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Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

ISSN 2278-4136
ISSN 2349-8234
JPP 2013; 2(4):149-157
© 2013 AkiNik Publications
Received: 16-10-2013
Accepted: 03-11-2013

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Spectral analysis of methanol extract of *Cissus quadrangularis* L. stem and its fractions

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ABSTRACT

Objective: The stem of *Cissus quadrangularis* L. (*Vitaceae*) is reported to have great medicinal value. The aim of the present study was to determine the chemical profile of methanol extract of *Cissus quadrangularis* and its fractions (by various spectral analysis) which will be useful for its proper identification when therapeutically used.
Methods: Spectral analysis was done by different analytical methods such as UV, IR, GC-MS and HPTLC studies in methanol extract (ME) of *Cissus quadrangularis* and its fractions (FS-I and FS-II).
Results: In HPTLC analysis, ME and its fractions showed the presence of different types of compound with different R_f values ranging from 0.01 to 1.05. Maximum number of compounds were in FS-I (24). The GC-MS analysis showed the presence of fatty acids such as hexadecanoic acid (CAS) palmitic acid, octadecanoic acid (CAS) stearic acid, 9, 12-octadecadienoic acid (Z, Z)-(CAS) linoleic acid, 2-hexadecenoic acid, methyl ester 7, 10-Octadecadienoic acid methyl ester, linoleic acid, butyl ester and 9, 12-octadecadienoic acid (Z, Z)-, 2, 3-dihydroxypropyl ester in ME and its fractions of *Cissus quadrangularis* L.
Conclusions: Spectral analysis is useful in differentiating the species from the adulterants and the results of this study may act as biochemical markers for this medicinally important plant in the pharma industry and plant systematic studies.

Keywords: *Cissus quadrangularis*, HPTLC, GC-MS, Spectral analysis

1. Introduction

Medicinal plants or their derived material, have been widely employed in all cultures, throughout history, for the prevention and treatment of diseases. The significant increase in the use of herbal medicines in recent decades may be attributed to popular wisdom, the costs of synthetic drugs and the resurgence of interest in the development of new drugs and the reestablishment of old ones from plant sources [1].

Medicinal plants are extensively studied for their large therapeutical interests and benefits. The complex mixture of bioactive compounds cover a large variety of demands for the human health [2, 3]. The discovery of novel drugs from nature is also important because many isolated molecules are quite complex and may not be potent enough to be drugs in their own right, but they can serve as pharmacophores for chemical modification and drug design, which often yield clinically useful drug [4].

Cissus quadrangularis Linn. belongs to the family Vitaceae, an edible plant found in India, Sri Lanka, Malaya, Java, West Africa and also found throughout Thailand [5]. It is commonly known as “bone setter”; the plant is referred to as “Asthisamdhani” in Sanskrit and “Hadjod” in Hindi because of its ability to join bones [6]. The plant has been documented in Ayurveda, an alternative system of medicine in India, for its medicinal uses in gout, syphilis, venereal diseases, piles, tumors, hemorrhoids peptic ulcers and leucorrhoea [7, 8].

The separation of plant constituents is generally performed using one, or a combination of chromatographic techniques like column chromatography and high pressure liquid chromatography, high-performance thin layer chromatography. The choice of technique depends largely on the solubility properties and volatilities of the compounds to be separated. The complexity of plant extracts can be simplified using different separation techniques based on size or polarity. The aim of the present study was to determine the chemical profile of ME of *Cissus quadrangularis* and its fractions which will be useful for its proper identification when therapeutically used.

2. Material and Methods

2.1 Collection of plant

The fresh stem of *Cissus quadrangularis* L. was collected from Jamnagar, Gujarat, in August, 2009. The plant was compared with voucher specimen (voucher specimen No. PSN127) deposited at Department of Biosciences, Saurashtra University, Rajkot. The stem was washed thoroughly with tap water, shade dried, homogenized to fine powder and stored in airtight bottles.

2.2 Fractionation

Ten grams of dried stem powder was extracted in 150 ml methanol using a Soxhlet apparatus. The extract was poured into evaporating dishes and the solvent was evaporated under reduced pressure. The solvent was then evaporated to dryness to give the dried crude extract. The percentage yield of methanol extract was 11.76%. Fractionation of the methanol extract was done by solvent-solvent partition [9]. Five grams of methanol extract of *Cissus quadrangularis* was dissolved in 200 ml hot methanol. Slight precipitation obtained was discarded as methanol insoluble matter. The methanol soluble fraction was filtered and collected. It was concentrated to about 50 ml volume and ethyl acetate was added to it till faint turbidity was obtained. Then it was allowed to settle down in a refrigerator. The settled gelatinous reddish mass and supernatant was separated and collected separately. The supernatant was further concentrated and ethyl acetate step was repeated till reddish gelatinous mass obtained. All the settled mass was collected together and dissolved in methanol. It was concentrated further to dryness and designated as Fraction I (FS I). The yield of FS I was 46.1%. The collected supernatant was concentrated further to near dryness and then dissolved in methanol. Then chloroform was added to it and cooled. Light yellow waxy sediment was separated and light buff coloured supernatant was collected. This supernatant was concentrated further to dryness and designated as Fraction II (FS II). The yield of FS II was 15.54%.

