Analysis and Characterization of Ethanol extract of Cissus quadrangularis L. by GC-MS, FTIR, and UV-Vis Spectroscopy

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Abstract: **Plants, since antiquity, have been a significant source of new chemical components useful in drug discovery. *Cissus quadrangularis* L. is a vine belonging to Vitaceae, well known for its bone-healing properties. The entire plant has multiple health benefits and forms a vital part of traditional medicine in many countries. The phytochemical analysis of the stem of *Cissus quadrangularis* L. dissolved in ethanol indicated the presence of many secondary metabolites like alkaloids, flavonoids, phenols, tannins, terpenoids, saponins, and steroids. The GC-MS analysis followed by the primary assessment revealed the chemical nature of the benzene compounds, some organic compounds, and three phytosterols in the ethanol stem extract. Furthermore, the FTIR study gave a clear insight into the functional groups of the active components, and the UV-Vis profile showed different peaks ranging from 190-1100 nm with different absorption, respectively. The occurrence of numerous bioactive constituents contributes to the plant's medicinal properties.**

***Keywords*: FTIR, UV-Vis Spectroscopy, *Cissus quadrangularis* L., phytosterols, and functional group.**

I.INTRODUCTION

In the contemporary era of an ever-rising increase in health disorders, the demand for the most effective drugs with the least number of side effects has also grown considerably. Phytotherapy has never been out of practice, especially in India, where traditional and indigenous medicine still constitutes the primary healthcare system in rural areas. Traditional medicinal plants offer a variety of chemicals that can be utilized to treat both chronic and infectious ailments (Eswaran et al. 139). Therefore, there is a need to conquer various diseases through herbal alternatives for synthetic drugs.

Cissus quadrangularis L is a succulent, perennial vine named Vitaceae. The names commonly known are Hadjod, Veld Grape, Veldt-grape, and Winged Treebine and are widely distributed across the tropical regions of India and Sri Lanka. In ethnobotanical surveys, the entire plant, including all parts such as stems, leaves, and roots, has been proven to exhibit therapeutic effects. One of the most remarkable uses for *Cissus quadrangularis* is, of course, as a bone healer (Kavitha and Manimekalai 15). No toxic effects were revealed upon the toxicological studies of the plant extract (Joseph et al. 596). The extracts from the plant's roots and stem contain various therapeutic properties, including antioxidants that speed up the healing of bone fractures, wounds, antibacterial activity, and antiulcer activity (Rex and Ravi 155).

More than 200 million people are estimated to be affected by osteoporosis at this time. According to the International Osteoporosis Foundation, one in every three women over the age of 50 and one in every five males will suffer an osteoporotic fracture during their lifetime (Sozen et al. 46). Treatment focuses on preventing bone fractures and treating the pain associated with the condition by delaying or stopping mineral loss (Shirwaikar et al., 245). The purpose of this research is to study the phytochemical constituents and chemical properties of the ethanol stem extract of *Cissus quadrangularis* L through GC-MS, FTIR, and UV-Visible Spectroscopy.

II. MATERIALS AND METHODS

Plant Collection and identification:

The pathogen-free and healthy stems of *Cissus quadrangularis* L. were collected from the Chevalla region of Telangana. The plant has been authenticated by the Herbarium facility of the Department of Botany, Osmania University, Hyderabad. The plant material was washed thoroughly first with tap water and then distilled water to remove the surface contaminants. Later, they are shade-dried for about two weeks at room temperature to remove the moisture content and then pulverized using a laboratory blender. The powder should be stored in an air-tight container.

Plant Extract Preparation:

To 30 grams of dried powder of stem taken in a conical flask, 200 ml of ethyl alcohol is added. It is then covered with aluminum foil and kept on the magnetic stirrer. The temperature of the stirrer is maintained between 25-30°C with medium stirring. The extraction process in this condition is continued for about 72 hours. The extract thus obtained is then filtered using the Whatman Filter Paper No.1. The filtered extract is concentrated with the rotatory evaporator, after which it can be stored at optimum temperature for further use.

Phytochemical Analysis:

The screening of the bioactive compounds in the stem extract of *Cissus quadrangularis* is carried out through a series of chemical tests. The tests are performed as per the standard procedures of Trease and Evans(2002).

