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Research article

Comprehensive Phytochemical Profiling and Molecular Characterization of *Cissus quadrangularis* Ethanolic Extract: Towards Unveiling Therapeutic Potentials.

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ABSTRACT

The stems of *Cissus quadrangularis* were collected systematically from the Coimbatore region of Tamil Nadu, India. Continuous ethanolic extraction using a Soxhlet apparatus yielded a crude ethanolic extract, which was subjected to percentage yield calculation for the extraction efficiency assessment. Phytochemical evaluation was performed using conventional thin-layer chromatography (TLC). Liquid Chromatography-Mass Spectrometry (LC-MS) and Gas Chromatography-Mass Spectrometry (GC-MS) analyses provided insights into the molecular constituents, whereas High-Performance Liquid Chromatography (HPLC) fingerprint analysis contributed to comprehensive characterization. The ethanolic extraction of *C. quadrangularis* using the Soxhlet technique resulted in a wet weight of the crude extract of 10 g, with a corresponding dry weight of 4.5 grams. The percentage yields calculated indicated that 2% of the wet ethanolic extract and 0.9% of the dry ethanolic extract were obtained from the crude drug. Phytochemical analysis revealed the presence of alkaloids, flavonoids, amino acids, carbohydrates, saponins, tannins, phenolic compounds, and more in the ethanolic extract, which is consistent with previous studies. Thin-layer chromatography (TLC) profiling further confirmed the presence of flavonoids, alkaloids, phenols, and tannins. LC-MS analysis identified a diverse array of compounds, including Quercetin-3-O-alpha-L-rhamnopyranoside, Vanilline, Beta-Amyrone, and various fatty acids, all of which have distinct pharmacological implications. The GC-MS analysis unveiled compounds such as B-Sitosterol, 4-Ethylbenzoic acid, and Vitamin E, contributing to the extract's potential health benefits. HPLC analysis provided a chromatographic profile indicating distinct peaks with varying intensities and area percentages, demonstrating the complexity of the *C. quadrangularis* ethanolic extract. This comprehensive characterization underscores the rich phytochemical diversity and potential bioactivity of this plant, paving the way for further exploration of its therapeutic applications.

Keywords: *Cissus quadrangularis*, Ethanolic extraction, Soxhlet extraction, LC-MS, Lyophilization.

INTRODUCTION

C. quadrangularis, commonly known as the Veldt grape or Devil's backbone, is a succulent plant belonging to the grape family (Vitaceae). Its square-shaped stem, characteristic of the species, has earned it the name "quadrangularis." This perennial plant, native to parts of Asia and Africa, has garnered attention for its traditional medicinal uses and potential health benefits [1]. In this discussion, we delve into the growth and development of *Cissus quadrangularis*, exploring its botanical features, cultivation practices, growth stages, and the factors influencing its overall well-being. *C. quadrangularis* exhibits a unique square-shaped stem, a feature from which it derives its name [2]. As a climber or creeper, the plant's succulent green stems are capable of storing water, enabling them to withstand periods of drought. The plant's native habitat spans tropical and subtropical regions in Asia and Africa, thriving in well-drained soils with moderate moisture levels. A slightly acidic to neutral pH range is considered optimal, and the plant displays adaptability to various soil types. *Cissus quadrangularis* is known for its hardness and resilience, making it suitable for cultivation in diverse environments. The cultivation of *C. quadrangularis* involves providing plants with suitable environmental conditions. It prefers tropical and subtropical climates, well-drained soils, and moderate moisture levels. Plants are versatile and can adapt to different soil types, with a preference for a slightly acidic to neutral pH range. Adequate sunlight is crucial for plant growth, and the plant can thrive in both full sun and partial shade. Propagation through stem cuttings is a common practice, and once established, *Cissus quadrangularis* can be cultivated in gardens, pots, or containers [3].

The life cycle of *C. quadrangularis* unfolds through distinct stages, commencing with germination of seeds under warm and moist conditions. As seedlings emerge, the development of cotyledons becomes crucial, serving as the initial leaves that provide essential nutrients to the young plant. Vigilant attention to watering and protection from extreme weather conditions is imperative during the vulnerable seedling stage. Transitioning into the vegetative growth phase, *C. quadrangularis* establishes trademark square-shaped stems and generates simple palmate leaves [4]. This stage is pivotal for structural development and biomass accumulation, thereby laying the foundation for the overall robustness of the plant. The subsequent flowering and fruit formation phases saw the production of small, greenish-yellow flowers in clusters, followed by the development of berries or drupes containing seeds, contributing significantly to the plant's reproductive success [5]. Various factors influence the growth and development of *C. quadrangularis*, including the need for adequate sunlight to facilitate photosynthesis and overall plant health, consistent and moderate watering during the establishment phase and dry periods, a preference for well-drained soil with a slightly acidic to neutral pH that directly

impacts nutrient uptake and root development, the thriving of the plant at warm temperatures while requiring protection in frost-prone areas during colder seasons, and the importance of providing a balanced fertilizer with essential nutrients to support healthy growth, particularly during vegetative and flowering stages. Each of these factors plays a pivotal role in shaping the life and vitality of this unique succulent plant [6,7].