2.3 Ultraviolet visible absorption (UV)

The methanol extract of *Cissus quadrangularis* and its fractions were analyzed in UV-Visible range between 200-800 nm using UV-Visible Spectrophotometer (UV-1800, Shimadzu). This method is useful for analyzing organic compounds viz. ketones, dienes etc.

2.4 Infra-red spectroscopy (IR)

Infrared spectroscopy is one of the powerful analytical techniques which offer the possibility of chemical identification. The technique is based on the simple fact that chemical substance shows selective absorption in infrared region. After absorption of IR radiations, the molecules vibrate, giving rise to absorption spectrum. It is an excellent method for the qualitative analysis because except optical isomers, the spectrum of compound is unique. It is most useful for the identification of purity and gross structural details. This method is useful in the field of natural products, forensic chemistry and in industrial analysis of competitive products. The IR spectra of methanol extract of *Cissus quadrangularis* L. stem and its fractions were scanned on FT-IR-Shimadzu-8400 over the frequency range from 4000-400 cm^{-1}.

2.5 HPTLC fingerprinting

CAMAG HPTLC system equipped with Linomat 5 applicator, TLC scanner 3, repro star 3 with 12 bit CCD camera for photo documentation, controlled by WinCATS-4 software were used. All the solvents used for HPTLC analysis were obtained from MERCK. A total of 100 mg extract was dissolved in 5 ml of methanol and used for HPTLC analysis as test solution. The samples (10 µl) were spotted in the bands of width 8 mm with a Camag microlitre syringe on pre-coated silica gel glass plate 60 F-254. The sample loaded plate was kept in TLC twin trough developing chamber (after saturated with Solvent vapor) with respective mobile phase and the plate was developed up to 83 mm in the respective mobile phase. The Toluene-Ethyl acetate-Glacial acetic acid (8:2:0.1) was employed as mobile phase for extract. Linear ascending development was carried out in 20 cm X 10 cm twin trough glass chamber saturated with the mobile phase and the chromatoplate development with the same mobile phase to get good resolution of phytochemical contents. The optimized chamber saturation time for mobile phase was 30 min at room temperature. The developed plate was dried by hot air to evaporate solvents from the plate. The plate was photo-documented at UV 366 nm and white light using photo documentation chamber. Finally, the plate was fixed in scanner stage and scanning was done at 366 nm. The plate was kept in photo-documentation chamber and captured the images under white light, UV light at 254 and 366 nm. Densitometric scanning was performed on Camag TLC scanner III and operated by CATS software (V 3.15, Camag) [10].

2.6 GC-MS spectroscopy

GC-MS analysis of methanol extract was performed using GC-MS instrument (GCMS-QP-2010) equipped with glass column SGE BPX5 and capillary dimension 30 m x 0.25 mm x 0.25 µ. The oven temperature was programmed from 80-260 °C. Inlet and interface temperature were 250 °C and 200 °C respectively. Carrier gas was helium at a flow rate of 1.0 ml/min. Ion source temperatures were maintained at 200 °C and spectra were measured [11].

3. Results

3.1 Ultraviolet visible absorption (UV)

UV-Visible spectra of ME and its fractions are shown in Fig. 1. The UV spectrum of ME of *Cissus quadrangularis* showed absorption maxima at 661, 273, 204 and 256 nm. The FS-I showed absorption maxima at 662.5, 272, 205 and 254.5 nm. While FS-II showed absorption maxima at 664.5, 532, 497.5, 401, 268, 203.5, 622, 577, 521, 489, 340.5 and 262 nm.

3.2 IR spectroscopy

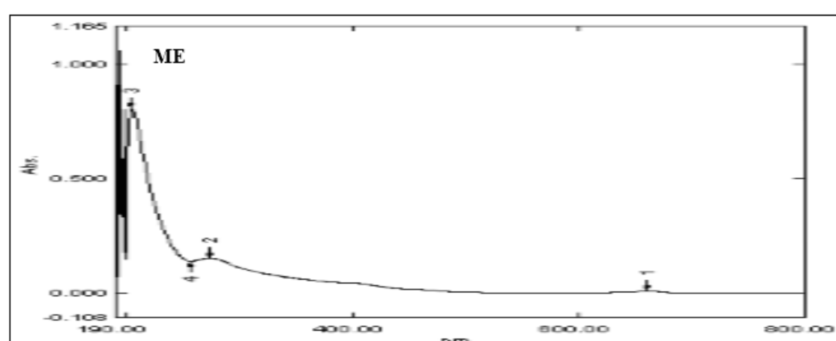
IR spectra of ME and its fractions are shown in Fig. 2 the mid-infrared, approximately 4000–400 cm^{-1} (2.5–25 µm) was used to study the fundamental vibrations and associated rotational-vibrational spectrum. Interpretation of ME and its fractions are given in Table-1.

