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Comprehensive Phytochemical Investigation And Lc-Ms Based Metaboloite Profiling Of Cissus Quadrangularis L. Stem

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ABSTRACT

Cissus quadrangularis Linn., a perennial medicinal plant in the family Vitaceae. This integrative review aims to unveil and synthesize current evidence on the phytochemical constituents of this plant part using qualitative phytochemical study and Liquid Chromatography-Mass Spectrometry analysis. Present study qualitatively analysed the Alkaloids, Carbohydrates Glycosides, Proteins, Flavonoid, Terpenoids, Phenols, Tannins, Saponins in petroleum ether (PECQ), Acetone (ACCQ), ethanolic (ETCQ) and water (WCQ) extracts of Cissus quadrangularis. LC–MS analysis revealed that the acetone extract of Cissus quadrangularis stem (ACCQ) is rich in phytosterols and diverse bioactive phytochemicals. The identified compounds included 4-vinylphenol, tyramine, 4-ethylphenol, glyceraldehyde-3-phosphate, shikimic acid, sinapyl alcohol, feruloyl aldehyde, coniferin, syringin, chrysoeriol, quercetin-3-O-glucoside, rutin, hesperidin, diosmin, and rosamultin. These findings demonstrate the rich phytochemical composition of the acetone extract and provide scientific evidence supporting the therapeutic potential of Cissus quadrangularis. The identified phytoconstituents may contribute to the plant's biological activities and warrant further investigation for pharmaceutical applications.....

Keywords: Cissus quadrangularis, Qualitative phytochemical, LC-MS study, Acetone extract..

INTRODUCTION

Cissus quadrangularis (L). is a perennial climber of family Vitaceae, that is widespread in tropical regions of India. It has been used to relieve dyspepsia, anorexia, flatulence, colic, seizures, tumors, epistaxis, asthma, abnormal menstrual disorders, inflammation, antibacterial infections and obesity (Nash et al., 2019, Sawangjit et al., 2017, Lee et al., 2018, Jainu and Devi, 2005). Toxicological reports demonstrate that the extract of Cissus quadrangularis (L). does not possess any adverse toxic effect (Zenebe et al., 2017, Jadhav et al., 2016, Tamburaci et al., 2018, Oben et al., 2008). Photochemical analysis showed to comprise of metal ions, resveratrol, piceatannol, pallidol, parthenocissus, 31 methyl triacontanoic acid, taraxeryl acetate, taraxerol, phenol, tannin, carotene, and vitamin. It also possesses novel flavonoids and indanes, phytosterols and keto-steroids which are useful and effective antioxidants (Zenebe et al., 2017, Sharp et al., 2007). For the analysis of whole plant extracts, the use of a UV-Visible spectrophotometer is limited by inherent difficulties in terms of assigning absorption peaks to particular constituents present in the system. UV-Visible findings must be supplemented with other analytical techniques such as LC/MS or GC/MS (Coppin et al., 2013; Kumar et al., 2020; Suleria et al., 2020). LC/MS is superior in its qualitative and quantitative capabilities. LC/MS has high detection selectivity, high sensitivity and high qualitative productivity. In LC/MS, Liquid chromatography is coupled with mass spectrometry (Pratima, 2018).

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MATERIALS AND METHODS

Sample collection and solvent extraction: The medicinal plant Cissus quadrangularis was selected in the present study. The plant material stem and leaf were collected from the home garden, Poovar, Trivandrum, Kerala. The plant material was dried under shade condition for one month and cut into small pieces, pulverized in a grinder and stored in a sterile container for further use (Helma Rachel et al., 2022)..

Preparation of solvent extract: The required quantity of the powder was weighted, transferred to the flask, treated with ethanol until the powder was fully immersed incubated overnight and filled through Whatman No.1 filter paper along with sodium sulphate to remove the sediments and traces of water in the filter paper. The filtrate is then concentrated to 1ml by budding nitrogen gas in the solution.

Qualitative phytochemical analysis

The phytochemicals like alkaloids (Mayer's test), carbohydrates (Fehling's test), glycosides (Killer-Killiani test), proteins (Biuret test), flavonoids (Alkaline reagent), terpenoids (Salkowski's test), phenols (Ferric chloride test), tannins (Lead acetate test), saponins (Froth test) and anthraquinones were analyzed. The phytochemical analysis was carried out using the extract of ethanol and aqueous. (Horborne, 1998; Trease and Evans, 1983; Adebayo and Ishola, 2009).