C. quadrangularis is a resilient succulent plant that contains a variety of phytochemicals that contribute to its medicinal properties. Phytochemical analysis identified the presence of compounds such as flavonoids, triterpenoids, carotenoids, and tannins in *C. quadrangularis*. These bioactive constituents are crucial for their traditional use in Ayurvedic medicine because they possess anti-inflammatory, antioxidant, and analgesic properties. Flavonoids play a significant role in the therapeutic effects of plants [8,9]. Identification of these phytochemicals has sparked interest in the potential health benefits of *C. quadrangularis*, including its use in managing joint and bone health, promoting weight loss, and supporting overall well-being. Ongoing research continues to explore the specific mechanisms and applications of these phytochemicals, highlighting the importance of plants in herbal medicine and their potential contribution to modern healthcare. The current work involved gathering, extracting, and identifying phytochemicals from *Cissus quadrangularis* using various analytical techniques.

MATERIAL AND METHODS

Plant collection and drying

The stems of *C. quadrangularis* were systematically collected from the Coimbatore region in Tamil Nadu, India on October 31, 2021 (Figure 1). Coimbatore, renowned for its rich biodiversity, serves as an optimal habitat for the flourishing of *Cissus quadrangularis*, a medicinal plant recognized for its potential therapeutic attributes. Following collection, rigorous processing protocols were used to safeguard the chemical composition of the plant. The harvested stems were subjected to a careful shade-drying technique, a widely utilized method that ensures the preservation of bioactive compounds inherent to the plant. This procedure involves exposing the plant material to a controlled environment with diminished sunlight, facilitating a gradual dehydration process without compromising the quality of its constituents.



Figure 1: *C. quadrangularis*

Continuous ethanolic extraction-Soxhlet

Two kilograms of *C. quadrangularis*, previously shade-dried, underwent initial crushing and was then placed into a filter paper thimble. This thimble, containing 500 g of ground *C. quadrangularis*, was positioned in chamber E within the Soxhlet apparatus (Figure 2), a widely used tool in chemical extraction. The setup involved applying heat to the ethanolic solvent in flask A, causing it to vaporize. The vapor ascended, condensed in condenser D, and then dripped into the thimble containing *C. quadrangularis*. The choice of an ethanolic solvent is strategic given its efficacy in dissolving a diverse range of phytochemicals present in the plant material. Continuous contact between the crude plant material and the dripping extractant ensures thorough extraction of bioactive compounds. A syphon mechanism was employed to control the liquid flow in chamber E. Once the liquid level in chamber E reached the top of syphon tube C, the liquid was syphoned into flask A, efficiently recycling the solvent and enhancing the extraction process. This iterative extraction continued until a drop of solvent

from the syphon tube evaporated without leaving any residue, indicating the completion of the extraction. The resulting crude ethanolic extract was carefully preserved for subsequent phytochemical analysis (Figure 2) [10].



Figure 2: Hot Continuous ethanolic extraction-Soxhlet

Percentage of yield of Ethanolic extract of *C. quadrangularis*

Determination of the percentage yield of the crude ethanolic extract of *C. quadrangularis* involves comparing the actual yield obtained through the experimental extraction process with the theoretical yield, which represents the maximum extractable amount under ideal conditions. Calculated using the formula (Percentage Yield = (actual yield/theoretical yield) × 100), this percentage is a crucial metric that indicates the efficiency of the extraction procedure.

Percentage yield of Wet and Dry extracts

The calculation of the percentage yield of the dried crude ethanolic extract of *C. quadrangularis* involves contrasting the actual yield of the crude wet extract obtained from the experimental extraction process with the practical dried yield achieved through lyophilization. The latter represents an estimate of the maximum amount of dry crude extract that is achievable under ideal conditions. Computed using the formula (Percentage Yield Dry Crude Extract = (Weight of Dry Crude Extract / Wet Crude Extract) × 100), this percentage serves as a crucial metric for evaluating the efficiency of the extraction procedure (Figure 3).



Figure 3: Lyophilization of crude ethanolic *C. quadrangularis* extract

Phytochemical evaluation

The ethanolic extract of *C. quadrangularis* was used for phytochemical screening using a standard procedure [10].

