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Pharmacognostic and phytochemical investigation of the aerial parts of *Cissus quadrangularis* Linn.

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Abstract

Aim: The aim of the present study is to investigate the pharmacognostic and phytochemical investigation of the aerial parts of the plant *Cissus quadrangularis* Linn. belonging to the family Vitaceae, which is greatly valued in Ayurveda for setting of bone fractures. **Methods:** In microscopic studies, transverse section (TS) of leaves, stem and powder characters of the plant were studied and characteristic features were established. Physicochemical parameters such as total ash value, acid insoluble ash value and water soluble ash value were determined. The alcohol soluble extractive and water soluble extractive were also determined. Preliminary phytochemical analysis of alcoholic extract was carried out. **Results:** The results of preliminary phytochemical screening were positive for flavonoids, carbohydrates, glycosides, proteins and saponins. The results obtained from standardization of aerial parts of *Cissus quadrangularis* established the macro and microscopical parameters, physicochemical parameters, TLC profiles that characterize the genuine plant drug. **Conclusion:** The present study provides pharmacognostical, physicochemical and phytochemical details of the aerial parts of *Cissus quadrangularis* which are useful in laying down standardization and pharmacopoeia parameters.

Keywords: *Cissus quadrangularis*, Flavonoids, Pharmacognostical, Phytochemical.

Introduction

Plant *Cissus quadrangularis* Linn. belonging to the family Vitaceae commonly known as Pirrandai, in Tamil.[1, 2] It is probably native to India or Sri Lanka, but also found in Africa, Arabia, and South East Asia. Traditionally, this drug is greatly valued in Ayurveda for setting of bone fractures, and indeed its Sanskrit name Asthishrinkhalaa means skeletal bones, while the trade name Hadjore implies one that joins bones.[3] The plant is used in the treatment of anorexia, dyspepsia, colic, flatulence, tumours, convulsions, asthma, epistaxis, otorrhoea, irregular menstruations, inflammations, pain and syphilitic infections. Powdered roots as well as the stem paste are very specific for bone fracture. Shoots are useful in colonopathy, scurvy, burns and wounds.[4] The literature survey revealed that the systemic evaluation including pharmacognostical study of this plant is still lacking. There is a need for documentation of research work carried out on traditional medicines. With this backdrop, it becomes extremely important to make an effort towards standardization of the material to be used as medicine. The main aim of the present work is to study the macro, microscopic, physico-chemical standards and phytochemical analysis of the aerial parts of the plant *Cissus quadrangularis*, which could be used for the proper identification of this drug.

Materials and Methods

Plant material

Cissus quadrangularis Linn (Vitaceae), distributed widely in India and Sri Lanka. The plants specimen for the proposed study was collected in Kancheepuram district, Tamilnadu. It was identified and authenticated by Dr. P. Jayaraman, Director, Plant Anatomy Research Centre (PARC), Tambaram, Chennai. A voucher specimen No. PARC/2012/1299 has been deposited for further references.

Microscopical characterization

The qualitative studies were performed. Free hand transverse sections of leaf and stem were, studied for different microscopic characters and photographs of different magnifications of the sections were taken with Nikon Lab Photo 2 (Two) Microscopic unit. For normal observations, bright field was used. For the study of crystal, starch grains and lignified cells, polarized light was employed.[5]

Powder analysis

The shade dried aerial parts of the plant were powdered and powder was passed through 100 # sieve. A small amount of powder was taken onto a microscopic slide, cleared from chlorophyll by heating with chloral hydrate solution and was mounted in 50% v/v glycerol in water. This was then observed under microscope to study the characteristic features.[6]

Physico-chemical evaluations

The ash values, extractive values and loss on drying were performed according to the officinal methods prescribed in Indian pharmacopeia and the WHO guidelines on quality control methods for medicinal plants materials.[7] Fluorescence analysis was carried out according to the method of Kokoski.[8]

Extraction

The collected aerials parts of plant was made thoroughly free from any foreign organic matter, dried under shade and powdered. The Hydroalcoholic extract was prepared using 70% aqueous ethanol by triple maceration process for 48 h each time. The extract was filtered and concentrated under vacuum. The concentrated extract was used for phytochemical screening and establishment of TLC profile.

