids, flavonoids, tannins, and phenolics. Antimicrobial assays showed notable activity against *Staphylococcus aureus*, *E. coli*, and *Candida albicans*, especially in methanol and ethyl acetate extracts. These findings support the traditional use of *C. quadrangularis* and its potential as a source of antimicrobial agents. *Cissus quadrangularis*, a medicinal plant traditionally used in Ayurvedic and folk medicine, was investigated for its phytochemical profile and antimicrobial efficacy. The study aimed to evaluate the antibacterial and antifungal activities of various solvent extracts (methanol, ethanol, ethyl acetate, and chloroform) of the plant stem and identify key bioactive compounds responsible for these activities. Soxhlet extraction methods were employed for extract preparation, followed by phytochemical screening using standard protocols. The presence of triterpenoids, flavonoids, steroids, phenolics, and tannins was confirmed across all extracts. Antimicrobial activity was assessed via the agar well diffusion method against a panel of bacterial and fungal pathogens including *Escherichia coli*, *Staphylococcus aureus*, *Enterobacter aerogenes*, *Leuconostoc mesenteroides*, and *Candida albicans*. Among the extracts, methanol and ethyl acetate demonstrated the highest inhibitory effects, particularly against gram-positive bacteria and fungal strains. The observed activity underscores the potential of *C. quadrangularis* as a source of natural antimicrobial agents. The results support the traditional use of the plant in treating infections and provide a scientific basis for its pharmacological applications in antimicrobial drug development.

Keywords: *Cissus quadrangularis*, antimicrobial activity, phytochemical screening, extract, agar diffusion, traditional medicine

Introduction

Cissus quadrangularis (Vitaceae) is traditionally used in Indian medicine for bone healing, obesity, and gastrointestinal disorders. Phytochemical studies have reported the presence of triterpenoids, flavonoids, and stilbene derivatives contributing to its therapeutic potential. The emergence of multidrug-resistant microorganisms has intensified the global search for novel antimicrobial agents, particularly from natural sources. Medicinal plants have long been used in traditional healthcare systems due to their therapeutic properties and minimal side effects. One such plant, *Cissus quadrangularis* Linn. (family: Vitaceae), commonly referred to as "Hadjod" or "Asthisamharaka," has been widely used in Ayurvedic medicine for its bone-healing properties. It is a succulent plant found in tropical regions, particularly in India, and is used for the treatment of a wide range of ailments including bone fractures, ulcers, obesity, gastrointestinal disturbances, and infections. Recent pharmacological investigations have revealed that *C. quadrangularis* possesses diverse biological activities such as anti-inflammatory, antioxidant, analgesic, anti-ulcer, anti-diabetic, anti-osteoporotic, and antimicrobial properties. These bioactivities are largely attributed to its rich phytochemical composition, which includes flavonoids, triterpenoids, stilbene derivatives (e.g., resveratrol), phenolic compounds, and steroids such as β -sitosterol. These constituents have been shown to modulate key biochemical pathways involved in inflammation, oxidative

stress, and microbial resistance. Given the increasing interest in plant-based therapeutics and the promising bioactivity profile of *C. quadrangularis*, the present study aims to evaluate the antimicrobial and phytochemical properties of various solvent extracts of its stem. The study focuses on identifying the efficacy of different extracts against a range of bacterial and fungal pathogens and correlates the findings with the presence of specific phytochemicals. By doing so, this work intends to provide scientific validation for the traditional medicinal uses of *C. quadrangularis* and explore its potential as a candidate for the development of natural antimicrobial agents. The rise of antimicrobial resistance and the diminishing efficacy of conventional antibiotics have become a pressing global health concern, prompting researchers to explore alternative therapeutic strategies. Natural products, particularly those derived from medicinal plants, have emerged as promising candidates for the discovery of novel bioactive compounds due to their structural diversity and historical relevance in traditional medicine. Over 80% of the world's population relies on traditional herbal remedies for primary healthcare, as recognized by the World Health Organization (WHO), highlighting the continued relevance of ethnopharmacological practices.

Among these plants, *Cissus quadrangularis* Linn., a member of the Vitaceae family, has garnered attention for its wide spectrum of pharmacological effects. Native to India and other tropical regions, it is colloquially known as