Separation using thin-layer chromatography

Aluminum sheets with were 7.5 cm × 10 cm were used for TLC (Thin Layer Chromatography) using TLC silica gel 60F254 (Merck, Germany). For TLC analysis, ethanolic extracts of *Cissus quadrangularis* were prepared at a concentration of 100 mg/mL in the appropriate solvents. Using a capillary tube, each extract (10 μ L) was manually placed in the form of dots on plates. There were four applications on each plate, with a 1.5 cm gap between each pair of spots, and the application took place 1 cm from the bottom and 1.5 cm from the side of the plate. All analyses used the same application parameters. The TLC plates were then placed in a developing glass chamber that had been saturated with various solvent systems for the mobile phase. Following development, the plates were removed from the chamber and dried on a hot plate to eliminate solvents from the mobile phase. The development distance is fixed at 80 mm. Through this procedure, distinct spots on the TLC plates were separated, each of which corresponded to a different chemical found in the ethanolic extracts of *C. quadrangularis* [11].

Analysis of the ethanolic extract by LC-MS

A Liquid Chromatography-Mass Spectrometry (LC-MS/MS-8045 Shimadzu) system was used to perform molecular weight analysis of the ethanolic extract from *C. quadrangularis*, as shown in Figure 4 [12]. A 10 μ l aliquot of the ethanolic extract was diluted and immediately vortexed after centrifugation at 10,000 rpm for ten minutes in order to prepare it for analysis. The resultant supernatant solution was then preserved at -18 $^{\circ}$ C for a later phytochemical examination after being filtered through Whatman filter paper (Grade 41). A solvent composition of 100% methanol and 0.5% (v/v) acetic acid was used for the chromatographic

separation. Acetic acid was used as the solvent in the elution process at the following rates: (i) 55% from 0 to 10 min, (ii) 65% from 11 to 20 min, and (iii) 35% from 21 to 30 min of the total run time. The column temperature was maintained at 30°C during the study and the Photodiode Array (PDA) detector was calibrated at a wavelength of 340 nm to monitor the chromatographic peaks. The system was operated in positive ionization mode for mass spectrometric analysis, covering a mass range of 150 m/z to 1000 m/z. The ionization parameters were as follows: capillary voltage, 3.50 kV, cone voltage, 30 V; extractor voltage, 3 V; gas flow rate, 30 L/Hr; and collision gas flow, 0.18 mL/Min were the ionisation parameters. This analytical approach covers a wide mass range and offers insights into the molecular constituents of the extract, making it easier to identify and characterize the molecular compounds in the ethanolic extract. As shown in Figure 4, the incorporation of LC-MS/MS techniques improved the sensitivity and precision of the analysis, leading to a thorough understanding of the phytochemical composition of the *C. quadrangularis* extract.

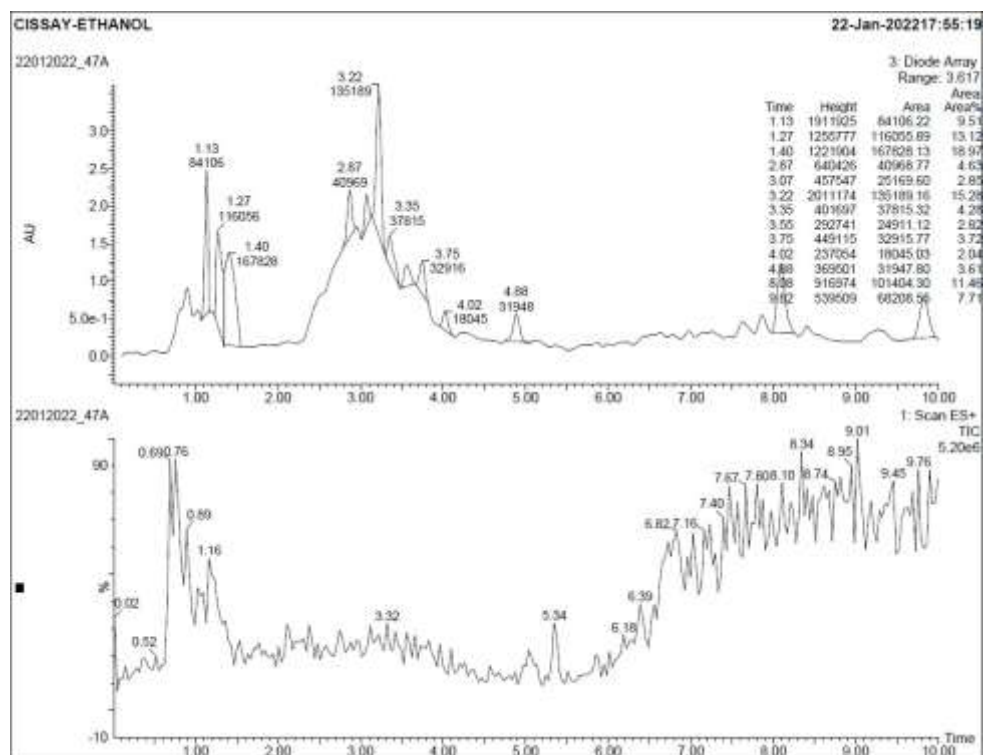


Figure 4: LC-MS analysis of ethanolic extract of *C. quadrangularis*

GC-MS analysis of ethanolic extract of *C. quadrangularis*

Gas Chromatography-Mass Spectrometry (GC-MS) was used to examine the phytochemicals present in the ethanolic extract of *C. quadrangularis*. The mobile phase flow rate was tuned to 0.1 ml/min for GC-MS analysis using the MS DSQ II GC-MS equipment from Thermo Scientific Co.. The temperature gradient ranged from 40°C to 250°C at a rate of 5°C/min, and