Preliminary Phytochemical Screening

The hydro-alcoholic extract were subjected to preliminary phytochemical screening for the detection of various phytoconstituents such as alkaloids, glycosides, tannins and phenolic compounds, flavonoids, steroids, saponins, proteins, amino acids, carbohydrates and triterpenoids.[9]

Thin Layer Chromatography

The ethanol extract was subjected to thin layer chromatography. Number of solvent system was tried. The solvent system which shows good resolution was used. The visualization of spot was done by exposing the plate to iodine vapour using the solvent system Benzene: Methanol: Ammonia (9:0.5:0.5).[10]

High performance thin layer chromatography

Chromatograph was performed on 10x10 cm aluminum packed TLC plate coated with 0.2 mm layer of silica gel 60F254 (E. Merck Ltd, Darmstadt, Germany) stored in a dessicator, application was done by Hamilton microsyringe (Switzerland), mounted on a Linomat V applicator. Spotting was done on the TLC plate, ascending development of the plate, migration distance 80 mm (distance to the lower edge was 10 mm) was performed at 25±20oC with Benzene: Methanol: Ammonia as a mobile phase in a camag chamber previously saturated for 30 min. After development the plate was dried at 60oC in an oven for 5 minutes. Densitometric scanning was then performed with a Camag TLC Scanner 3 equipped with win CATS Software and the chromatograms were recorded.[11]

Results and Discussion

Macroscopical Study

The morphological studies showed that the plant is a perennial climber, conspicuous by its jointed, succulent, cactus like, have four angled stems. The Leaves were broadly ovate, crenate-serrate, alternate, short petioled (Figure 1, A). Stems were dark green, slender, dichotomously branched, much contracted at the nodes, with tendril (opposite to the leaves) at some nodes; internodes 5-15 cm long. Old stems are leafless and brown (Figure 1, A). Flowers were small, dull white, borne in short umbellate cymes (Figure 1, B). Fruits were one seeded, succulent acrid, obovoid berries, 4-6 mm long, pale brown to red when ripe (Figure 1, C).

Microscopical Study

T.S. of leaf in the midrib exhibits convex adaxial side 660 µm wide and short conical abaxial side 400 µm wide of the mid rib. The mid rib has thin epidermal layer of small thick walled squarish cells. The ground tissues in the adaxial part are small circular compact thick walled. In the other regions of the midrib, the ground tissue is disintegrated leaving only small lobed parenchyma cells. The vascular system of the mid rib consists of four radiating arms of vascular strands. Of the four strands one on the abaxial side is smaller than others. The vascular strand are collateral, having two or three rows of xylem elements with phloem located on the outer ends of the xylem segments. In the xylem, the cells are circular, narrow and thick walled (Figure 2.1).

T.S. of leaf in the lamina is fairly dorsiventral with adaxial palisade and abaxial spongy mesophyll differentiation. The leaf consists of adaxial epidermal layer of spindle shaped thick walled cells and the abaxial epidermal cells are thin walled and rectangular in shape. The lamina is 120µm thick. In the adaxial part of lamina occur single row cylindrical palisade cells, in the abaxial part are 6 or 7 layers of spherical spongy parenchyma cells. Two or three layers of cells on the adaxial part of the lamina are slightly elongated in vertical plane and form the palisade cells. Remaining parenchyma cells are circular and less compact, forming spongy mesophyll tissue. Dispersed in the mesophyll tissue are thick bundles of calcium oxalate crystals called raphides (Figure 2.2).