LC-MS analysis

The phytochemical constituents of *Cissus quadrangularis* extract were analysed using LC-MS. The analysis was performed using a Mariner Bio spectrometry system equipped with a binary HPLC pump and a Q-TOF mass spectrometer with an electrospray ionization (ESI) source. Separation was carried out on a Phenomenex C8 column (150 × 2 mm, 5 µm) using acetone containing 0.3% formic acid as the mobile phase at a flow rate of 0.1 mL/min under isocratic conditions. Mass spectra were recorded in positive ion mode over an m/z range of 100–1200 with a source temperature of 140°C. Compound identification was based on matching the observed m/z values with reported secondary metabolites of *Cissus quadrangularis*.

RESULTS AND DISCUSSION

Phytochemical analysis

The phytochemical investigation of *Cissus quadrangularis* stem was performed using different solvent extracts acetone, ethanol, petroleum ether, and aqueous. The qualitative analysis focused on detecting and assessing the presence of phytochemicals such as alkaloids, carbohydrates, glycosides, proteins, flavonoids, terpenoids, phenols, tannins, saponins, and anthraquinones.

Table 1. Phytochemical analysis of *Cissus quadrangularis* L. stem using different solvents

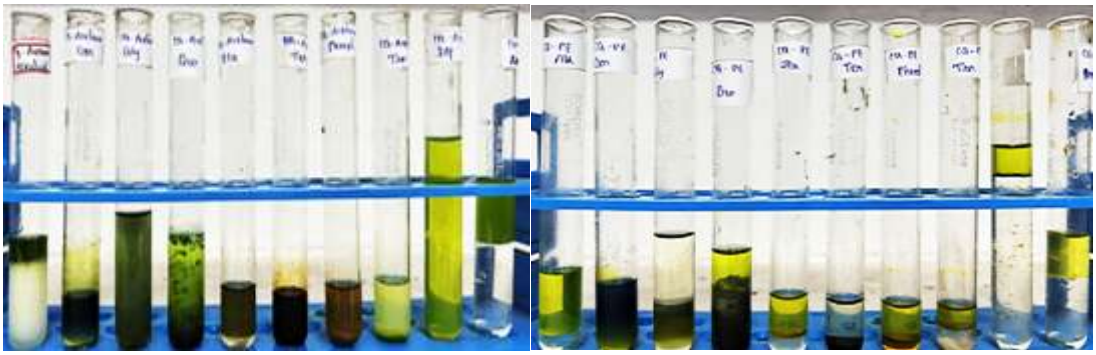
Phytochemicals	Aqueous	Ethanol	Acetone	Petroleum ether
Alkaloids	+	+	+	_
Carbohydrates	+	+	+	+
Glycosides	+	+	+'	+
Proteins	+	+	+	_
Flavonoids	+	+	+	+
Terpenoids	_	_	+	_
Phenols	+	_	+	_
Tannins	+	+	+	+
Saponins	+	_	+	_
Anthraquinones	_	_	_	_

(+) present; (-) absent

Figure 1. Phytochemical analysis of *Cissus quadrangularis* L. stem using different solvent extracts

Aqueous **Ethanol**

Acetone **Petroleum ether**



Preliminary phytochemical screening of *Cissus quadrangularis* stem extracts revealed the presence of a wide range of bioactive secondary metabolites (Table, Figure 1). Glycosides, proteins, flavonoids, and tannins were detected in all solvent extracts, indicating their widespread distribution in the plant. Alkaloids and carbohydrates were identified only in the ethanol and acetone extracts, while they were absent in the petroleum ether and aqueous extracts. Terpenoids and phenolic compounds were detected exclusively in the ethanol extract and were not observed in the aqueous, acetone, or petroleum ether extracts. Saponins were present in the aqueous and acetone extracts but absent in the ethanol and petroleum ether extracts. Anthraquinones were not detected in any of the solvent extracts. These findings demonstrate that the phytochemical composition of *Cissus quadrangularis* varies with the extraction solvent, with ethanol exhibiting the broadest spectrum of phytoconstituents.

Nature's green wealth is a rich reservoir of bioactive phytochemicls. Phytochemicals are the natural compound occur in plants, vegetables and fruits that work with nutrients and fibres to act ,against disease or more specifically to act against disease. The stem of *Cissus quadrangularis* plant has been documented to contain a rich array of major phytochemicals including Alkaloids, Saponins, Flavonoids, Terpenoids, Phenols, Glycosides and Phytosteroids (Kumar 2018).

LC-MS Analysis

The results on LC-MS analysis of *Cissus quadrangularis* stem using acetone extract with their retention time were showed in Table 2 and Figure 1. Thirteen compounds were detected with the retention time 1.13, 1.27, 1.40. 2.87, 3.07, 3.22, 3.35, 3.55, 3.75, 4.02, 4.88, 8.08, 9.82 minutes. The LC-MS data from the *Cissus quadrangularis* acetone extract yielded prominent mass-to-charge ratios (*m/z*) of 120.19, 129.23, 147.18, 192.32, 208.32, 236.28, 267.29, 282.30, 316.40, 357.41, 454.70, 496.70, 569.67, 622.76, 683.06, 829.30, and 903.07. These *m/z* values, representing ionised molecular fragments or intact molecules, suggest the presence of diverse compounds within the sample, which may include various phenolic compounds, flavonoids, and glycosides commonly found in plants.