1. Test for Alkaloids: On a hot water bath, 3ml of the extract was mixed with 3ml of 1 percent HCL. Then 1ml of the liquid was split between two test tubes. 1 mL of Dragendroff's reagent was added to the first test tube. The presence of orange-red precipitate was regarded as a good sign.
***2. Test for Flavonoids*:** 2 ml of each extract was added with a few drops of 20 percent sodium hydroxide, and an intense yellow color was formed. A few drops of 70 percent dilute hydrochloric acid was added, and the yellow color disappeared. The formation and disappearance of yellow indicate the presence of flavonoids in the sample extract. Trease and Evans (1983 and 1996).
***3. Test for Saponins*:** First, 0.5ml of plant extract was diluted with 5ml of distilling water. The suspension was shaken vigorously for a few minutes. And then check if the foam developed and persisted for about 10 minutes it showed the presence of saponin.
***4. Test for Steroids*:** One ml of extracts of the plant was dissolved in 10ml of chloroform and added an equal volume of concentrated sulphuric acid by the sides of the test tubes. The upper layer of the solution turns red, and the sulphuric acid layer shows yellow color with green fluorescence. This change in color shows the presence of steroids.
5. Test for Terpenoids: 1 ml of concentrated sulphuric acid added to 1 ml crude extract and heated for 2 minutes. Furthermore, the grayish color would show the presence of terpenoids.
6. Test for Quinines: 1 ml of extract added to 1 ml of 1% NaOH and adequately mixed. The appearance of bluish-green or red indicates the presence of Quinines.
7. Test for Phenols: To 1gm of the extract, 5ml of distilled water was added, and this solution was then treated with 5% ferric chloride solution. The development of dark green color confirms the presence of phenols.

GC-MS Analysis:
The GC-MS analysis of the ethanolic stem extract was done at the Central Analytical Facility, University College of Technology, Osmania University using the standard GCMS model.
The GC-MS analysis is carried out using the instrument SHIMADZU, model GCMSQP2010. The carrier gas used is helium, whose flow rate is fixed at 1 ml/min. The HP5 column is employed, which has a length of 30 mm, an internal diameter of 0.32 mm, a film thickness of 0.25 mm, and a temperature range of -60 to 325 degrees Celsius (350 degrees Celsius). GC had a total run time of 35 minutes. The oven temperature was raised from 70 degrees Celsius to 280 degrees Celsius at a pace of 8 degrees Celsius per minute. The volume of the sample injected is 1µl through the injector. The MS was carried out at 70eV. The name, molecular weight, and structure of unknown compounds were identified by comparing their spectrum to the spectrum of known compounds in their library.
Fourier Transform Infrared Spectrometer Analysis:
Fourier transform infrared spectrophotometer (FTIR) was employed to identify the functional groups present in the compounds of the plant extract. The annotated spectrum gives information regarding the wavelength of absorbed light, characteristic of the chemical bond. The chemical bonds can be inferred by analyzing the infrared absorption spectra.
The ethanol extract was centrifuged for 30 minutes at 12,000 rpm for FTIR analysis. The pellet was washed three times with 25 mL de-ionized water (Muthukrishnan et al. 123). The dried powder of the ethanolic stem extract of *Cissus quadrangularis* L. is used for the FTIR analysis. A translucent sample disc is prepared using 10mg of the sample, encapsulated in 100mg of KBr pellet (Perkin Elmer). Then the ethanolic extract of the *Cissus quadrangularis* L. stem is loaded into an FTIR spectroscope.
UV-VIS Spectroscopy:
The UV-Visible spectrophotometric analysis is carried on the ethanolic stem extract of *Cissus quadrangularis* L. using the UV-Visible spectrophotometer (UV-1800 Series) with a slit width of 1.0 nm. The examination was done under UV and Visible light in the wavelength ranging between 190.00 to 1100.00 nm.
The extract is first subjected to centrifugation at 3000 rpm for approximately 10 minutes and then filtered through Whatman filter paper No.1. It is followed by diluting the sample with the same solvent (ethanol) in the ratio of 1:10.

III. RESULTS AND DISCUSSION:
The preliminary phytochemical analysis of the ethanolic stem extract of *Cissus quadrangularis* L. revealed the presence of alkaloids, flavonoids, saponins, tannins, terpenoids, steroids, and phenols. However, quinines were absent in the ethanol extract, as summarized in Table 1.