a 1 µl injection volume was used for the analysis. The mass values obtained during the analysis were cross-referenced with the Willy spectral library [13] for comparison and identification. This analytical approach enables a detailed exploration of the chemical composition of the ethanolic extract, providing valuable insights into the specific phytochemicals present. The use of the advanced MS DSQ II GC-MS instrument, coupled with the Willy spectral library, enhances the accuracy and reliability of compound identification and contributes to a comprehensive understanding of the phytochemical profile of *C. quadrangularis*.

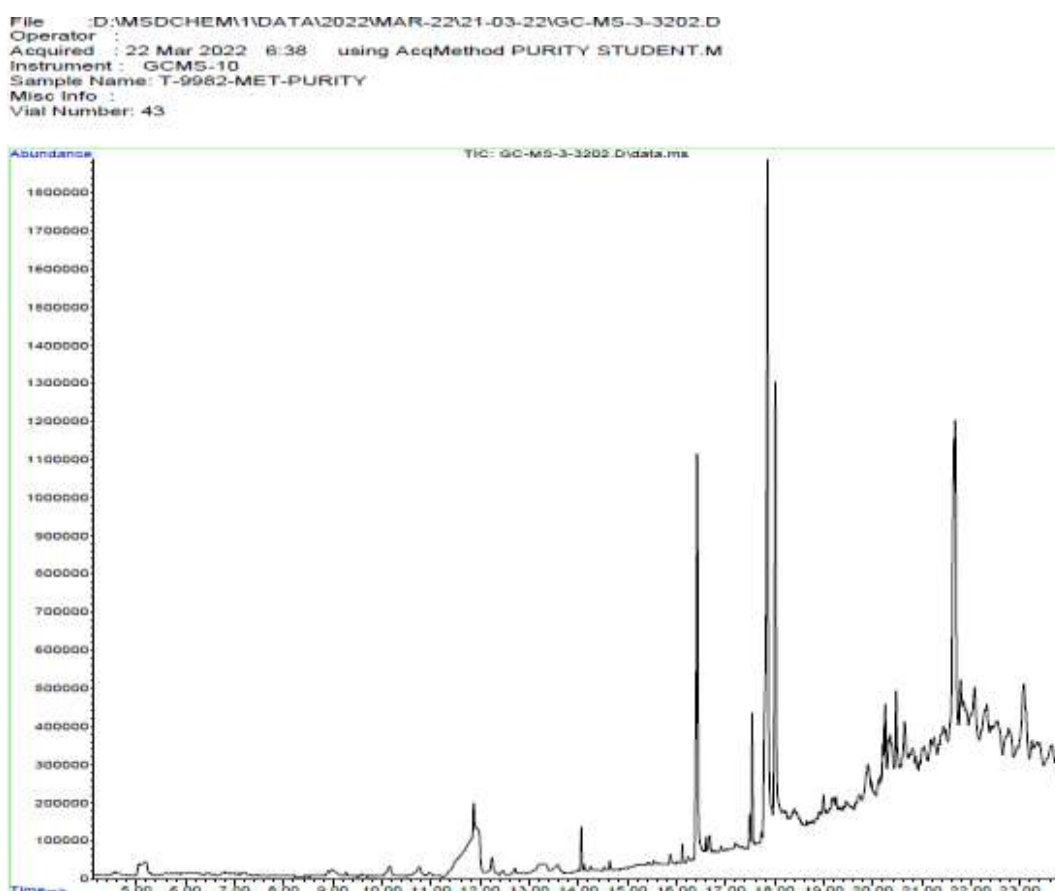


Figure 1: GC-MS chromatogram ethanolic extract of *C. quadrangularis*

HPLC phytochemical analysis

Shah et al. (2010)'s methodology was applied to the ethanolic extract of *C. quadrangularis* before it was subjected to HPLC analysis (Figure 5). High-Performance Liquid Chromatography (HPLC) was used to analyse fingerprints. A SHIMADZU 15C HPLC-DAD system with an ODS-3v of 4.6150 mm and 5 µm was used. The mobile phase employed a gradient elution using solutions A and B containing 0.1% TFA in water and acetonitrile, respectively, at a flow rate of 1 mL/min. Between 0 and 25 min, the gradient program was as follows: 0–6 minutes, 0–7% B; 6–16 min, 7–30% B; 16–31 minutes, 30–80% B; and 31–40 min, 80–90% B. At 210 nm, the chromatogram was seen, and the column temperature was

continuously kept at 37 °C. An injection volume of 10 µL was used for the analysis. Using the different elution profiles of the sample's constituents during the designated gradient elution programme, this method makes it easier to characterise the sample's chemical composition in detail [14, 15].

