



Received:

2025/12/20

Accepted:

2026/01/26

Published:

2026/01/27



RESEARCH PAPER

OPEN ACCESS

Qualitative and Quantitative Analysis of Phytometabolites Present in Stem and Leaf of *Cissus quadrangularis* (L.): A Therapeutic Herb

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*Correspondence for materials should be addressed to SP (email: smita.purohit@iisuniv.ac.in)**Abstract**

The present work was conducted to evaluate the phytochemical constituents and to screen the methanolic and ethyl acetate extracts of the stem and leaves of *Cissus quadrangularis* (Linn) for the presence of bioactive compounds. Methanol and ethyl acetate were used as solvents to extract the biocompound according to their polarity. The solvents were used to extract into it by the extraction method at optimized conditions. Crude protein, crude fat, and carbohydrate content were determined as primary metabolites. The chemical composition in methanolic and ethyl acetate extracts of *Cissus quadrangularis* L. revealed the presence of alkaloids, saponins, phytosterols, phenolic compounds, flavonoids, terpenoids, protein, amino acids, fixed oils, fats, tannins and cardiac glycosides. This work could be further useful to the structural evaluation and further quantification of bioactive compounds for further studies in medical, pharmaceutical fields and formation of many drugs in therapeutic fields.

Keywords: *Cissus quadrangularis*; Methanolic extract; Phytoconstituent; Phytosterols; Saponin**Introduction**

The plant kingdom has been the best source of therapies for curing a variety of ailments. The drug discovery industry is equally dependent on natural sources for new medicines, mainly because existing remedies exhibit many side effects that result in the recall of drugs, bringing vast losses to the pharmaceutical industries (Newman and Cragg, 2007). Recently, there has been a tremendous growth in the use of herbal health products in developing as well as developed countries resulting in an exponential progress of plant-based products worldwide (EL-Kamali and EL-Amir, 2010). One such set of plants that is used in all the continents and is implicated to treat different treatments is plants belonging to the genus *Cissus* (Tasadduq et al., 2017). Genus *Cissus* consist many therapeutic plants of vitaceae family having many medicinal and curative properties due to the presence of many phytochemicals in their plant parts. The genus *Cissus* shows its ethnomedicinal and pharmacological value for the treatment of many diseases. Over the past few decades, there has been a surge in global interest in medicinal plants as alternatives to synthetic medicines. According to the World Health Organisation (WHO), more than 80% of the world's population still relies on traditional medicines obtained from plants to meet their basic medical needs (Ng et al., 2025). Plants medicinal by herbs are more focused because of their major practice and less harmful effects in medicinal handling. In the new time, the focus on medicinal plants, research has supremacy worldwide and enormous potential of medicinal plants used in various traditional medication schemes (Khatri, 2025). A collection of plants with unique qualities or attributes that make them suitable as therapeutic agents and medical products that are employed for health-related purposes could also be referred to as medicinal plants (Akter and Azad, 2024). More than 15000 floras have been stated during the last 5-year period for traditional and herbal medication.



Scientists are forwarding these renewable foundations of drugs to produce a new generation of treatments and therapies. Crude medicines, which are frequently utilised in their natural state to treat a variety of ailments, can be derived from these plants. The potential of medicinal plants in medication development and discovery has been acknowledged more and more by contemporary scientific research, especially when it comes to disorders for which there are few available treatments. Many plant extracts have been reported to have pharmacological uses in many therapies (Alam et al., 2021). Manufacturing and pricing of herbal pharmaceuticals can make them more favourable to be exploited in research and progress of new treatment approaches, permitting patients to connect to new medications which are faster and safer for relexification (Mishra et al., 2010). It is a requirement of time to preserve and file this traditional and advanced information of plants proven through various studies that it will serve as a lighthouse for future researchers (Bunalema et al., 2014). Outmoded formulas for the treatment of physical and mental conditions exist in all major ancient civilisations of the earth (Rao et al., 2007). One such set of plants that is used in all the continents and is implicated to treat different treatments is plants belonging to the genus *Cissus* and one of its herb *Cissus quadrangularis* (Linn) has been used commonly used as therapeutic purpose such as bone setter, for maintaining female hormonal balance, weight loss, antidiabetic, antiulcer and so on treatments due to the presence of phytochemical compounds such as primary and secondary metabolites in plant (Khatri, 2025). *Cissus quadrangularis* is well known for its nutritional value and bone healing properties. It is a biological matrix of excellence. In 1963, it was first used for bone fracture healing due to its significant effects in the bone remodelling. This review article explores the potential and efficacy of *Cissus quadrangularis* towards human health. This study reveals the evaluation and presence of phytochemicals in methanol and ethyl acetate solvents; this analysis helps in medicinal and therapeutic fields.