T.S. of stem is four angled with four thick and long wings. The central part of the stem is 1.8 mm thick; the wings are 1.4 mm thick and 6 mm long. The stem consists of an epidermal layer of circular, thick walled, darkly stained and with thick cuticle (Figure 3.1). The sub epidermal cells at certain places have undergone tangential divisions forming periderm like layer of cells. The ground tissue is parenchymatous, circular thin walled and compact. Scattered in the ground tissue are wide, circular secretory cavities. The extreme margins of the wings have sclerenchyma cells. The vascular system consists of multistranded bundles located in groups within the outer part of the wings. In each wing there is an arc of 3 radially stretched collateral vascular bundles. Each bundle has a having a thick cap of sclerenchyma cells (Figure 3.2). The xylem strand includes wide, circular, angular thin walled vessels surrounded by thick walled xylem fibres.

Powder microscopy: Microscopic study of powder revealed the presence of spherical or elliptical parenchyma cells either in solitary or in aggregates of two or more cells, narrow lignified xylem fibres gradually tapering at the ends. Vessel elements with some unique characters are often seen in the powder. The vessel elements are long and narrow with uniseriate horizontally elliptical wide lateral wall pits and partly scalariform pits. The stomata are cyclocytic type, in which the stoma was surrounded by one or two circular layers of subsidiary cells. Raphides and druses of calcium oxalate crystals were seen in the powder (Figure 4).

Physicochemical Parameters

Various physicochemical parameters viz., ash, extractive values and loss on drying were determined. The results were summarized in Table 1. The fluorescence analysis of the powder was also done and results were given in Table 2. The powder was treated with various reagents and the mixture was observed under UV light (366 nm) to see the type of fluorescence. These data's were helpful for identifying and ascertaining the quality of the collected crude drug.

A **B** **C**

Figure 1: Aerial Parts of *Cissus quadrangularis* Linn. A: Stem with leaves; B: Flowers; C: Fruits

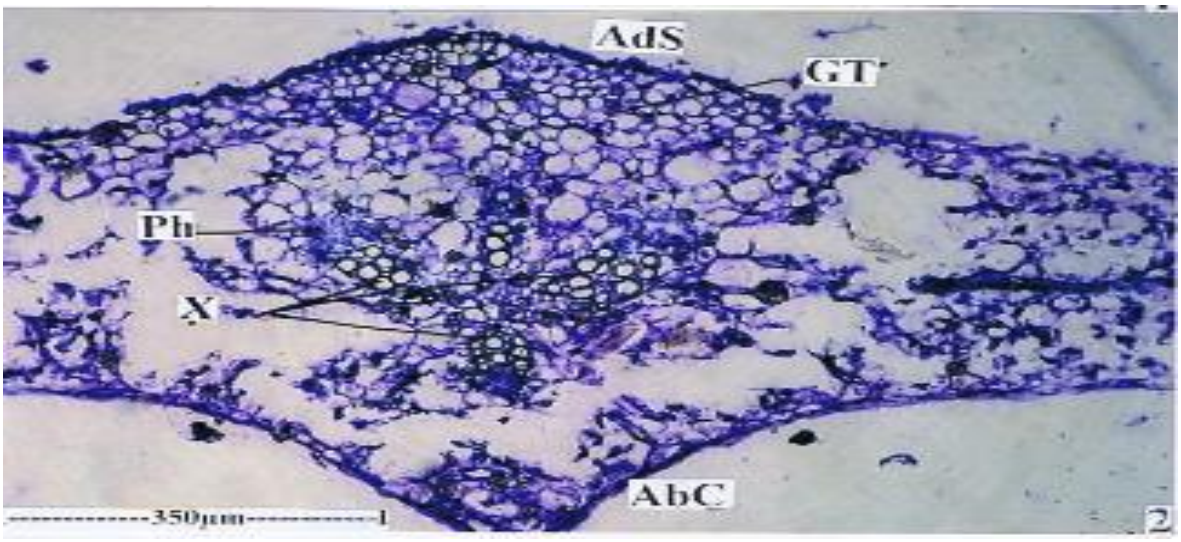

Figure 2.1: Transverse section of leaf through mid rib
Abc: Abaxial cone; Ads: Adaxial side; GT: Ground tissue; La: Lamina; Ph: Phloem;
VS: Vascular strand; X: Xylem.