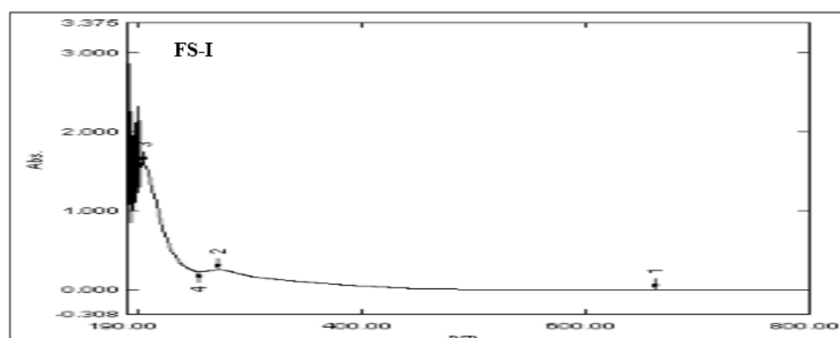
3.3 HPTLC fingerprinting

High Performance Thin Layer Chromatography (HPTLC) technique is most simple and fastest separation technique available today which gives better precision and accuracy with extreme flexibility for various steps. The results showing number of peaks, maximum R_f value and total % area are given in Table 2. The HPTLC fingerprinting of ME and it fractions are shown in Fig. 3. The ME showed 21 peaks in 200-800 nm spectral range. In ME, the maximum percentage area covered was by peak No. 1, 6, 19 and 21 (R_f value 0.03, 0.31, 0.78 and 0.94 respectively) (Fig. 4). The FS-I showed 24 peaks in 200-800 nm spectral range.

Table 1: Interpretation of IR of ME of *C. quadrangularis* and its fractions FS-I and FS-II

Wavelength (cm^{-1})	Interpretation
ME	
3381	-C≡C-H stretching vibration band
2922	Alkane asymmetric C-H stretching vibration band
2850	Alkane symmetric C-H stretching vibration band
1709	C=O stretching vibration band
1636	Aromatic carbon skeleton C=C stretching vibration band
1512	
1384	Alkane C-H bending vibration band
1256	C-C stretching vibration band
1050	C-O-C stretching vibration band
FS-I	
3445	Inter molecular H-bond O-H stretching vibration band
2921	Alkane asymmetric –C-H stretching vibration band
1634	Aromatic carbon skeleton C=C stretching vibration band
1385	Alkane C-H bending vibration band
1057	C-O-C stretching vibration band
921	Vinylic C=C bending vibration band
FS-II	
3397	Inter molecular H-bond O-H stretching vibration band
2925	Alkane asymmetric C-H stretching vibration band
2852	Alkane symmetric C-H stretching vibration band
1735	C=O stretching vibration band
1464	Alkane $-CH_2-$ bending vibration band
1384	Alkane $-CH_3$ bending vibration band
1246	C-C stretching vibration band
1075	C-O-C stretching vibration band