“Hadjod” (bone setter) in Hindi, referencing its well-documented use in accelerating bone fracture healing. Traditional healers have also employed this plant for treating gastrointestinal disorders, piles, asthma, obesity, and menstrual irregularities. The plant is characterized by its quadrangular, fleshy stem, which is the primary source of its therapeutic compounds. Phytochemical analyses of *C. quadrangularis* have confirmed the presence of a variety of secondary metabolites, including triterpenoids (e.g., amyriins), flavonoids (e.g., quercetin and kaempferol), stilbenes (e.g., resveratrol), tannins, and phenolic acids, many of which are known for their antimicrobial, antioxidant, and anti-inflammatory properties. Some studies have suggested that these bioactive compounds act synergistically, enhancing the plant’s therapeutic potential against various pathogens and chronic diseases. In recent years, studies have begun to scientifically validate the antimicrobial activity of *C. quadrangularis*, yet comparative evaluations of its different solvent extracts remain limited. The polarity of solvents used during extraction significantly influences the yield and type of phytochemicals obtained, which in turn affects bioactivity. Therefore, evaluating and comparing the antimicrobial efficacy of multiple extracts can provide insights into the optimal extraction conditions and therapeutic potential. This study aims to investigate the phytochemical composition and antimicrobial activity of methanol, ethanol, ethyl acetate, and chloroform extracts of *C. quadrangularis* stem. Antibacterial and antifungal effects were tested against clinically relevant bacterial strains such as *Escherichia coli*, *Staphylococcus aureus*, *Leuconostoc mesenteroides*, *Enterobacter aerogenes*, and the fungal pathogen *Candida albicans*. The findings are intended to contribute to the growing body of evidence supporting *C. quadrangularis* as a valuable medicinal plant with potential application in natural product-based antimicrobial therapies.

Materials and Methods

1. Collection and Identification of Plant Material

The fresh, healthy, and disease-free stems of *Cissus quadrangularis* were collected from the Botanical Garden of Punjabi University, Patiala, Punjab, India. The plant was authenticated by a qualified botanist, and a voucher specimen was preserved for future reference. The collected stems were thoroughly washed under running water to remove dust, soil, and other contaminants, followed by rinsing with distilled water. The cleaned stems were cut into small pieces and shade-dried for 15–20 days at ambient temperature until they became brittle. The dried material was ground into a fine powder using a mechanical blender and stored in airtight containers for further use.

2. Preparation of Extracts

Sequential extraction was carried out using Soxhlet apparatus with solvents of increasing polarity—chloroform, ethyl acetate, ethanol, and methanol. The powdered plant material (8–15 g per extraction) was loaded into the main chamber of the Soxhlet apparatus, and 70–120 mL of each solvent was used.

Each extraction was performed at a controlled temperature range of 60–80°C for 8–10 hours. The extracts were filtered and concentrated using a rotary evaporator or dried in a hot air oven at 40°C to obtain solid residues. The percentage yield of each extract was calculated using the following

formula:

$$\text{Yield (\%)} = (\text{Weight of dried extract} / \text{Weight of plant material}) \times 100$$

3. Phytochemical Screening

Qualitative phytochemical screening of the prepared extracts was performed to detect the presence of various secondary metabolites such as flavonoids, alkaloids, tannins, saponins, steroids, glycosides, and phenolic compounds. Standard methods were employed for the detection of each class of compounds:

- **Flavonoids:** Alkaline reagent test
- **Tannins:** Ferric chloride test
- **Alkaloids:** Dragendorff’s and Mayer’s reagent tests
- **Saponins:** Frothing test
- **Steroids:** Liebermann–Burchard test
- **Phenols:** Ferric chloride test

4. Test Microorganisms

The antimicrobial potential of the extracts was assessed against five microbial strains:

- **Bacterial strains:** *Escherichia coli* (MTCC 1678), *Staphylococcus aureus* (MTCC 3160), *Leuconostoc mesenteroides* (MTCC 107), *Enterobacter aerogenes* (MTCC 111), *Staphylococcus xylosum* (MTCC 7443)
- **Fungal strain:** *Candida albicans*

All microbial cultures were procured from the Institute of Microbial Technology (IMTECH), Chandigarh, and maintained on appropriate media prior to testing.

5. Antibacterial and Antifungal Assay

The antibacterial and antifungal activities of each extract were evaluated using the Agar Well Diffusion Method on Mueller-Hinton Agar (MHA) for bacteria and Sabouraud Dextrose Agar (SDA) for fungi. The media were prepared according to standard protocols and autoclaved at 121°C for 15–20 minutes.

Petri plates were poured with 20–25 mL of sterile molten agar and allowed to solidify. Microbial suspensions were prepared by adjusting the turbidity to match 0.5 McFarland standard and uniformly swabbed onto the surface of solidified agar. Wells of 6 mm diameter were punched aseptically into the agar using a sterile cork borer. Each well was loaded with 50 µL of plant extract at different concentrations (ranging from 5–50 mg/mL depending on solvent).

6. Controls

- **Positive controls:** Chloramphenicol and Streptomycin for bacteria; Fluconazole for fungi
- **Negative control:** Dimethyl sulfoxide (DMSO), the solvent used to dissolve the extracts

All plates were incubated at 37°C for 24 hours (bacteria) and at 25°C for 72 hours (fungi). The diameter of the zone of inhibition around each well was measured in millimeters to assess antimicrobial activity.

Results

The percentage yield of *Cissus quadrangularis* stem extracts varied depending on the solvent used. The highest yield was observed with methanol, followed by ethanol, ethyl acetate, and chloroform.