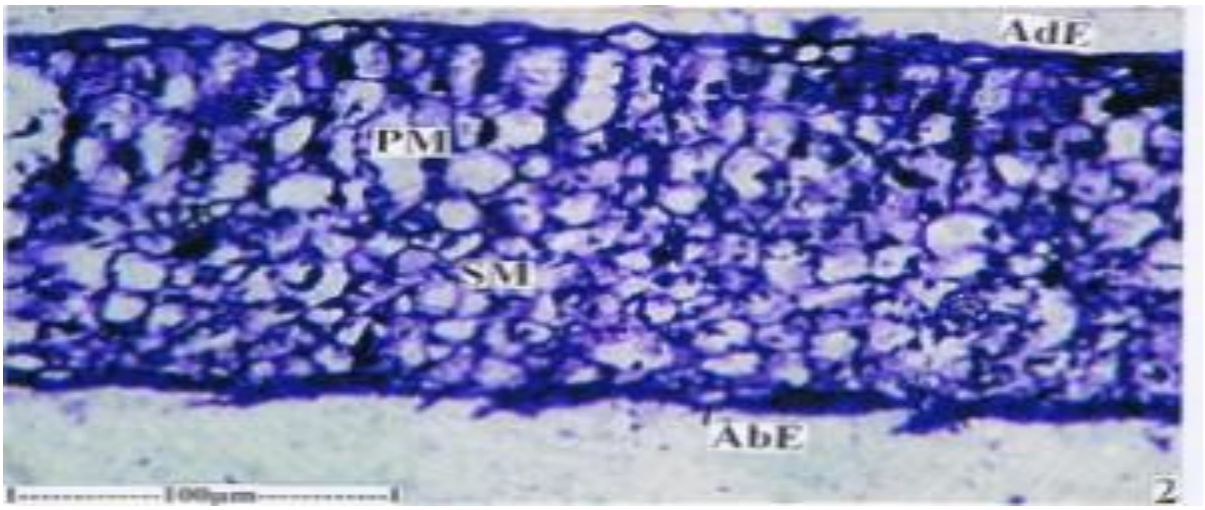

Figure 2.2: Transverse section of Lamina showing the mesophyll tissues
AbE: Abaxial Epidermis; AdE: Adaxial Epidermis; PM: Palisade mesophyll; SM: Spongy Mesophyll,