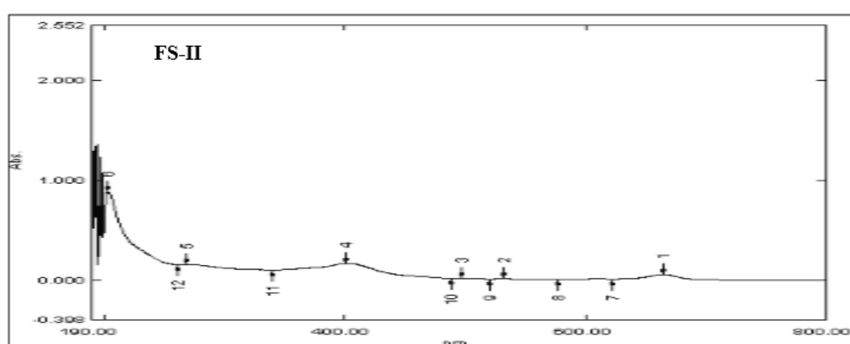


Fig 1: UV spectra of ME of *C. quadrangularis* and its fractions FS-I and FS-II

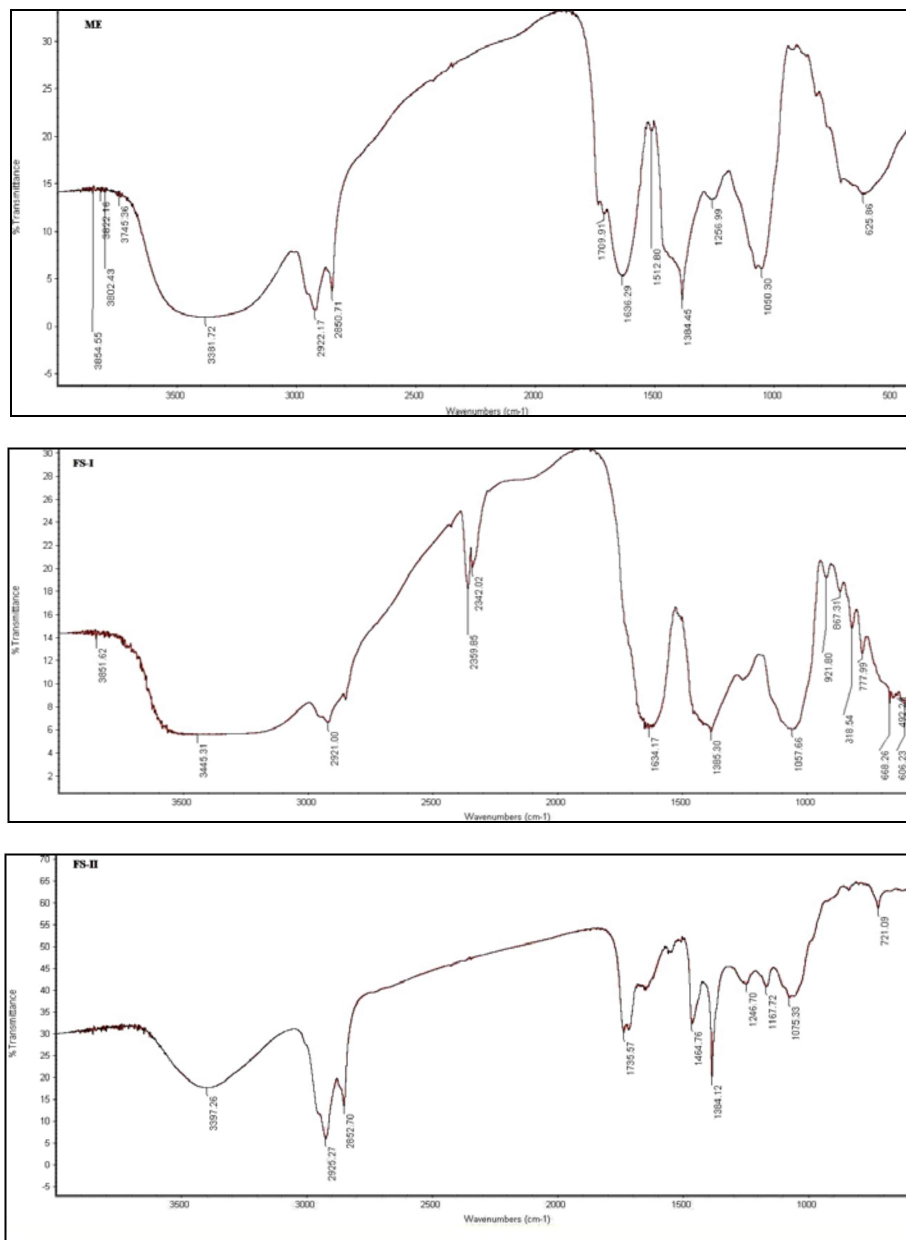


Fig 2: IR spectra of the methanol extract of *C. quadrangularis* L. stem and its fractions FS-I and FS-II

The maximum percentage area covered was by peak No. 1, 5, 19 and 20 (R_f value 0.03, 0.32, 0.76 and 0.80 respectively) (Fig. 5). The FS-II showed 17 peaks in 200-800 nm spectral range. The maximum percentage area covered was by peak No. 1, 4 and 12 (R_f value 0.02, 0.29 and 0.78 respectively) (Fig. 6). This HPTLC technique may be useful for both the identification and the quality-evaluation of preparations containing *Cissus quadrangularis* L. stem.

3.4 GC-MS spectroscopy

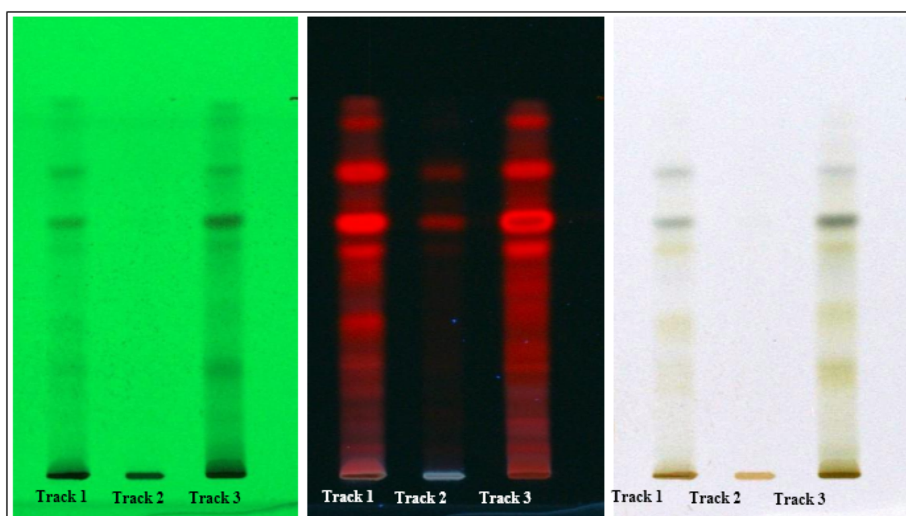
GC-MS analysis has found a variety of analytical uses, including quality control analysis in both the pharmaceutical and food product industries. The detail tabulation of the GC-MS analysis of ME and its fractions is given in Table 3.

4. Discussion

The ultraviolet spectroscopy method is very much useful for identification of unsaturated bonds present in a plant component, which can be used to distinguish between conjugated and non-conjugated system. Using the principle of absorption maxima, the structure of compounds can be deduced. Gas chromatography-mass spectrometry (GC-MS) is a method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. Applications of GC-MS include drug detection, fire investigation, environmental analysis, explosives investigation, and identification of unknown samples [12]. The presence of fatty acids and aromatics in ME and its fractions shows the pharmacological properties of the plant. In the presence study, GC-MS data showed the highest presence of saturated and unsaturated fatty acids like, hexadecanoic acid (CAS) palmitic acid, octadecanoic acid (CAS) stearic acid, 9, 12-octadecadienoic acid (Z, Z)-(CAS) linoleic acid, 2-Hexadecenoic acid, methyl ester 7,10-octadecadienoic acid methyl ester, linoleic acid, butyl ester and 9,12-Octadecadienoic acid (Z, Z)-2, 3-dihydroxypropyl ester in ME and its fractions of *Cissus quadrangularis* L. In all, 29 compounds were present in ME and its fractions; some of them are reported earlier [13, 14] and some we are reporting for the first time.