Taxonomical classification

Division: Magnoliophyta

Class: Magnoliopsida

Order: Vitales

Family: Vitaceae

Genus: *Cissus*

Species: *Cissus quadrangularis*



Fig. 1. *Cissus quadrangularis* plant



Fig. 2. Stem and leaf of *Cissus quadrangularis*

Material and Methods

Sample collection and authentication

Stems and leaves of *Cissus quadrangularis* were collected from Muhana, Jaipur, Rajasthan, where it grows as a medicinal plant. The plant was authenticated at the Department of Botany, University of Rajasthan, Jaipur with the authenticated no. 21800. The leaves and stems were collected, separated, washed with Milli Q water and dried under shade for about 15-20 days and grind as a fine powder with the help of an electric blender. Store the powder at a low temperature for further use.

Extraction Procedure

To investigate the bioactive components of *Cissus quadrangularis*, crude extraction was done using methanol and ethyl acetate separately for the stem and leaf parts. 20 grams of powder extracted in 200 ml in each solvent for each plant part. Crude extraction is done by using soxhlet apparatus under optimal conditions for 12 to 15 hours. After extraction, collect the extract and store it in a cool and dry place for further analysis.

Qualitative phytochemical analysis

Test for primary metabolite

Test for protein

Ninhydrin test to the test solution added 1 ml of 0.2 % ninhydrin solution, violet color indicate the presence of amino acids in the sample (Talreja et al., 2016).

Test for carbohydrate

Filtrate (1 ml) was boiled on a water bath with 1ml each of Fehling solution A & Fehling solution B; a coloured product indicates the presence of sugar (Talreja et al., 2016) chemical analysis.

Test or secondary metabolite**Test for Saponin**

An amount of 5 mL of each extract was added to 5 mL of distilled water and shaken vigorously. It was warmed in a water bath, and the formation of a stable, persistent froth indicates the presence of saponins (Rohit, 2015).

Test for tannin

This was carried out using Braymer's test. An amount of 2 mL of each extract was dissolved in 5 mL of distilled water, it was filtered, and to the filtrate, 2-3 drops of 5 % FeCl_3 were added. The presence of green precipitate indicates the presence of tannins (Mir et al., 2016; Yadav et al., 2014).

Test for flavonoids

A few Magnesium turnings and 5 drops of concentrated Hydrochloric acid were added dropwise to 1 ml of the test solution. A crimson red colour appeared after a few minutes confirmed the test (Talreja et al., 2016).

Test for alkanoids

An amount of 2 mL of each extract was added to a few drops of Hager's reagent, and a yellow precipitate shows positive result for the presence of alkaloids (Yadav et al., 2014).

Test for Phenols

An amount of 2 mL of each extract was added to 2 mL of 5 % aqueous ferric chloride. Appearance of blue colour indicates the presence of phenols (Prabhavathi et al., 2016).

Test for steroids

The Salkowski test was used to determine the presence of steroids. An amount of 2 mL of each extract was added to 2 mL of chloroform and 2 mL of conc. H_2SO_4 . Production of a reddish-brown ring at the junction shows the presence of steroids (Yadav et al., 2014).

Quantitative phytochemical analysis**Test for primary metabolite****Protein**

BSA is used as a standard reagent for preparing the standard curve against which the unknown concentration of proteins is estimated. 4.5 ml of reagent 1 (48 ml of 2% sodium carbonate in 0.1N sodium hydroxide + 1ml of 1% sodium potassium tartrate + 1ml of 0.5% copper sulphate) was added to the sample extracts and incubated for 15 min. After this, 0.5 ml of freshly prepared reagent 2 (1 part Folin-Ciocalteu: 1 part water) was mixed with each sample and left for 30 min of dark incubation. After that, the absorbance was measured at 660 nm, and the amount of protein was expressed as mg BSAE/ g of fresh weight (Lowry et al., 1951; Sapan et al., 1999; Shakir et al., 1994; Peterson et al., 1983).