Figure 2. LC-MS Analysis of *Cissus quadrangularis* L. stem using acetone extract.

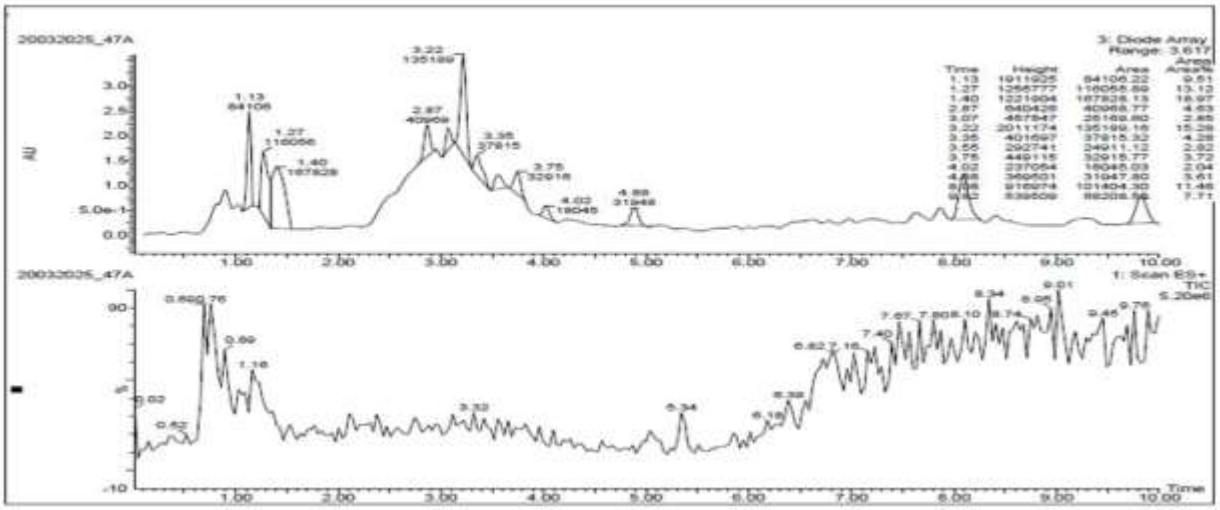


Table 2. LC-MS Analysis of *Cissus quadrangularis* L. stem using acetone extract

Peak No:	Retention Time (min)	Mass values (m/z)	Height	Potential Compound	Molecular formula
1	1.13	120.19	1911925	4-vinylphenol, Tyramine, 4-Ethylphenol	$C_8H_{11}NO$
2	1.27	147.18	1255777	Glyceraldehyde-3-phosphate	$C_3H_7O_6P$
3	1.40	192.32	1221904	Shikimic Acid	$C_7H_{10}O_5k$
4	2.87	236.28	640426	Sinapyl alcohol.	$C_{11}H_{14}O_4$
5	3.07	267.29	457547	Feruloyl aldehyde	$C_{10}H_{10}O_3$
6	3.22	282.30	2011174	Coniferin	$C_{16}H_{22}O_8$
7	3.35	316.40	401697	Syringin	$C_{17}H_{24}O_9$
8	3.55	357.41	292741	Chrysoeriol	$C_{16}H_{12}O_6$
9	3.75	454.70	449115	Quercetin-3-O-glucoside	$C_{21}H_{20}O_{12}$
10	4.02	496.70	237054	Rutin	$C_{27}H_{30}O_{16}$
11	4.88	569.67	369501	Hesperidin	$C_{28}H_{34}O_{15}$
12	8.08	622.76	916974	Diosmin	$C_{28}H_{32}O_{15}$
13	9.82	683.06	539509	Rosamultin	$C_{36}H_{58}O_{11}$

CONCLUSION

The present study demonstrated that *Cissus quadrangularis* stem is a rich source of bioactive phytochemicals, with the phytochemical profile varying according to the extraction solvent. Preliminary phytochemical screening showed that the acetone extract possessed the broadest spectrum of secondary metabolites, while LC-MS analysis further confirmed the presence of diverse phytoconstituents through distinct retention times and characteristic mass-to-charge (m/z) values. These findings provide scientific evidence supporting the medicinal potential of *Cissus quadrangularis* and highlight its importance as a promising natural source of pharmacologically active compounds for future isolation, characterization, and therapeutic investigations.

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