Table 1 Phytochemicals present in the ethanol extract of *Cissus quadrangularis* L. stem.

S.No.	Name of Compound	Presence/Absence in Stem
1.	Alkaloids	Present
2.	Flavanoids	Present
3.	Saponins	Present
4.	Tannins	Present
5.	Terpenoids	Present
6.	Steroids	Present
7.	Quinines	Absent
8.	Phenols	Present

The GC-MS chromatogram of the ethanolic stem extract of *Cissus quadrangularis* L. showed a total of 52 peaks depicting various bioactive components. These compounds were identified based on their retention time, area percentage, the height of the peaks, fragmentation patterns of the mass spectra. The resultant features are related to the already known compounds of the National Institute of Standards and Technology (NIST). The 14 phytocompounds and the peaks depicting them are mentioned in Table 2 and Fig.1 respectively.

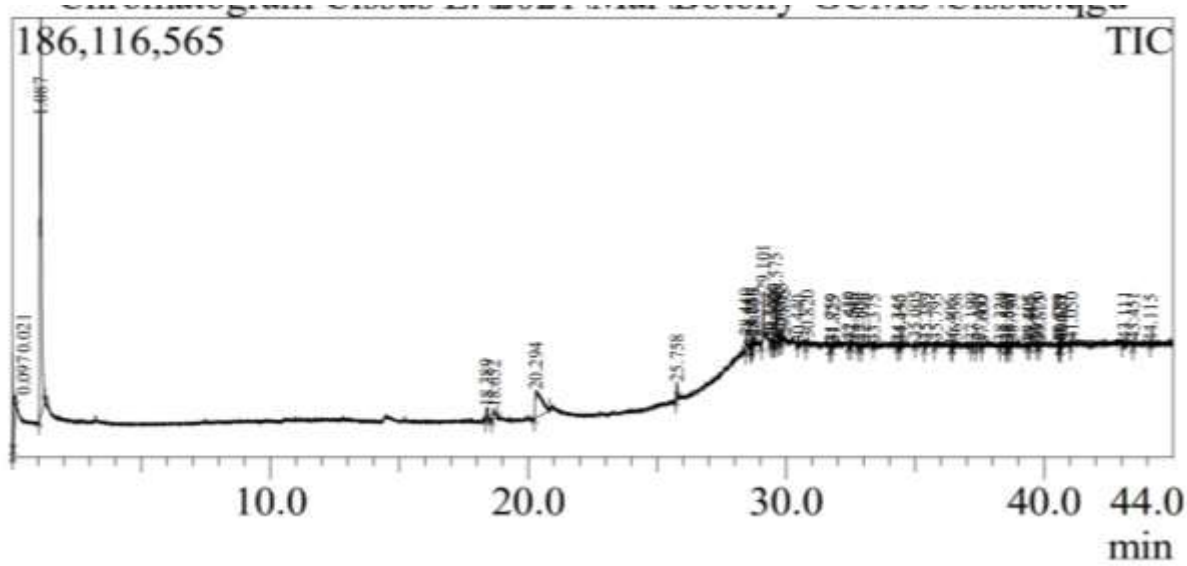

Figure 1 GC-MS chromatogram of the ethanolic stem extract of *Cissus quadrangularis* L.

Table 2 Phytocompounds from GC-MS analysis of the ethanolic stem extract of *Cissus quadrangularis* L