Table 1: Yield Percentage of Different Solvent Extracts

Solvent	Weight of Extract (g)	Yield (%)
Methanol	1.74	11.6%
Ethanol	1.36	9.0%
Ethyl Acetate	1.28	8.5%
Chloroform	0.96	6.4%

1. Phytochemical Screening

Qualitative analysis revealed the presence of multiple phytochemicals known for antimicrobial properties. All extracts contained flavonoids and phenols, while steroids, tannins, and saponins were variably distributed.

Table 2: Phytochemical Constituents Detected in Extracts of *C. quadrangularis*

Phytochemical	Methanol	Ethanol	Ethyl Acetate	Chloroform
Flavonoids	+	+	+	+
Tannins	+	+	+	-
Saponins	+	+	-	-
Steroids	+	+	+	+
Alkaloids	-	-	-	-
Phenols	+	+	+	+

2. Antibacterial Activity

The antibacterial activity of each extract was tested against five bacterial strains. Methanol and ethyl acetate extracts exhibited the strongest activity, particularly against *Staphylococcus aureus* and *Leuconostoc mesenteroides*.

Table 3: Antibacterial Activity (Zone of Inhibition in mm)

Extract	<i>S. aureus</i>	<i>E. coli</i>	<i>L. mesenteroides</i>	<i>E. aerogenes</i>	<i>S. xylosum</i>
Methanol	18.5	16.2	17.4	14.3	15.8
Ethanol	17.3	14.9	15.2	13.0	14.5
Ethyl Acetate	16.8	13.7	16.5	12.1	13.9
Chloroform	14.6	11.2	13.8	10.5	12.0
Positive Ctrl.	22.0	21.5	23.1	20.3	21.7
Negative Ctrl.	0.0	0.0	0.0	0.0	0.0

3. Antifungal Activity

Methanol and aqueous extracts showed moderate inhibition against *Candida albicans*.

Table 4: Antifungal Activity Against *C. albicans*

Extract	Zone of Inhibition (mm)
Methanol	13.4
Ethanol	11.7
Ethyl Acetate	10.3
Chloroform	9.1
Positive Ctrl.	19.6
Negative Ctrl.	0.0

The results indicate that the methanol extract of *Cissus quadrangularis* stem possesses the most potent antimicrobial activity, likely due to its higher yield and rich phytochemical content. Ethyl acetate extracts also showed consistent bioactivity, particularly against gram-positive bacteria.

Discussion

The present study evaluated the phytochemical composition and antimicrobial potential of *Cissus quadrangularis* stem extracts obtained using various solvents. The findings confirm that *C. quadrangularis* is a rich source of bioactive

compounds and exhibits promising antimicrobial activity, particularly in its methanol and ethyl acetate extracts. The high extractive yield in methanol (11.6%) aligns with previous studies that have reported methanol as an effective solvent for extracting polar phytoconstituents such as flavonoids, phenolics, and tannins [Kashikar & George, 2006]. These classes of compounds are well-known for their antimicrobial, antioxidant, and anti-inflammatory effects. Phytochemical screening revealed that all extracts contained flavonoids, steroids, and phenols, while tannins and saponins were predominantly found in the methanol and ethanol extracts. The presence of these secondary metabolites provides a strong chemical basis for the observed biological activities. The methanol and ethyl acetate extracts demonstrated broad-spectrum antibacterial activity, particularly against *Staphylococcus aureus*, *Leuconostoc mesenteroides*, and *Escherichia coli*. These results are consistent with prior reports which have shown that extracts of *C. quadrangularis* possess potent antibacterial properties against both gram-positive and gram-negative bacteria. The greater susceptibility of gram-positive bacteria observed in this study may be attributed to their simpler cell wall structure, which allows better penetration of plant-based antimicrobial agents. The effectiveness of the methanol extract across both antibacterial and antifungal assays suggests that it contains

the highest concentration of bioactive constituents. This highlights the importance of solvent polarity in phytochemical extraction and reinforces the need for optimized extraction protocols in plant-based drug development. Overall, the study validates the ethnomedicinal use of *C. quadrangularis* in treating infections and supports its potential for the development of natural antimicrobial formulations. Further work, including isolation and structural elucidation of individual compounds and mechanistic studies, is recommended to better understand the antimicrobial pathways and to assess clinical applicability.

Conclusion

Extracts of *Cissus quadrangularis* demonstrate promising antimicrobial and phytochemical profiles. Future work may isolate active compounds for drug development. This study highlights the significant antimicrobial potential and phytochemical richness of *Cissus quadrangularis* stem extracts, particularly those obtained using methanol and ethyl acetate. The extracts exhibited considerable activity against both gram-positive and gram-negative bacteria, as well as the fungal pathogen *Candida albicans*. Qualitative phytochemical screening confirmed the presence of flavonoids, phenolics, steroids, tannins, and saponins, which likely contribute to the observed bioactivity. The results support the traditional medicinal use of *C. quadrangularis* in treating infections and reinforce its relevance in the ongoing search for natural alternatives to synthetic antimicrobial agents.

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