Carbohydrate

About 50 mg of each powdered sample was taken, ground in a mortar and pestle with 20 ml of 80% ethanol, and left overnight to determine the total soluble sugars (Ext. 1). The supernatants were gathered and concentrated in a water bath after the centrifugation of the homogenate for 12 minutes at 1500 rpm. Distilled water was used to make up the remaining 50 millilitres of the resulting concentration. The remaining pellet from the centrifugation procedure was collected for the starch (ext. 2), suspended in 6.5 mL of distilled water and 5 ml of 52% perchloric acid, agitated continuously for 5 minutes and centrifuged for 20 minutes at 2500 rpm. After three iterations of the procedure, the supernatant was gathered. Using distilled water, the volume of the collected supernatants was increased to 100 ml. Each test sample (ext. 1 & 2) was estimated using the phenol-sulfuric acid reagent for quantification. Using a glucose stock solution (1 mg/ml), the standard regression curve was created. One millilitre of the test sample was placed in a test tube and stored in an ice chest. After adding 1 millilitre of 5% aqueous phenol, the mixture was gently shaken. After quickly adding 5 ml of concentrated H_2SO_4 , the mixture was allowed to rest for 20 minutes at 25–300 C in a water bath. The sample's OD was measured at 490 nm. Various aqueous glucose concentrations were used for the standard curve. (Khandelwal et al., 2025).

Test for secondary metabolite**Test for Saponin**

This was conducted using a method in which 50 mL of 20 % aqueous methanol was added to 10 g of the sample in a conical flask. This was placed on hot water bath (about 55 °C) for 4 h with continuous stirring. It was then filtered, and the residue re-extracted using 100 mL of 20 % aqueous methanol. The extracts were combined and reduced to 40 mL on a water bath at 90 °C. The concentrate was poured into a separating funnel, and 10 mL of diethyl ether was added to it and shaken vigorously. The ether layer was discarded while the aqueous layer was recovered, and the purification process was repeated. 30 mL of n-butanol was added, and the n-butanol extracts were washed twice with 10 mL of 5 % aqueous NaCl. The remaining solution was heated to evaporation on a water bath, the samples were then dried in the oven to a constant weight, and the saponin content was calculated as a percentage (Biradar and Rachetti, 2013; Obadoni and Ochuko, 2002).

Test for tannin

The tannin content was determined according to the modified vanillin-HCl method described by (Lawal et al., 2015) and (Omoruyi et al., 2012). The vanillin-HCl reagent was prepared just before use by mixing equal volumes of 8 % HCl and 1 % vanillin in methanol. An amount of 10 mL of 1 % concentrated HCl in methanol was added to about 0.2 g of ground sample in a conical flask. The flask was stoppered and continuously shaken for 20 minutes; the content was further centrifuged at 2500 rpm for 5 min. About 1.0 mL of the supernatant was transferred into a test tube containing 5 mL of vanillin-HCl reagent. It was incubated at 30 °C for 20 minutes, and absorbance was read at 450 nm. The tannin content was expressed as mg of catechin equivalents per 100 mg sample (Lawal et al., 2015; Omoruyi et al., 2012).

Test for flavonoids

This was measured using the aluminum chloride colorimetric assay. Amount of 1 mL of extract and 4 mL of distilled water were added to a volumetric flask; 0.3 mL of 5 % NaNO₂ was added and after 5 minute, 0.3 mL of 10 % AlCl₃ was also added. 2 mL of 1 M NaOH was added after 5 minutes and the content of the flask was made up to the 10 mL mark with distilled water. Standard solutions of quercetin at 20, 40, 60, 80 and 100 µg/mL were prepared in the same manner. The absorbance for test and standard solutions was read against the blank at 510 nm on a spectrophotometer. Total flavonoid content was expressed as mg of quercetin equivalents per gram of extract (Mythili et al., 2014).

Test for alkaloids

This was conducted using the method of (Harborne, 1984) as described by (Biradar and Rachetti, 2013) with slight modifications. To 5 g of sample in a 250 mL beaker, 200 ml of 10 % acetic acid in methanol was added; it was covered and allowed to stand. After 4 h, it was filtered, and the filtrate was concentrated on a water bath to about one-quarter of its original volume. Conc. NH₄OH was added to the extract dropwise until precipitation stopped. The solution was allowed to settle, the precipitate was collected and washed with dilute NH₄OH and then filtered. The residue was dried and weighed as the alkaloid.

