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ORIGINAL ARTICLE

Scientific Standardization of Different Stem extracts of *Cissus quadrangularis*

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Abstract

Background: Perennial herb known as *Cissus quadrangularis* is found across the tropical world and has medicinal effects. It is one among the plants that is most regularly used as medicine in India.

Aim: The present study was aimed to study the scientific standardization of different stem extracts of *Cissus quadrangularis.*

Methodology: The determination of the physicochemical constants was done using powdered, shade dried *Cissus quadrangularis* Linn. stem material. Aqueous, chloroform, ethanol, and pet ether were used to create the solvent extracts.

Results: The preliminary phytochemical screening showed presence of carbohydrates, proteins, amino acids, glycosides like saponin and flavonoids, tannins, phenols, alkaloids, and steroids. On physiological evaluation, loss of drying was found to be 6.4% W/W, extractive value of pet ether extract was found to be 3.6% W/W, chloroform extract 4.7% W/W, ethanol extract 7.2% W/W and water 8.9% W/W. The total flavonoid content of pet ether extract was found to be 19.2537±0.0632 µg/mL, chloroform extract 33.7294±0.0574 µg/mL, ethanol extract 59.8601±0.0363 µg/mL, water extract 45.6305±0.0735 µg/mL. The total phenolic content of pet ether extract was found to be 19.867±0.0565 µg/mL, chloroform extract 19.669±0.0654 µg/mL, ethanol extract 404±0.0199 µg/mL and water extract 25.658±0.0343 µg/mL. TLC of pet ether extract showed constituents having retention factor (Rf) values, chloroform extract showed constituents having Rf values 0.55, 0.46; ethanol extract showed constituents having Rf values 0.815 and 0.584; and water extract showed constituents having Rf values 0.234.

Conclusion: In the current study, *Cissus quadrangularis* plants were examined for pharmacognostic characterization, physiochemical parameter measurement, phytochemical screening, and TLC examinations of the crude extracts.

Keywords: Histological examination, Physiological evaluation, Phytochemical screening, *Cissus quadrangularis*

Introduction

Succulent *Cissus quadrangularis L.* is a member of the Vitaceae family and is typically found in tropical and subtropical xeric forests. It is a husky desert vine-like plant that is typically consumed as natural food in India. It is said to have a variety of medical applications. Cell reinforcement, free radical scavenging, antimicrobial activity, bone regeneration, ulceration, pain relief, mitigation, and diuretics are some of the stated medical uses of the plant. The tropical world is home to the

evergreen perennial herb *Cissus quadrangularis*, which has a number of therapeutic uses. In India, it is one of the most widely usedmedicinal plants. The plant is said to be native to West Africa, Malaysia, Java, Sri Lanka, and India.[1] An essential species is *Cissus quadrangularis Linn*.[2]

The mending of broken bones is accelerated by the indigenous medicinal herb *Cissus quadrangularis Linn*., which is primarily grown in India.[3] It is also known as a perennial fleshy quadrangular climber with tendrils that stand out from the stem. This ancient medicinal plant is grown in India's tropical regions as well as those of its neighbors like Pakistan, Bangladesh, Sri Lanka, Malaysia, etc. It can also be grown in plains, coastal regions, shrub jungles, and western terrain at elevations of up to 500 metres.[4]

Materials and Methods

Collection, identification, and authentication of plant material

The plant *Cissus quadrangularis* leaves was collected from surrounding areas of Chennai. It was dried under shade and made into coarse powder. The plant material collected was identified and authenticated by Scientist (Dr) S. Mutheeswaran, MSc, MPhil, PhD, Xavier Research foundation, St Xavier's College, Tamil Nadu, India.

Morphologic and macroscopic features

The fresh leaves of *Cissus quadrangularis* were examined for various macroscopic features like colour, odour and taste of leaves. Other external morphologic characters like surface, base, margin, size, and shape of leaves were also studied. The microscopic examination of *Cissus quadrangularis* was done with the help of microscope. The air-dried plant material was then pulverized into a coarse powder and used for research work. The stomatal number and stomatal index of leaves of *Cissus quadrangularis* was also determined and evaluated by the methods referred from textbook authored by Pulok Mukherjee and Kokate.[5]

Physicochemical constants

The pharmacopoeial standards of the medicine were established using physical constants like ash and extractive values. According to a method outlined in pharmacopoeias and previously reported, the physico-chemical constants of *Cissus quadrangularis* leaf sections were determined for loss of drying, Ash value, and Extractive value.[6,7]

Preparation of extracts

Successive solvent extraction: *Cissus quadrangularis* stem fragments were dried and ground into a coarse powder. Petroleum ether was used to grind and defat one kilogram of fresh plant material. The air dried, powdered, defatted plant material weighed around 50 g. It was extracted afterwards using a Soxhlet device using chloroform, pet ether, ethanol, and water. The marc was air dried each time below 50°C before extraction with the subsequent solvent. After the solvent was allowed to evaporate at room temperature, the extracts were accurately weighed. The air dried medication was used to calculate the extractive value (%).