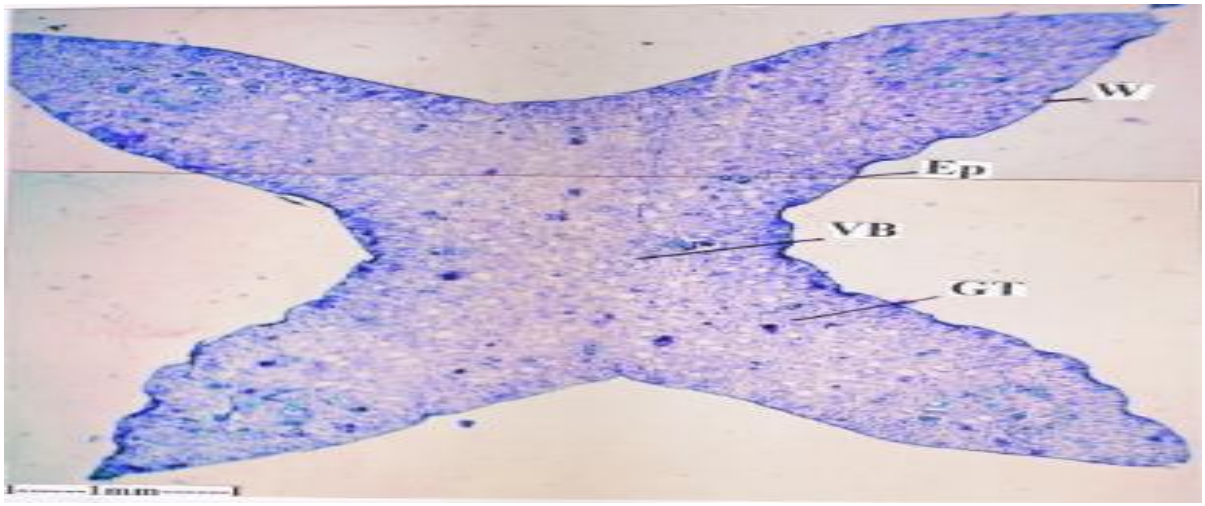

Figure 3.1: Transverse section of stem entire view

Ep: Epidermis; GT: Ground Tissue; VB: Vascular Bundle; W: Wings

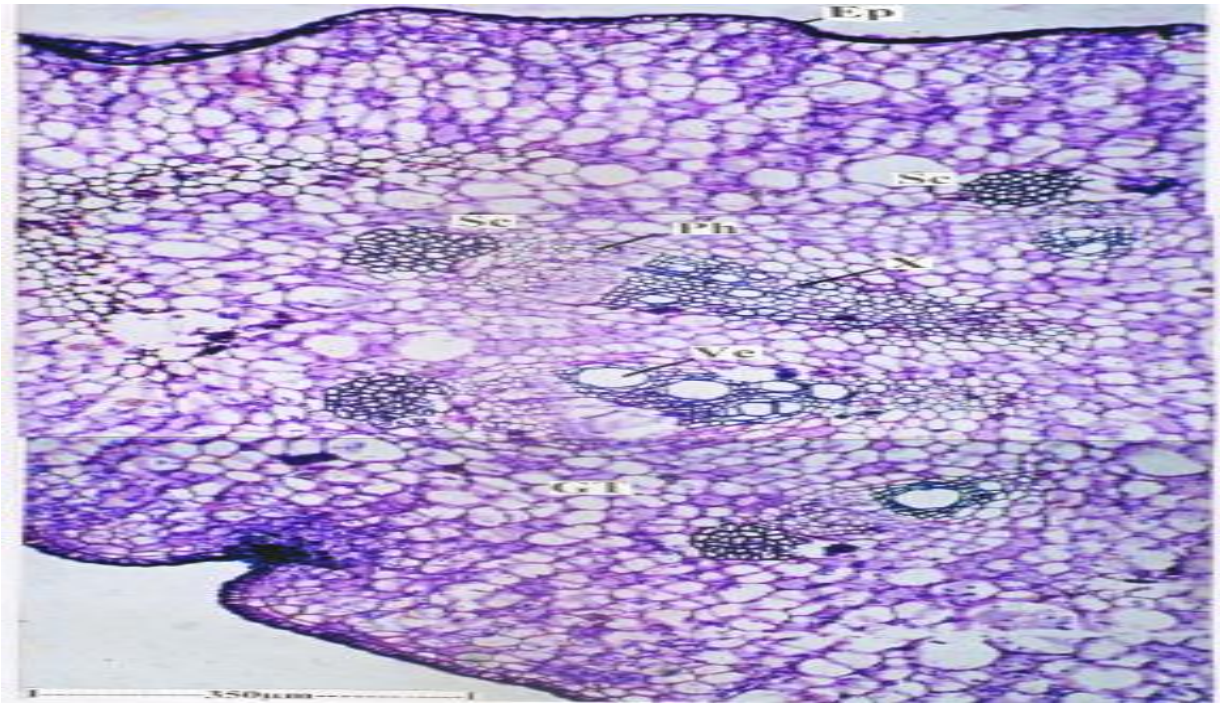

Figure 3.2: Vascular strands of the stem enlarged
Ep: Epidermis; GT: Ground tissue; Sc: Sclerenchyma; Ve: Xylem vessel; Ph: Phloem; X: Xylem