Test for Phenols

The total phenols present in both extracts were calculated according to the methods of Slinkard and Singleton 1977. A quantity of 100 mg of the sample in 0.5 ml of water is mixed with Folin–Ciocalteu's reagent (diluted 1:10v/v) and 2 ml of Na₂CO₃ (7.5%, v/v) solution. The whole setup was incubated for 90 min at 30°C, and the resultant colour was measured at 765 nm with results expressed as gallic acid equivalents (Slinkard and Singleton, 1977).

Test for phenolics

The total phenolic content was determined using the method of (Singleton et al., 1999) as described by (Stankovic, 2011). An amount of 0.5 mL of the methanolic solution of the extract was mixed with 2.5 mL of 10 % Folin–Ciocalteu's reagent dissolved in water and 2.5 mL of 7.5 % NaHCO₃. The blank was made up of 0.5 mL methanol, 2.5 mL of 10 % Folin–Ciocalteu's reagent dissolved in water and 2.5 mL of 7.5 % NaHCO₃. They were incubated at 45°C for 45 minutes, and absorbance was read on a spectrophotometer at 765 nm. A similar procedure was carried out for standard solutions of gallic acid, and a calibration curve was constructed. The concentration of phenolics was read from the calibration curve, and total phenolics present in the extract were expressed in terms of mg of gallic acid equivalent per gram of extract (mg GAE/g) (Stankovic, 2011; Singleton et al., 1999).

Results

The yield shows the presence of primary and secondary metabolites in the exact yield. The table below shows the yield of extract of both plant parts in both solvents (methanol and ethyl acetate). Extraction was done in a Soxhlet apparatus for some hours.



Fig. 3. Soxhlet apparatus

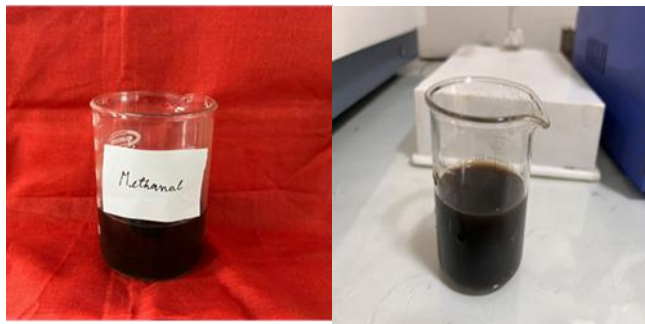


Fig. 4. Extracts in methanol and ethyl acetate

Table 1. Yield obtained from stem and leaf parts in both solvents

Leaves		Stem	
Methanol	Ethyl acetate	Methanol	Ethyl acetate
1.54	1.03	1.57	1.02

Yield in mg/ml

Qualitative estimation results of phytochemicals

Quantitative analysis of primary as well secondary phytochemical present in both methanol and ethyl acetate solvent shows the presence of protein, carbohydrate, alkaloid, flavonoid, saponin, tannin and phenol except steroid. The phytochemical analysis were estimated by ninhydrin test, felhing test, hager's test, hydrochloric test, froth test, braymer's test, libermann's test, folin-ciocalteu's test respectively. The analysis was carried out by standard protocols in both solution.

Table 2. Qualitative results of phytochemical in present both solvent

S.No.	Phytochemical	Applied test	Stem		leaves	
			Methanol	Ethyl acetate	Methanol	Ethyl acetate
	Primary metabolite					
1.	Protein	Ninhydrin test	+	+	+++	+++
2.	Carbohydrate	Felhing test	+	+	+++	++
	Secondary metabolite					
3.	Alkaloid	Hager's reagent test	+	+	+	+
4.	Flavonoid	Hydrochloric test	+	+	+++	++
5.	Saponin	Froth test	+	+	+	+
6.	Tannin	Braymer's test	+	+	+	+
7.	Steroids	Libermann's test	-	-	-	-
8.	Phenols	Folin-Ciocalteu's	+	+	++	+