RESULTS AND DISCUSSION

Ethanollic extraction of *C. quadrangularis*

Crude ethanollic extract was obtained from *C. quadrangularis* using a Hot Continuous Extraction method, specifically the Soxhlet extraction technique. Wet weight of the crude *C. quadrangularis* ethanollic extract (10 g) and dry weight of the crude *C. quadrangularis* ethanollic extract (4.5 g). 2% of percent of *C. quadrangularis* wet cured ethanollic extract obtained from the crude drug and 0.9 % of dry crude *C. quadrangularis* ethanollic extract obtained from the crude drug.

Table 1: weight of crude ethanollic extract was obtained from *C. quadrangularis*

Crude drug	Wet weight of crude extract of <i>C. quadrangularis</i>	Dry weight of crude extract <i>C. quadrangularis</i>	Color
500 gm	10	4.5	Dark colour



Figure 1: Cure ethanollic extract of *C. quadrangularis*

Phytochemical analysis of extract *C. quadrangularis* by conventional method

Table 1 displays the findings of the phytochemical screening of the *C. quadrangularis* ethanollic extract. Alkaloids, flavonoids, amino acids, carbohydrates, saponins, tannins, and phenolic compounds were among the numerous phytochemical components discovered.

Table 1: Investigating an ethanollic *C. quadrangularis* extract using phytochemical screening.

S.NO	Phytochemical Test	Ethanollic extract
1	Alkaloids	+
2	Carbohydrate	-
3	Glycosides	+
4	phenolic compounds	+
5	Protein and amino acid	+
6	Gum and mucilage	+
7	Flavones and flavonoids	+
8	Saponins	+
9	Steroids and sterols	+
10	Tannins	+

Phytochemical examination of *C. quadrangularis* extract using TLC

Thin-layer chromatography was used to separate the phytochemical components of the *C. quadrangularis* ethanolic extract (TLC). For the various phytochemicals, TLC profiling of the ethanolic extract was carried out utilising a variety of solvent systems (Table 2).

Table 2: Phytochemical contents of an ethanolic extract of *C. quadrangularis*-TLC

S.NO	Phytochemical Test	Ethanollic extract
1	Flavonoids	+
2	Alkaloids	+
3	Phenols	+
4	Tannins	+

LC-MS phytochemical study of *C. quadrangularis* extract

By using liquid chromatography (LC-MS), the phytochemical composition of the ethanolic extract of *C. quadrangularis* was determined. 100% methanol and 0.5% (v/v) acetic acid were used as the solvent system for the LC-MS profiling of the ethanolic extract.

Table 3: Phytochemical contents of an ethanolic extract of *C. quadrangularis*-LC-MS

S. NO	Compound Name	Molecular Weight
1.	Quercetin-3-O-alpha-L-rhamnopyranoside	448.4
2.	Vanilline	152.36
3.	Beta-Amyrone	424.43

4.	Beta-Sitosterol acetate	456.73
5.	Cholesterin	385.42
6.	Quinoline	129.16

7.	Phytol	295.86
8.	Erucic acid	338.20
9.	Hexadecanoic acid	258.24
10.	Carotene	535.59
11.	Linoleic acid	280.13
12.	Vaccenic acid	282.29
13.	Oleic acid, 10-octadecenoic acid	282.30
14.	Tetradecanoic acid	228.31
15.	Pentadecanoic acid	242.29
16.	Isopropyl stearate	326.83

LC-MS analysis of the ethanolic extract of *Cissus quadrangularis* revealed a diverse array of phytochemical constituents, each identified by its compound name and corresponding molecular weight. Notable compounds include Quercetin-3-O-alpha-L-rhamnopyranoside (448.4), known for its antioxidant properties, vanilline (152.36), recognized for its aromatic characteristics, and beta-amyrone (424.43), which may contribute to the bioactivity of the extract. Beta-sitosterol acetate (456.73) and cholesterol (385.42) are sterols with potential health benefits. Quinoline (129.16), Phytol (295.86), and Erucic acid (338.20) add to the chemical diversity, each with distinct pharmacological implications. Fatty acids such as hexadecanoic acid (258.24), linoleic acid (280.13), and oleic acid (282.30) present in various forms contribute to the lipid profile of the extract. Carotene (535.59) is a precursor of vitamin A, and may contribute to the antioxidant capacity of the extract. Vaccenic acid (282.29) and tetradecanoic acid (228.31) further enhanced the fatty acid composition of the extract. Pentadecanoic acid (242.29) and isopropyl stearate (326.83) were additional components with potential bioactive properties. This comprehensive phytochemical profiling underscores the richness and complexity of *C. quadrangularis* ethanolic extract. The identified compounds spanned a spectrum of chemical classes with diverse biological activities, suggesting the potential therapeutic significance of the plant. Further studies are warranted to elucidate the specific health-promoting properties and synergistic effects of these phytochemicals in *C. quadrangularis*.