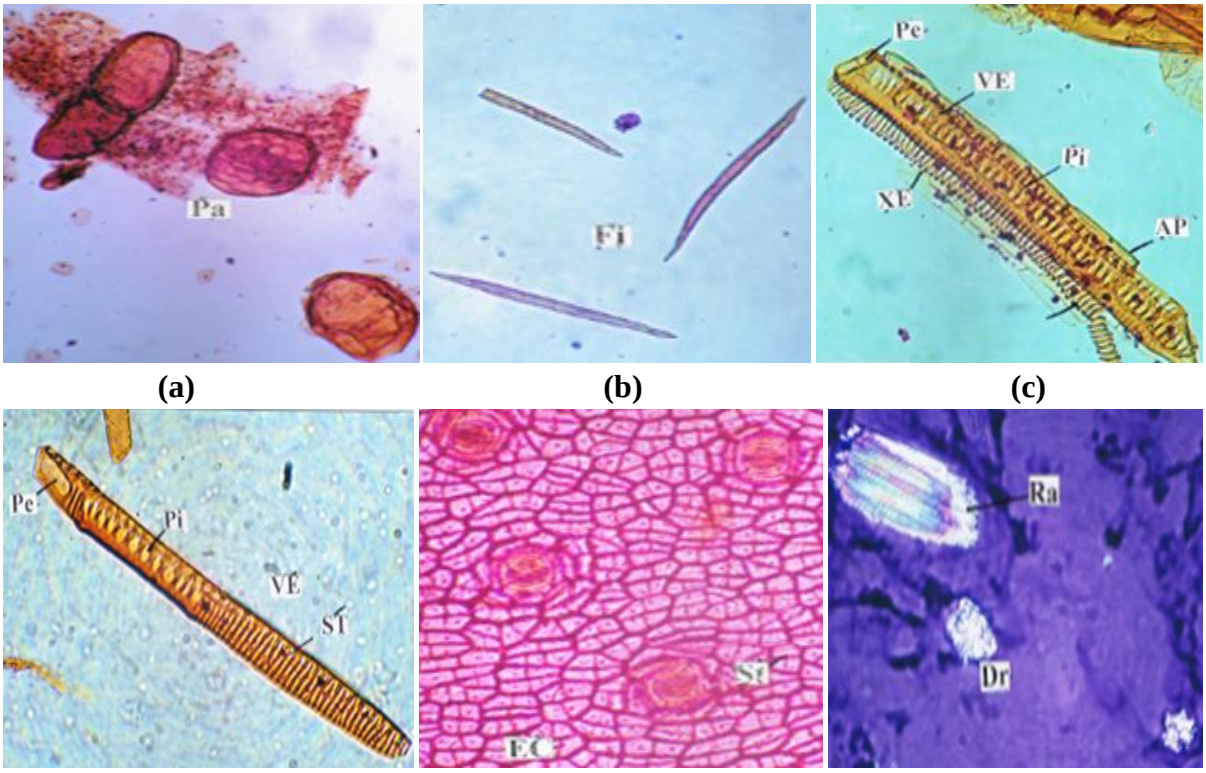

Figure 4: Powder microscopy: (a) parenchyma; (b) Xylem fibres; (c) Spiral Xylem element; (d) Vessel element with sclariform pits; (e) Cyclocytic stomata; (f) Calcium oxalate crystals

Table 1: Physico chemical analysis of the aerial parts of *Cissus quadrangularis*

S. No	Parameters	Values (%w/w)
1.	**Ash values**	
	Total ash	12.51%
	Water soluble ash	8.16%
	Acid insoluble ash	2.5%
2.	**Extractive values**	
	Water soluble extractives	19.18%
	Alcohol soluble extractives	7.91%
	Ethyl acetate soluble extractives	3.09%
3.	**Loss on drying**	3.5%