Preliminary phytochemical screening

Extracts underwent preliminary phytochemical analysis to identify the presence of several secondary metabolites. The methodologies used for phytochemical screening were taken from the textbook written by Pulok Mukherjee and Kokate.[8,9]

Fluorescent analysis

According to the procedures of Chase and Pratt (1949) and Kokoshi *et al* (1958), fluorescent analyses were performed in both day light and UV light. Different solvents were used to treat the plant powders and extracts, and daytime near- and far-UV light were used to observe the fluorescence. A petri dish containing 10 g of powdered medication was treated with various reagents and illuminated with ultraviolet and visible light to see the results (254 nm and 365 nm).[10]

Total phenol content determination

Using gallic acid as the reference, the total phenol content was calculated using the Folin-Ciocalteu test. In the process, 1.5 mL of Folin-reagent Ciocalteu's (FCR) in 1:10 v/v dilution was combined with 0.5 mL of plant extracts.. Five minutes later, 1.5 mL of solution containing 7% sodium carbonate was added. With distilled water, the tubes' ultimate volume was increased to 10 mL, and they were then left to remain at room temperature for 90 minutes. A spectrophotometer was used to test the sample's absorbance at 750 nm in comparison to the blank. The entire experiment was done three times for accuracy, and values stated as phenol content (Gallic acid equivalent, GAE) per g of dry weight were expressed as mean + standard deviation.[11]

Total flavonoid content determination

Using quercetin as a reference, the total flavonoid concentration was calculated using the aluminum

chloride technique. A volumetric flask was filled with 4 mL of water and 1 mL of test sample (10 mL volume). After five minutes, 0.3 mL of 10% Aluminum chloride and 0.3 mL of 5% Sodium nitrite were added. 1 mL of 1 M Sodium hydroxide was added to the reaction mixture after the mixture had been incubated at room temperature for six minutes. With pure water, the final volume was quickly increased to 10 mL. A spectrophotometer was used to test the sample's absorbance at 510 nm in comparison to the blank. The entire experiment was done three times for accuracy, and values were represented in terms of flavonoid content (Quercetin equivalent, QE) per g of dry weight and were expressed as mean standard deviation.[11]

Thin-layer Chromatography (TLC)of extracts

For TLC experiments, one gram of extract was dissolved in methanol, filtered, and used. On aluminum plates that had been coated with silica gel G F254 beforehand, about 6 l of *C. album* extracts were applied using a CAMAG Linomat 4 (Muttenz, Switzerland) TLC applicator. The plate was created in a CAMAG twin trough chamber using a separate solvent system. Using a CAMAG Photo documentation equipment, produced plates were seen and documented in both short and long UV.[12]

Results

Morphology of *Cissus quadrangularis* stems (Figure 1)

The quadrangular-sectioned branches of the perennial, evergreen climber *Cissus quadrangularis* has internodes that are 1.2-1.5 cm (0.5-0.6 in) wide and 8-10 cm (3-4 in) long, growing to a height of 1.5 m (4.9 ft). A leathery edge runs along each angle. The nodes have toothed trilobe leaves that are 2–5 cm (0.8–2.0 in) broad. Each has a tendril coming out of the node's other side; small white, yellowish, or green blooms in racemes with globular berries that become red when ripe.

Cissus quadrangularis is an evergreen climber that expands quickly to reach heights of 5 m (16 ft) by .5 m (1.6 ft). It can withstand zone (UK). It may grow in nutrient-poor soil and is suitable for light (sandy), medium (loamy), and heavy (clay) soils. It favours well-drained soil. It may grow in highly acidic and very alkaline soils and is best in soils with an acidic, neutral, or basic pH. In the shade, it cannot grow. It enjoys moist or dry soil, and it can withstand drought.

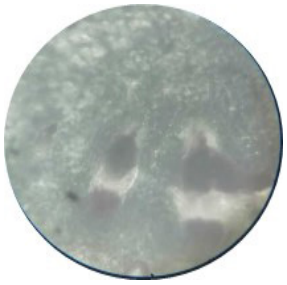
Whole plant

Stems and flowers Leaves

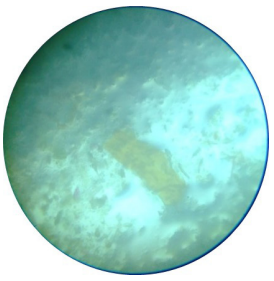
Figure 1: Morphological characters of stems of *Cissus quadrangularis*

Microscopical features of *Cissus quadrangularis* stems

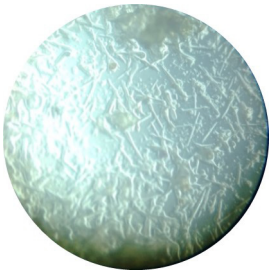
With the aid of a microscope, the microscopic examination of *Cissus quadrangularis* stems was completed. Diagrammatic transverse section (TS) of the stem is given in Figure 2.