Phytochemical evaluation of ethanolic extract of *C. quadrangularis* by GC-MS

The chemicals identified by GC-MS analysis of the ethanolic extract of *C. quadrangularis* were tentatively identified. The chemicals were identified using the NIST collection in conjunction with their fragmentation patterns. The compounds were identified based on peak area and retention duration (Table 2).

Table 2: Phytochemical screening of an ethanolic extract of *C. quadrangularis* by GC-MS analysis

S.NO	Phytoconstituents	Mass (M/Z)
1	B-Sitosterol	414
2	4-Ethylbenzoic acid	150
3	Antra-9,10-quinone	343
4	2-p-Nitrophenyl-oxadiazol-1,3,4-one-5	207
5	1,4-Phthalazinedione, 2,3-dihydro-6-nitro	207
6	5-Methyl-2-phenylindolizine	207
7	2-Nitro-4-(trifluoromethyl)phenol	207
8	Octanoic acid	298
9	Vitamin E	431.50

GC-MS analysis of the ethanolic extract of *C. quadrangularis* revealed a diverse array of phytoconstituents, each identified by its mass-to-charge ratio (M/Z). Among the notable compounds detected were B-Sitosterol, known for its potential health benefits, 4-Ethylbenzoic acid, a derivative of benzoic acid with diverse biological activities; and Antra-9,10-quinone, which contributes to its potential antioxidant properties. Additionally, the presence of various derivatives such as 2-p-Nitrophenyl-oxadiazol-1,3,4-one-5, 1,4-Phthalazinedione, 2,3-dihydro-6-nitro, and 5-Methyl-2-phenylindolizine, suggests a chemical complexity that may have pharmacological significance. Compounds like 2-Nitro-4-(trifluoromethyl)phenol, octanoic acid, and Vitamin E further enriched the extract composition. This comprehensive profiling highlights the potential health-promoting properties of the *C. quadrangularis* extract, motivating further exploration of its biological activities and therapeutic applications. The compounds identified from the ethanolic extract of *Cissus quadrangularis* exhibit diverse biological applications. B-Sitosterol, a plant sterol, may contribute to cardiovascular health by lowering LDL cholesterol levels [16]. 4-Ethylbenzoic acid, a derivative of benzoic acid, has antimicrobial properties. Quinones, such as Antra-9,10-quinone, often exhibit antioxidant properties and potentially influence cellular redox processes [17]. Nitrophenyl compounds like 2-p-Nitrophenyl-oxadiazol-1,3,4-one-5 may find applications as fluorescent probes in

biological imaging. Nitro-substituted phthalazinediones, including 1,4-Phthalazinedione, 2,3-dihydro-6-nitro, have medicinal applications and may display antimicrobial or cytotoxic activity [18]. The indolizine derivative 5-Methyl-2-phenylindolizine has pharmacological activities, including anti-inflammatory and antimicrobial effects [19]. 2-Nitro-4-(trifluoromethyl)phenol, a nitrophenol, may possess antimicrobial properties and serve as a precursor for organic synthesis. Octanoic acid, also known as caprylic acid, exhibits antimicrobial properties, and has potential applications in the management of infections. Vitamin E, a fat-soluble antioxidant, plays a crucial role in cellular protection from oxidative stress, supporting skin health and the immune system, and potentially preventing chronic diseases [20]. These compounds show pharmacological diversity within the *C. quadrangularis* extract, suggesting their potential significance in various health-related applications.

Phytochemical evaluation by HPLC

HPLC analysis of the ethanolic extract of *Cissus quadrangularis* revealed a chromatographic profile with distinct peaks identified by their Retention Time (RT), corresponding height (μV), and relative area percentages. Notable peaks occurred at RT values of 1.646, 9.645, 10.501, and 15.539 min, with varying intensities and contributions to the overall composition, as indicated by the area percentages. Additionally, novel compounds seem to be present, as suggested by the peaks at RT values of 16.935 and 17.156 min. These compounds, marked by their unique RTs and corresponding chromatographic characteristics, may signify the presence of unidentified constituents in the ethanolic extract. Specifically, the chromatogram illustrates the complexity of the extract, with peaks displaying diverse intensities, as reflected by the height (μV) values. This comprehensive HPLC analysis provided valuable insights into the composition of the *C. quadrangularis* extract, providing a foundation for further exploration and identification of novel compounds that contribute to the overall bioactive profile of the plant.