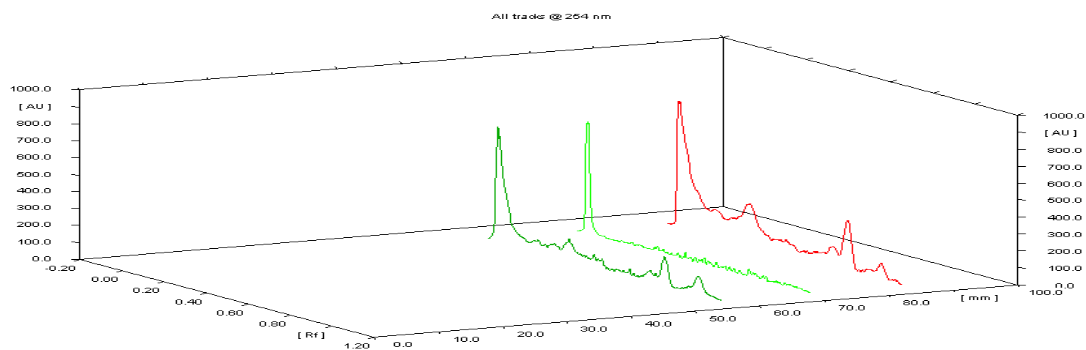
Table 2: HPTLC spectral analysis of ME of *C. quadrangularis* and its fractions FS-I and FS-II

ME			FS-I			FS-II		
Peak	**Max R_f**	**Area (%)**	**Peak**	**Max R_f**	**Area (%)**	**Peak**	**Max R_f**	**Area (%)**
1	0.01	48.41	1	0.02	65.63	1	0.02	43.45
2	0.18	2.28	2	0.17	0.91	2	0.19	7.56
3	0.20	2.25	3	0.23	1.11	3	0.28	4.04
4	0.24	1.63	4	0.26	1.78	4	0.36	16.40
5	0.29	5.32	5	0.35	2.89	5	0.49	1.86
6	0.36	8.51	6	0.38	1.36	6	0.53	1.92
7	0.41	1.32	7	0.44	1.62	7	0.62	0.99
8	0.43	1.68	8	0.48	1.84	8	0.63	0.60
9	0.47	2.07	9	0.50	1.13	9	0.68	1.65
10	0.49	0.92	10	0.53	1.16	10	0.71	1.40
11	0.51	1.45	11	0.56	1.44	11	0.75	4.56
12	0.56	0.23	12	0.57	1.72	12	0.82	10.56
13	0.58	0.68	13	0.59	1.98	13	0.88	0.27
14	0.62	0.45	14	0.63	1.48	14	0.89	0.31
15	0.64	0.79	15	0.67	1.69	15	0.93	0.91
16	0.66	0.32	16	0.69	1.14	16	0.98	2.91
17	0.74	3.59	17	0.71	0.88	17	1.05	0.61
18	0.77	1.24	18	0.74	1.03			
19	0.81	8.60	19	0.78	2.22			
20	0.92	1.31	20	0.81	2.93			
21	0.97	6.97	21	0.85	1.18			
			22	0.86	0.95			
			23	0.92	1.10			
			24	0.95	0.83			

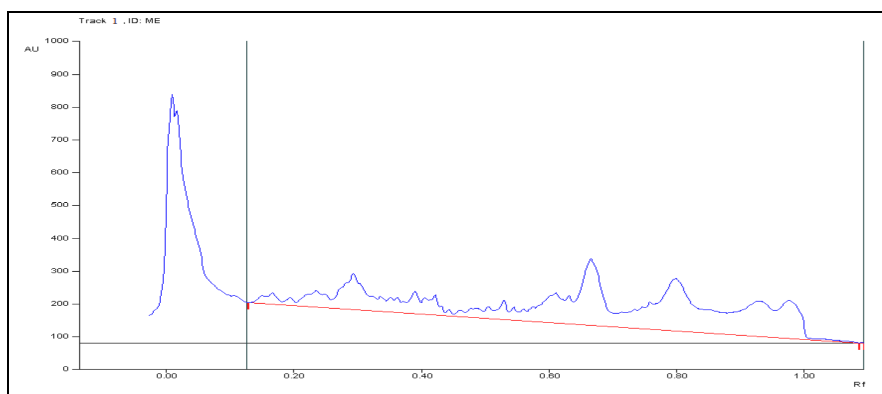


TLC chromatogram visualized in various lights representing separated compounds

Plant name:	***Cissus quadrangularis* L.**
Plant part used:	Stem
Spotting of samples:	
Track 1	ME
Track 2	FS-I
Track 3	FS-II
Band width	8 mm
TLC plate size	10 cm ×10 cm
Two bands distance	23.3 mm
Sample conc.	20 mg/ml
Sample volume	10 µl
Solvent system	Toluene : Ethyl acetate: Glacial acetic acid (8:2:0.1) v/v
Slit dimension	6.00 mm × 0.30 mm, Micro