+ indicates presence - indicates absence

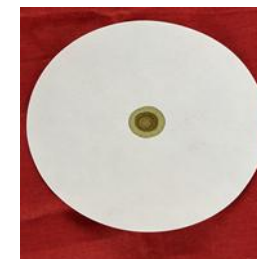
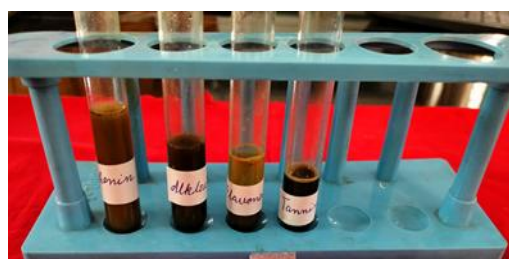
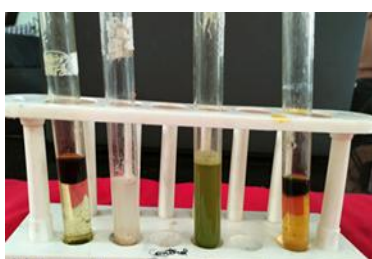
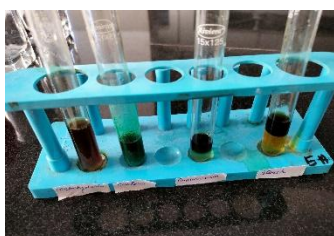


Fig. 5. Pictures of the primary quantitative test of primary and secondary metabolites

Quantitative estimation results of phytocompounds

The table below reveals the quantity of primary and secondary metabolites such as protein, carbohydrate, alkaloid, flavonoid, saponin, tannin, and phenol present in both solvents (methanol and ethyl acetate). The quantity yielded in the extractions given below is in the table.

Table 3. Quantitative results of phytometabolite

S. No.	Phytocompound	Stem		Leaves	
		Methanol	Ethyl acetate	Methanol	Ethyl acetate
1.	Protein	0.95±0.25	0.75±0.03	0.99±0.31	0.45±0.21
2.	Carbohydrate	0.99±0.11	0.89±0.01	0.84±0.54	0.51±0.12
	Secondary metabolite				
3.	Alkaloid	0.98±0.34	0.95±0.02	0.84±0.25	0.75±0.11
4.	Flavonoid	0.91±0.57	0.92±0.07	0.65±0.41	0.88±0.14
5.	Saponin	0.94±0.74	0.96±0.03	0.57±0.17	0.25±0.16
6.	Tannin	0.85±0.24	0.87±0.04	0.77±0.19	0.54±0.14
7.	Phenol	0.84±0.21	0.88±0.02	0.89±0.57	0.49±0.54