Table 2: Fluorescence analysis of powder of the aerial parts of *Cissus quadrangularis*

Particulars	White light	UV light
Powder	Whitish yellow	Light green
Powder + aqueous Sodium hydroxide	Light green	Pale brown
Powder + hydrochloric acid	Light brown	Deep brown
Powder + 50% sulphuric Acid	Blackish green	Reddish brown
Powder + 50% Nitric Acid + ammonia	Pale green	Pale brown
Powder + methanol	Fluorescent green	Pale brown
Powder + Iodine	Brown	Black
Powder + 5 % ferric chloride	Pale green	Black
Powder + Acetic acid	Pale green	Pale brown
Powder + NaOH + methanol	Fluorescent green	Yellowish brown

Preliminary Phytochemical Screening

The hydro-alcoholic extract of plant aerial parts shows the presence of carbohydrates, alkaloids, flavonoids, glycosides, saponins, steroids, tannins, triterpenoids.

Thin Layer chromatography (TLC)

To support phytochemical screening, the ethanol extract was subjected to thin layer chromatography. Number of solvent system was tried. The solvent system which shows good resolution was used. The ethanol extract showed seven spot with Rf value 0.16, 0.25, 0.33, 0.41, 0.50, 0.53 and 0.66 using the solvent system Benzene: Methanol: Ammonia (9:0.5:0.5). TLC findings were in agreement with the data of qualitative chemical tests and reported in Figure 5.

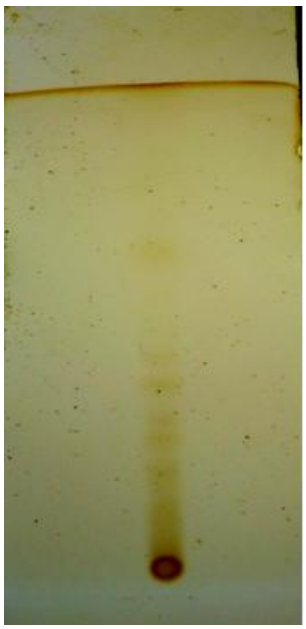

Figure 5: TLC of ethanolic extract of the aerial parts of Cissus quadrangularis

High performance thin layer chromatography

The ethanolic extract was further subjected to HPTLC for the conformation of the active constituents. The ethanolic extract showed nine resolutions of spot with the solvent system Benzene: Methanol: Ammonia (9:0.5:0.5). Out of 9 components, the component with Rf values 0.04, 0.16, 0.30, 0.53 and 0.77 were found to be more predominant as the percentage area was more with 15.06%, 20.55%, 22.04%, 15.08% respectively. The remaining components were found to be very less in quantity as the percent area for all the spots were less than 9.0%. The Rf values were correspondingly showed in the Table 3 and Peak densitogram in Figure 6. Thus the developed chromatogram will be specific with selected solvent system Benzene: Methanol: Ammonia (9:0.5:0.5), Rf value and serve the better tool for standardization of the drug. Characteristic TLC/HPTLC fingerprinting of particular plant species will not only help in the identification and quality control of a particular species but also provide basic information useful for the isolation, characterization and identification of marker compounds of the species.

Table 3: Data densitogram of ethanolic extract of the aerial parts of *Cissus quadrangularis*

Peak	Start Rf	Start height	Max Rf	Max height	Max %	End Rf	End height	Area	Area %
1.	0.03	1.0	0.03	82.8	9.39	0.03	81.2	2306.2	8.51
2.	0.04	81.2	0.06	213.6	24.23	0.09	92.7	4078.2	15.06
3.	0.16	91.8	0.19	103.3	11.72	0.25	58.3	5566.5	20.55
4.	0.26	66.8	0.29	76.3	8.99	0.30	74.6	1674.5	6.18
5.	0.30	75.0	0.32	87.3	9.90	0.43	17.1	5968.5	22.04
6.	0.53	0.3	0.58	140.1	15.90	0.60	124.2	4085.1	15.08
7.	0.60	124.2	0.61	131.2	14.89	0.65	23.9	2235.7	8.25
8.	0.65	24.2	0.66	25.2	2.86	0.69	16.3	558.8	2.06
9.	0.71	16.1	0.73	21.5	2.44	0.76	10.1	612.5	2.26