3-D graphical display of absorbance peaks (100- 1000 nm)
Fig 3: HPTLC fingerprinting of ME of *C. quadrangularis* and its fractions FS-I and FS-II



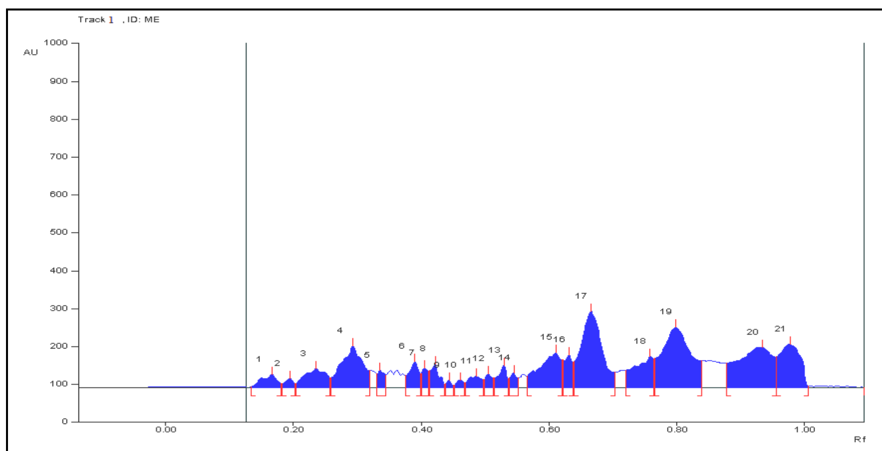
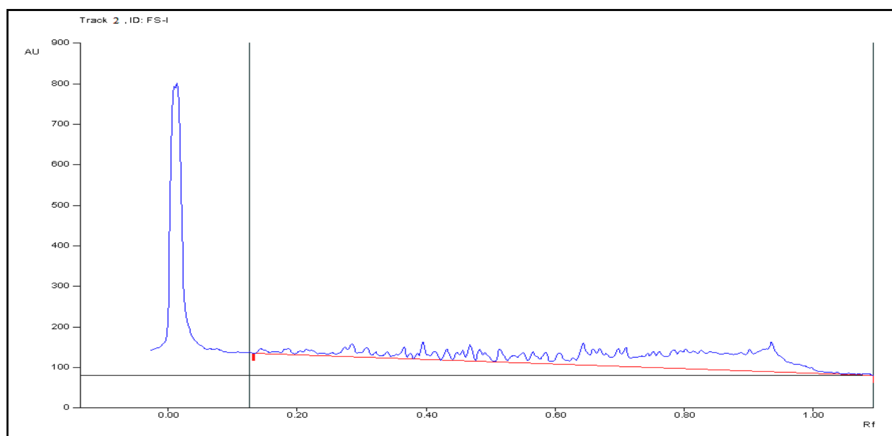


Fig 4: HPTLC spectra of ME of *C. quadrangularis* L. stem



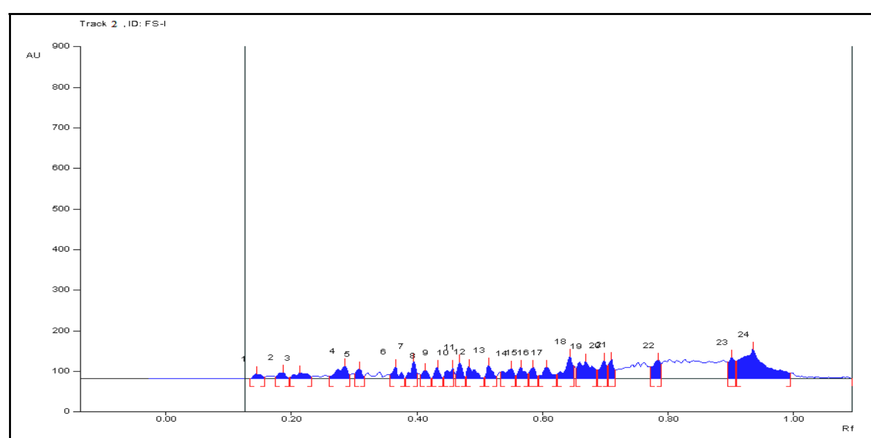


Fig 5: HPTLC spectra of FS-I of *C. quadrangularis* L. stem

Table 3: Phytocomponents identified in the ME of *C. quadrangularis* and its fractions FS-I and FS-II by GC-MS peak report