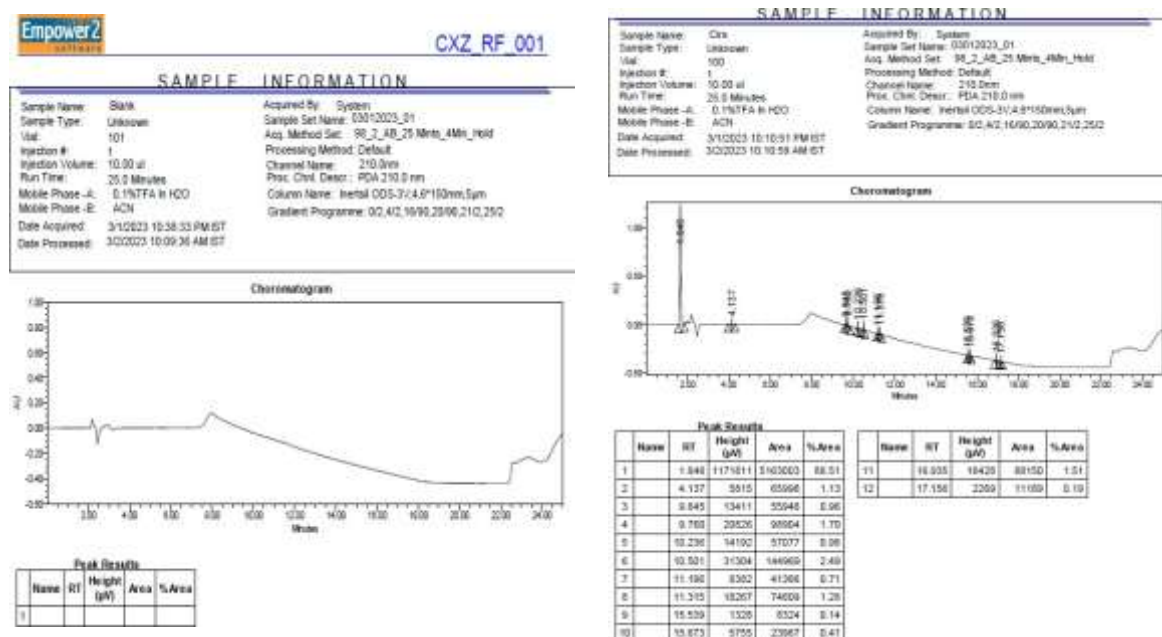


Figure 5: HPLC analysis of ethanolic extract of *C. quadrangularis* A: control and B: ethanolic extract of *C. quadrangularis*.

CONCLUSION

In conclusion, the ethanolic extraction of *Cissus quadrangularis* using the Soxhlet technique yielded a crude extract with a wet weight of 10 g and a corresponding dry weight of 4.5 grams. The percentage yields calculated indicated that 2% of the wet ethanolic extract and 0.9% of the dry ethanolic extract were obtained from the crude drug, reflecting the efficiency of the extraction process. Phytochemical analysis confirmed the presence of various bioactive compounds, including alkaloids, flavonoids, amino acids, carbohydrates, saponins, tannins, phenolic compounds, and more, aligning with prior studies. Thin Layer Chromatography (TLC) profiling further substantiated the presence of specific phytochemicals, such as flavonoids, alkaloids, phenols, and tannins. Liquid Chromatography-Mass Spectrometry (LC-MS) analysis revealed a diverse array of compounds, each identified by its name and molecular weight. Noteworthy compounds, such as Quercetin-3-O- α -L-rhamnopyranoside (448.4), vanilline (152.36), beta-amyrone (424.43), and various fatty acids, demonstrate the chemical complexity of the extract with distinct pharmacological implications. Gas Chromatography-Mass Spectrometry (GC-MS) analysis provided further insights, identifying compounds like B-Sitosterol, 4-Ethylbenzoic acid, and Vitamin E, each contributing to the potential health benefits of the extract. High-Performance Liquid Chromatography (HPLC) analysis provided a detailed chromatographic profile, revealing distinct peaks and suggesting the presence of novel compounds. This comprehensive characterization underscores the rich phytochemical

diversity and potential bioactivity of the *C. quadrangularis* ethanolic extract. These findings provide a valuable foundation for future research and the exploration of plant therapeutic applications in various health-related contexts. The identified compounds, with their diverse biological applications, highlight the potential of the extract for cardiovascular health, antimicrobial activities, antioxidant processes, and other pharmacological effects. Further studies are essential to elucidate the specific health-promoting properties, potential synergies, and overall therapeutic potential of *Cissus quadrangularis*.