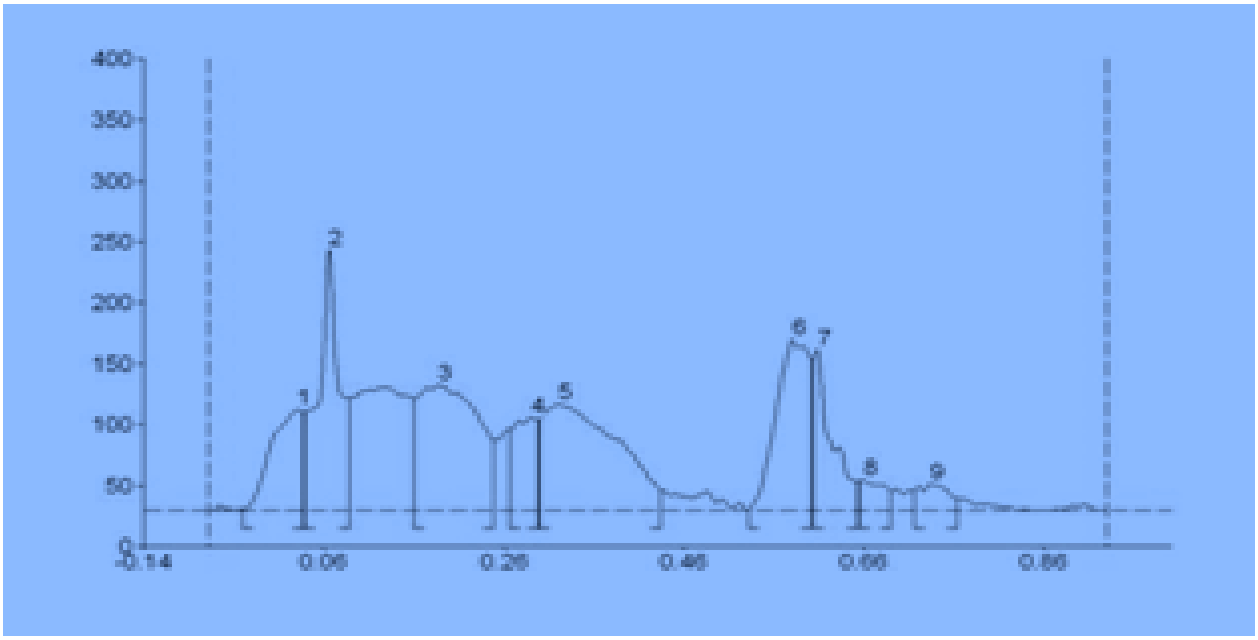

Figure 6: Peak densitogram display of ethanolic extract of the aerial parts *Cissus quadrangularis*

Conclusion

In the present investigations, the pharmacognostical and physicochemical characteristics of *Cissus quadrangularis* Linn. (Aerial parts) were studied. Various parameters established in the present study will help in controlling the standards and quality of the raw material of *Cissus quadrangularis*. Moreover, the plant has been traditionally used for its anti-inflammatory activity. The preliminary phytochemical analysis showed the presence of various phytoconstituents which may contribute to the anti-inflammatory activity of this plant. All the pharmacognostical characters and physico-chemical parameters have been reported for the first time. The present investigation adds to the existing knowledge of *Cissus quadrangularis* Linn. and will be quite useful to pharmaceutical industries for quality control, ensuring batch to batch consistency of raw drug and in the field of medical, pharmacological evaluation and development of a formulation for treating various ailments.

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