Sr. No.	Retention time	Area	Area %	Molecular formula	Mole. Wt.	Extracts	Compounds
1	9.33	84865	2.82	$C_{20}H_{38}$	278.52	ME,FS-I, FS-II	Neophytadiene
2	9.55	34304	1.14	$C_{18}H_{34}$	250.46	ME,FS-II	1-Octadecyne
3	9.79	43550	1.45	$C_{17}H_{33}O_2$	269.44	ME	Methyl palmitate
4	10.64	37490	1.25	$C_{17}H_{32}$	236.25	ME	3-Methyl-Z,Z-4,6-hexadecadiene
5	10.68	14885	0.50	$C_7H_{12}S$	128.24	ME	7-Thiabicyclo[4.1.0.] heptanes, 1-methyl
6	10.75	82921	2.76	$C_{20}H_{40}O$	296.53	ME	Phytol
7	10.88	298180	9.92	$C_{18}H_{32}O_2$	280.45	ME, FS-I	9, 12-Octadecadienoic acid (Z, Z)-(CAS) Linoleic acid
8	10.94	67043	2.23	$C_{18}H_{32}O$	264.45	ME	9, 17- Octadecadienal
9	12.11	120698	4.02	$C_{20}H_{36}O_3$	324.50	ME	Z-6, 17-Octadecadien-1-ol acetate
10	12.31	116493	3.88	$C_{16}H_{28}O$	236.39	ME	8-Cyclohexadecen-1-one
11	15.53	497816	16.56	$C_{28}H_{48}O$	400.68	ME	Ergost-5-en-3.beta.-ol
12	15.68	52499	1.75	$C_{18}H_{38}O$	270.29	ME	7-Methyl-7-heptadecanol
13	15.99	991687	32.99	$C_{29}H_{50}O$	414.71	ME, FS-I, FS-II	Gamma-Sitosterol
14	16.40	208066	6.92	$C_{30}H_{48}O$	424.70	ME, FS-II	Lup-20(29)-en-3-one
15	17.57	355102	11.81	$C_{30}H_{50}O$	426.72	ME, FS-II	Friedeline
16	12.52	310535	0.73	$C_{16}H_{32}O_2$	256.42	FS-I	Hexadecanoic acid (CAS) Palmitic acid
17	13.48	236167	0.55	$C_{18}H_{32}O_2$	284.45	FS-I	Octadecanoic acid (CAS) Stearic acid
18	14.60	232407	0.55	$C_{21}H_{38}O_4$	354.52	FS-I, FS-II	Beta Monolinolein
19	14.80	169831	0.40	$C_{17}H_{32}O$	252.44	FS-I, FS-II	(R)-(-)-14-Methyl-8-hexadecyn-1-ol
20	15.58	66610	0.16	$C_{19}H_{34}$	262.47	FS-I	1,3,12-Nonadecatriene
21	18.000	8908951	20.90	$C_{56}H_{95}O_6$	864.35	FS-I	Trilinolein
22	23.44	28719398	67.37	$C_{21}H_{38}O_4$	354.52	FS-I	9, 12-Octadecadienoic acid (Z,Z)-, 2, 3-dihydroxy-propyl ester
23	9.46	24157	0.67	$C_{20}H_{38}$	278.52	FS-II	1-Eicosyne (CAS) Eicosyne-1
24	9.80	24382	0.67	$C_{17}H_{32}O_2$	268.43	FS-II	2-Hexadecenoic acid, methyl ester
25	10.64	39789	1.10	$C_{19}H_{34}O_2$	294.47	FS-II	7, 10-Octadecadienoic acid methyl ester
26	10.76	104008	2.87	$C_{22}H_{40}O_2$	336.55	FS-II	Linoleic acid, butyl ester
27	15.52	596845	16.44	$C_{28}H_{48}O$	400.68	FS-II	Campesterol
28	15.69	77323	2.13	$C_{10}H_{18}$	138.25	FS-II	2,6,6-Trimethyl-bicycle [3.1.1] heptane
29	16.14	57140	1.57	$C_{30}H_{50}O$	426.72	FS-II	Beta.-Amyrene