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REFERENCES

1. Sundaran J, Begum R, Vasanthi M, Kamalpathy M, Bupesh G, Sahoo U. A short review on pharmacological activity of *Cissus quadrangularis*. *Bioinformation*. 2020;16(8):579-585.
2. Shirwaikar A, Khan S, Malini S. Antiosteoporotic effect of ethanol extract of *Cissus quadrangularis* Linn. on ovariectomized rat. *J Ethnopharmacol*. 2003;89(2-3):245-250.
3. Purohit S, Bohra MK, Jain R. Identification of Bioactive Pentacyclic Triterpenoids and Fatty Acid Derivatives from *Cissus quadrangularis* and *C. rotundifolia* Through Untargeted Metabolite Profiling. *Appl Biochem Biotechnol*. 2023;195(4):2235-2251.
4. Dhanasekaran S. Phytochemical characteristics of aerial part of *Cissus quadrangularis* (L) and its *in-vitro* inhibitory activity against leukemic cells and antioxidant properties. *Saudi J Biol Sci*. 2020;27(5):1302-1309.
5. Maunder A, Bessell E, Lauche R, Adams J, Sainsbury A, Fuller NR. Effectiveness of herbal medicines for weight loss: A systematic review and meta-analysis of randomized controlled trials. *Diabetes Obes Metab*. 2020;22(6):891-903.
6. Zhao X, Wang Y, Zhang Z, Velu P, Liu R. In-vitro Antioxidant, In-vitro and In-silico Ovarian Anticancer Activity (Ovarian Cancer Cells-PA1) and Phytochemical Analysis

- of *Cissus quadrangularis* L. Ethanolic Extract. *Comb Chem High Throughput Screen.* Published online October 10, 2023.
7. Lekshmi RK, Divya BT, Mini S. *Cissus quadrangularis* extract attenuates hyperglycaemia-mediated oxidative stress in streptozotocin-induced diabetic rats. *Redox Rep.* 2014;19(5):214-220.
 8. Syed AA, Reza MI, Singh P, Husain A, Dadge S, Gayen JR. Polyphenolic-rich *Cissus quadrangularis* extract ameliorates insulin resistance by activating AdipoR1 in peri-/post-menopausal rats. *Exp Gerontol.* 2022; 159:111681.
 9. Swamy AH, Kulkarni RV, Koti BC, Gadad PC, Thippeswamy AH, Gore A. Hepatoprotective Effect of *Cissus quadrangularis* Stem Extract Against Rifampicin-induced Hepatotoxicity in Rats. *Indian J Pharm Sci.* 2012;74(2):183-187. doi:10.4103/0250-474X.103859.
 10. Shilpa VP, Muddukrishnaiah K, Thavamani BS, Dhanapal V, Arathi KN, Vinod KR, Sreeranjini SR. In vitro immunomodulatory, antifungal, and antibacterial screening of *Phyllanthus niruri* against to human pathogenic microorganisms. *Environmental Disease.* 2018;3(3):63.
 11. Sureshkumar V. Phytochemical Screening and Thin Layer Chromatography Profiling of Various Extracts of *Achyranthes aspera* and *Cissus quadrangularis*. *J Phytopharmacol* 2021; 10(4):225-229.
 12. Göger G, Köse YB, Demirci F, Göger F. Phytochemical Characterization of Phenolic Compounds by LC-MS/MS and Biological Activities of *Ajuga reptans* L., *Ajuga salicifolia* (L.) Schreber and *Ajuga genevensis* L. from Turkey. *Turk J Pharm Sci.* 2021;18(5):616-627.
 13. Castellaneta A, Losito I, Leoni B, et al. A targeted GC-MS/MS approach for the determination of eight sterols in microgreen and mature plant material. *J Steroid Biochem Mol Biol.* 2023; 232:106361.
 14. Shah UM, Patel SM, Patel PH, Hingorani L, Jadhav RB. Development and Validation of a Simple Isocratic HPLC Method for Simultaneous Estimation of Phytosterols in *Cissus quadrangularis*. *Indian J Pharm Sci.* 2010;72(6):753-758.
 15. Li W, Zhang X, Chen R, et al. HPLC fingerprint analysis of *Phyllanthus emblica* ethanol extract and their antioxidant and anti-inflammatory properties. *J Ethnopharmacol.* 2020; 254:112740

16. Huang M, Zhang L, Mesaros C, et al. Metabolism of a representative oxygenated polycyclic aromatic hydrocarbon (PAH) phenanthrene-9,10-quinone in human hepatoma (HepG2) cells. *Chem Res Toxicol*. 2014;27(5):852-863.
17. Chung CY, Tseng CC, Li SM, Tsai SE, Lin HY, Wong FF. Structural Identification between Phthalazine-1,4-Diones and *N*-Aminophthalimides via Vilsmeier Reaction: Nitrogen Cyclization and Tautomerization Study. *Molecules*. 2021;26(10):2907.
18. Dawood KM, Abbas AA. Inhibitory activities of indolizine derivatives: a patent review. *Expert Opin Ther Pat*. 2020;30(9):695-714.
19. Rizvi S, Raza ST, Ahmed F, Ahmad A, Abbas S, Mahdi F. The role of vitamin e in human health and some diseases. *Sultan Qaboos Univ Med J*. 2014;14(2):e157-e165.
20. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacogn Rev*. 2010;4(8):118-126.