S.No,	RT Time	Name of Compound	Formula	MW	Peak Area(%)	Nature of Compound
1.	0.021	Dextroamphetamine	$C_9H_{13}N$	135	1.95	Amine
2.	1.087	Tetraborane(10)	B_4H_{10}	54	44.09	Inorganic metal
3.	1.087	Thiazolidinedione	$C_3H_3NO_2S$	117	44.09	Heterocyclic Compounds
4.	18.652	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	1.77	Fatty acid
5.	18.652	Phthalic Acid	$C_{20}H_{30}O_4$	334	1.77	Organic Compound
6.	18.60	D-Glucopyranoside,	$C_7H_{14}O_6$	320	1.77	Carbohydrate
7.	20.29	E-1,9-Hexadecadiene	$C_{16}H_{30}$	222	13.35	Hydrocarbon
8.	28.62	Trimethylsilyl acetylsalicylate	$C_{12}H_{16}O_4Si$	252	0.32	Benzene Compound
9.	28.73	9-Tetradecenoic acid, trimethylsilyl ester	$C_{17}H_{34}O_2Si$	298	0.31	Ester
10.	29.10	Stigmasterol	$C_{29}H_{48}O$	414	4.02	Phytosterol
11.	28.44	Campesterol	$C_{28}H_{48}O$	400	1.74	Phytosterol
12.	29.1	Beta.-Sitosterol	$C_{29}H_{50}O$	414	4.03	Phytosterol
13.	29.57	Lupeol	$C_{30}H_{50}O$	426	4.03	Carbon Compound
14.	37.60	Dimethyl hydrostatic	$C_{11}H_{10}O_6$	238	0.26	Methyl Ester
15.	38.73	1,3-Bis(trimethylsilyl)benzene	$C_{12}H_{22}Si_2$	222	0.37	Benzene Compound

The Fourier transform infrared spectrophotometer (FTIR) spectra reported the presence of functional groups in the ethanolic stem extract of *Cissus quadrangularis* L. Based on the peak value in the infrared radiation region, the FTIR spectrum indicated the functional group of the active components. The functional groups of the components were separated based on the peak ratio when extract passed through FTIR (Table 3 and Fig.2). The functional groups reported at between 400-4000 cm^{-1} were Alkanes (C-H), Alcohol (O-H), Alkenes (C=C-H), Alkynes, Aromatics, Primary Amines, Aldehydes (CHO), and Esters (RCOOR).

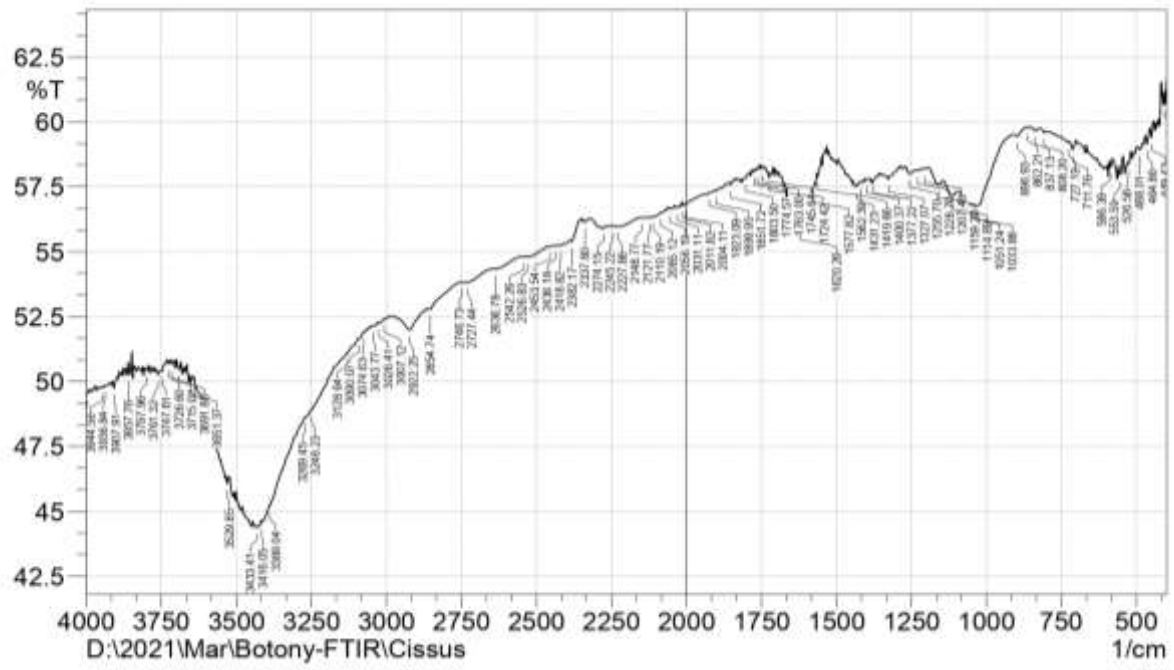

Figure 2 FTIR spectrum of the ethanolic stem extract of *Cissus quadrangularis* L