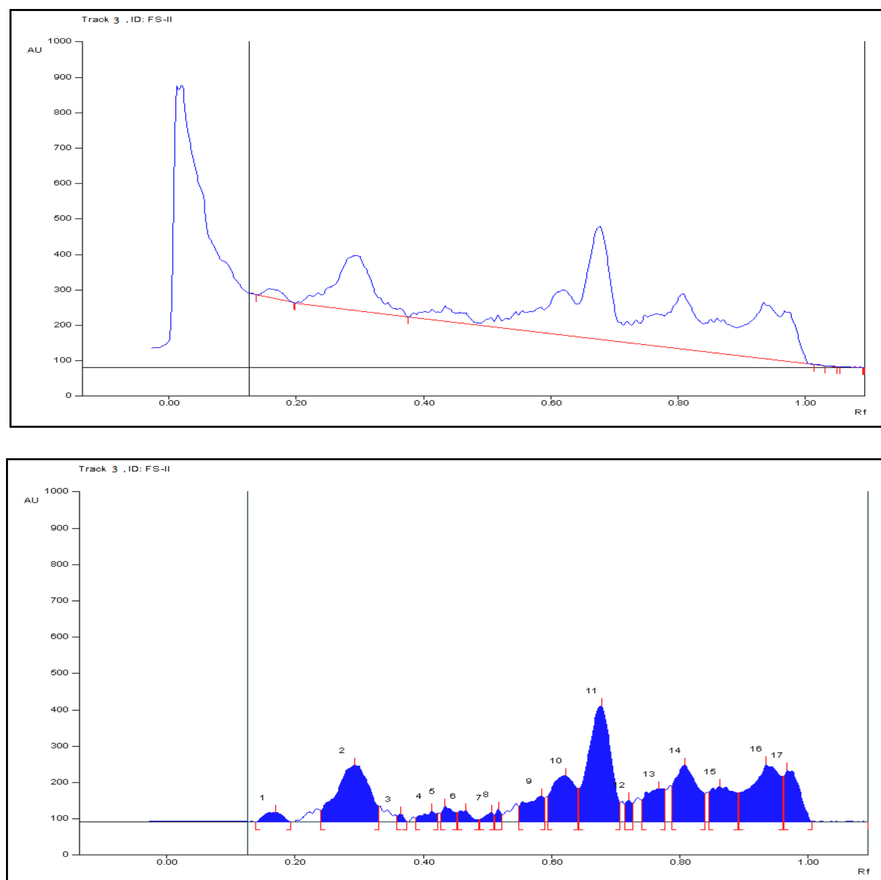


Fig 6: HPTLC spectra of FS-II of *C. quadrangularis* L. stem

This is quite interesting HPTLC is a valuable tool for reliable identification. It can provide chromatographic fingerprints that can be visualized and stored as electronic images [15]. HPTLC determination using a modern scanner with DAD makes it possible to apply the extract containing solution directly on the HPTLC plates without prior preparation steps. The optimum wavelength for quantification can easily be chosen via the DAD spectrum. The full spectrum is taken at once and allows to judge the resulting densitogram [16]. HPTLC method has emerged as an important tool for the qualitative and quantitative phytochemical analysis of herbal drugs and formulations [17]. This analysis is the first step towards understanding the nature of active principles and their detailed phytochemistry. In the presence study, ME and its fractions (FS-I and FS-II) showed 21, 24 and 17 peaks respectively in chromatogram which has been produced by HPTLC. Therefore, HPTLC fingerprinting is proved to be a linear, precise, accurate method for herbal identification and can be used further in authentication and characterization of the medicinally important plant.

The spectral analysis will help the manufacturer for quality control and standardization of herbal formulations. Such type of analysis is useful in differentiating the species from adulterants and other sub species and the result of this study will become the finger print of this plant and it may act as a biochemical marker which will be useful for pharma industry and systematic studies.

5. Reference

1. Rodrigues MVN, Reyes FGR, Magalhaes PM, Rath S. GC-MS determination of organochlorine pesticides in medicinal plants harvested in Brazil. J Braz Chem Soc 2007; 18:135-142.
2. Miyazawa M, Watanabe H, Kameoka H. Inhibition of Acetylcholinesterase activity by monoterpenoids with a p-menthane skeleton. J Agric Food Chem 1997; 45:677-679.
3. Gherman C, Culea M, Cozar O. Comparative analysis of some active principles of herb plants by GC/MS. Talanta 2000; 53:253-262.
4. Kingston DGI. Recent advances in the chemistry of taxol. J Nat Prod. 2000; 63:726-734.
5. Pongboonrod S. Mai Thed Mung Muang Thai. Bangkok: Kaeseam-bunnakit Printing, Thailand, 1995, 428-429.
6. Shirwaikar A, Khan S, Malini S. Antiosteoporotic effect of ethanol extracts of *C. quadrangularis* Linn. on ovariectomized rat. J Ethnopharmacol 2003; 89:245-250.
7. Yoganarisimhan SN. Medicinal Plants of India. Cyber Media Bangalore 2000; 2:136-137.
8. Nagani KV, Kevalia J, Chanda SV. Pharmacognostical and phytochemical evaluation of stem of *C. quadrangularis* L. Int J Pharmaceut Sci Res 2011; 2:2856-2862.
9. Tang J, Meng X, Liu H, Zhao J, Zhou L, Qiu M, Zhang X, Yu Z, Yang F. Antimicrobial activity of Sphingolipids isolated from the stems of Cucumber (*Cucumis sativus* L.). Molecules 2010; 15:9288-9297.
10. Sethi PD. HPTLC. In: Quantitative analysis of pharmaceutical formulations. Edn 1, New Delhi: CBS Publishers and Distributors. 1996, 3-73.
11. Adams RP. Identification of essential oil components by gas. Allured publishing corporation, IL, USA, 2007.
12. Manjamalai A, Narala Y, Haridas A, Grace VMB. Antifungal, anti-inflammatory and GC-MS analysis of methanolic extract of *Plectranthus amboinicus* leaf. Int J Current Pharmaceut Res 2011; 3:129-136.
13. Rao GV, Annamalai T, Mukhopadhyay T, Madhavi MSL. Chemical Constituents and Melanin Promotion activity of *Cissus quadrangularis* Linn. Res J Chem Sci 2011; 1:25-29.
14. Thakur A, Jain V, Hingorani L, Laddha KS. Phytochemical studies

on *Cissus quadrangularis* L. Phcog Res 2009; 1:213-215.
15. Manikandan A, Doss AVA. Evaluation of biochemical contents, nutritional value, trace elements, SDS-PAGE and HPTLC profiling in the leaves of *Ruellia tuberosa* L. and *Dipteracanthus patulus* (Jacq.). J Chem Pharm Res. 2010; 2: 295-303.
16. Pozharitskaya ON, Kosman VM, Shikov AN, Demchenko DV, Eschenko AY, Makarov VG. Comparison between HPLC and HPTLC densitometry for the determination of icariin from *Epimedium koreanum* extracts. J Sep Sci 2007; 30:708-712.
17. Sajeeth CI, Mann PK, Manavalan R, Jolly CI. Quantitative estimation of gallic acid, rutin and quercetin in certain herbal plants by HPTLC method. Der Chimica Sinica 2010; 1:80-85.