Mean ± SD [n=3] Quantity estimated in mg/ml

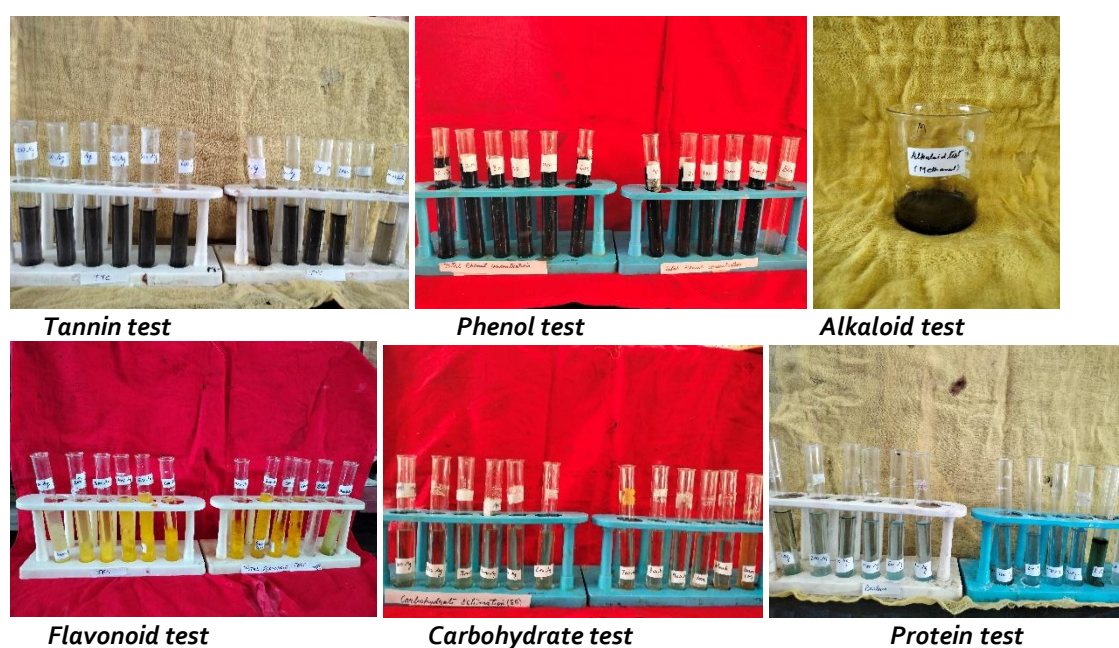


Fig. 6. Pictures of quantitative test in CQ sem exacts

Discussion

Throughout the world, plants are considered an important source of raw material for the synthesis of ancient as well as modern drugs. Research on medically important species of plants plays a vital role in the confirmation of plants to treat disorders, and it is probably a better solution to develop low-cost and effective medicines from available raw material by plant production. In Ayurvedic, Chinese and Unani ancient remedial systems of medicines, herb, shrub and trees are broadly used in crude form or medicines. The preliminary phytochemical screening was carried out, and result showed the presence of protein, carbohydrate, alkaloids, steroids, saponins, flavonoids, phenolic compounds, and triterpenoids in both methanolic and ethyl acetate extracts of leaves and the stem part of the plant. The phytochemical screening for leaves and stems of *Cissus quadrangularis* has played an important role in the pharmacological investigation to prove that this plant and its plant parts are useful in many therapies or medications. Qualitative results reveal the presence of carbohydrate, protein, saponin, alkaloid, tannin, phenol and flavonoid in methanol as well as in ethyl acetate solvents in both stem and leaf parts. Quantitative results show that carbohydrate shows more yield compared to protein in both solvents. Alkaloid and saponin are present in higher quantities in methanol compared to ethyl acetate, while flavonoid, phenol and tannin show lesser quantity in methanol and ethyl acetate solvent. Phytocompound as carbohydrates, proteins, alkaloid and saponins are present in high quantities in the stem compared to the leaves part as confirmed by results, while phenol and tannin are present in both solvents in both parts of the plant (Yadav et al., 2014).

These observations of experiments clearly indicate that the higher concentration of phytocompounds was present in the extract of plant *C. quadrangularis*, which is responsible for many pharmacological activities. Extractive values are also useful to evaluate the chemical composition present in the crude extract, which also helps in the estimation of specific constituents soluble in particular solvents.

Conclusion

The current study showed that *Cissus quadrangularis* L. has a wide display of phytochemicals, which are known to possess numerous medicinal properties. This plant was found to be therapeutically important. The phytochemical analysis is very important to evaluate the possible medicinal utilities of a plant and also to determine the active principles responsible for the known biological activities exhibited by the plants. Further, it provides the base for targeted isolation of compounds and for performing more precise investigations. Extraction of a phytochemical from the plant material is mainly dependent on the type of solvent used. Similarly, the test applied for phytochemical analysis determines the presence or absence of a phytochemical in the sample. Hence, this phytochemical screening helps to analyze its high potency due to the presence of primary and secondary metabolites in the plant parts of this plant. Both the qualitative and quantitative analysis of phytochemicals of *Cissus quadrangularis* was done in order to find out the active bioconstituents against various diseases and their therapies, which play a resourceful need for mankind in their medication. Further studies are needed to evaluate and understand a clearer picture of this plant's utilization against diseases and their remedies.

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Author Contributions

RK and SP conceived the concept, wrote and approved the manuscript.

Acknowledgements

The authors would like to deliver grateful thanks to Dr. Rohit Jain, Manipal University, Jaipur for his helpful guidance and support to perform this research work.

Funding

Not applicable.

Availability of data and materials

Not applicable.

Competing interest

The authors declare no competing interests.

Ethics approval

Not applicable.



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Citation: Khatri R and Purohit S (2026) Qualitative and Quantitative Analysis of Phytometabolites Present in Stem and Leaf of *Cissus quadrangularis* (L): A Therapeutic Herb. *Environmental Science Archives* 5(1): 75-82.