Table 3 FTIR Peak values and functional groups of the ethanolic stem extract of *Cissus quadrangularis* L

S.No.	Wavenumber (cm^{-1})	Functional Groups
1.	2854.74 - 2922.25	Alkanes (C-H)
2.	3416.05 - 3691.88	Alcohol (O-H)
3.	3026.41 - 3090.07	Alkenes (C=C-H)
4.	2110.19 - 2245.22	Alkynes
5.	1724.42 - 1923.09	Aromatics
6.	1033.88	Primary Amines
7.	1724.42	Aldehydes (CHO)
8.	1745.64	Ester (RCOOR)
9.	1562.39 - 1577.82	Carboxylic Acid

Because of the clarity of the peaks and a suitable baseline, the UV-Vis spectrum profile of the ethanolic stem extract of *Cissus quadrangularis* L. was chosen at a wavelength of 190 nm to 1100 nm. Peaks with the absorption of 1.845, 1.840, and 1.837 were found at 268.60 nm, 263.10 nm, and 264.20 nm, respectively, as depicted in Fig 3 and Table 4.

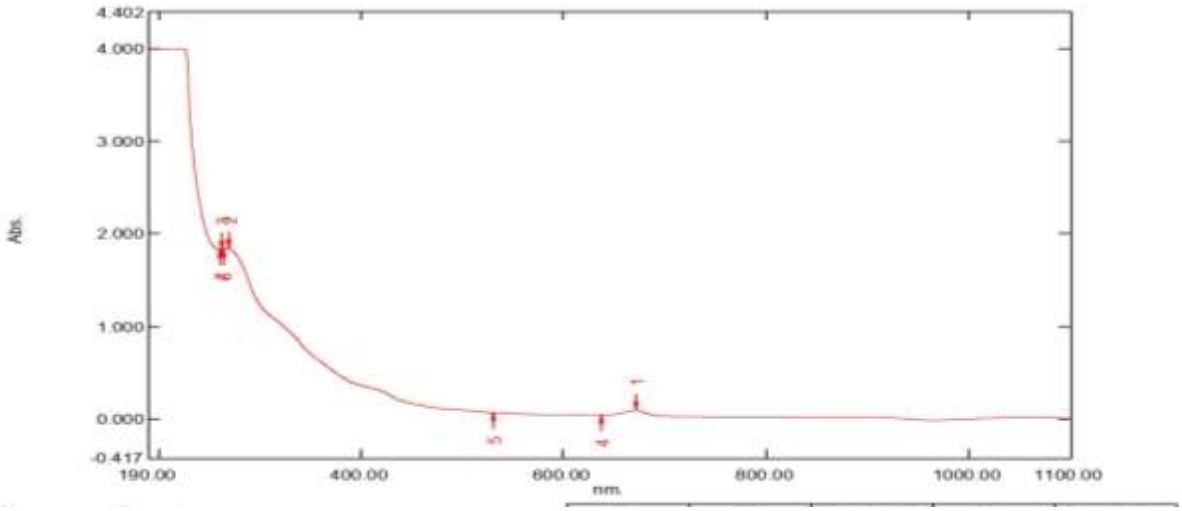

Figure 3 UV-VIS Spectrum of the ethanolic stem extract of *Cissus quadrangularis* L

Table 4 UV-VIS Spectrum peak values of the ethanolic stem extract of *Cissus quadrangularis* L

S.No.	Wavelength(nm)	Absorbance
1.	671.20	0.093
2.	268.60	1.845
3.	263.10	1.840
4.	638.00	0.042
5.	530.90	0.067
6.	264.20	1.837
7.	260.60	1.832

IV. CONCLUSION:

The previous study highlighted the presence of various phytochemical components in the methanol and ethyl acetate extract of *Cissus quadrangularis* L. The researchers have attempted to identify the metabolites present in the different parts of the plant. Apart from its bone healing properties, the selected plant appears to be a source of promising active components like Thiazolidinedione, used in another metabolic disorder- diabetes mellitus. The range of inquisitive analyses like the GC-MS, FTIR, and UV-Vis spectroscopy employed in the present study reveals the occurrence of pharmaceutically important secondary metabolites in the ethanol extract of the aerial part of *Cissus quadrangularis* L.

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