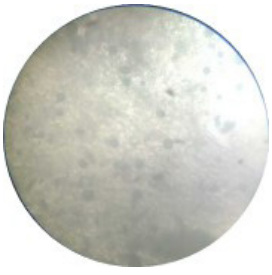
a) TS *of Cissus quadrangularis* stem



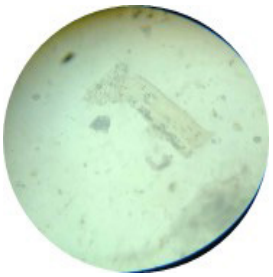
b) Vascular bundles



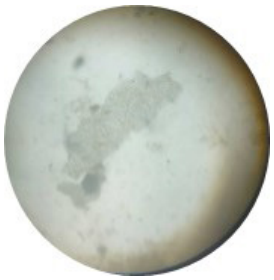
c) Acicular crystals



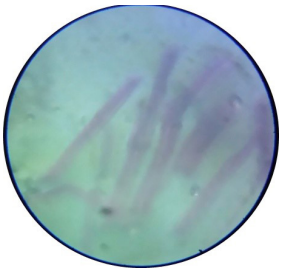
d) Calcium oxalate crystals



e) Vascular strands



f) Mucilage cells



g) Fibres

h) Spiral and annular vessel

Figure 2(a-h): Powder microscopy of stems of *Cissus quadrangularis*

Determination of % yield of various extracts

The appearance and yield of different extracts are mentioned in Table 1.

Table 1: Yield of extracts obtained from successive extraction of stems of *Cissus quadrangularis*

Plant name	Type of Extract	Appearance/ State	Yield (% w/w)
***Cissus quadrangularis* stem**	Pet ether	Yellowish green/ Semisolid	4.5%
	Chloroform	Greenish black/ Semisolid	5.2%
	Ethanol	Darkgreen, black/ Semisolid	6.5%
	Water	DarkBrown black/ Semisolid	9.3%

Preliminary phytochemical screening of extracts

Table 2: Preliminary phytochemical screening of leaf extracts of *Cissus quadrangularis*

Chemical tests	*Cissus quadrangularis* stem extracts			
	Pet ether	Chloroform	Ethanol	Water
Proteins & Amino acid	-	-	+	+
Carbohydrates	-	-	+	+
Steroids	+	+	-	-
Phenols	-	-	+	+
Saponins	-	-	+	+
Flavonoids	-	-	+	+
Alkaloids	-	-	+	+
Tannins	-	-	+	+

Fluorescent analysis

The selected plant was made into course powder and treated with required chemical reagents and observed under visible and ultraviolet rays. The results are given in Table 3 and 4.

Table 3: Fluorescence analysis of powder of leaves of *Cissus quadrangularis*

Sl. No.	Treatment	Day Light	Short UV (254 Nm)	Long UV (366 Nm)
1.	Powder	Yellowish buff	Yellowish buff	Yellowish buff
2.	Powder + Water	Yellowish buff	Yellowish buff	Yellowish buff
3.	Powder + 1NHCl	Yellowish buff	Yellowish buff	Yellowish buff
4.	Powder + $1NH_2SO_4$	Green	Green	Dark green
5.	Powder + 1NHNO3	Brown	Green	Green
6.	Powder + Acetic acid	Brown	Green	Green
7.	Powder + 1NnaOH	Green	Green	Dark green
8.	Powder + 1N Alc.NaOH	Green	Green	Yellowish green
9.	Powder + 1NKOH	Green	Green	Dark green
10.	Powder +1N Alc.KOH	Green	Green	Yellowish Green
11.	Powder + Ammonia	Yellowish green	Green	Dark green
12.	Powder + Iodine	Yellowish brown	Dark green	Dark green
13.	Powder + Fecl3	Yellowish brown	Dark green	Dark green
14.	Powder + Ethanol	Green	Green	Yellowish green

Table 4: Fluorescence analysis of extracts of *Cissus quadrangularis*

Sl. No	Extracts	Daylight	Uvlight	
			Short 254 Nm	Long 365 Nm
1	Pet.ether	Brown	Brown	Yellowish
2	Chloroform	Greenish black	Dark green	Reddish brown
3	Ethanol	Greenish black	Greenish black	Reddish brown
4	Water	Brownish dark	Green	Greenish black

Total phenolic content and Flavanoid content

The total phenolic and flavonoid content for aqueous, ethanol, chloroform, and petroleum ether extracts of *Cissus quadrangularis* were estimated and results are given in Table 5.

TLC studies of extracts

To determine the type of phytochemicals present, the extracts underwent TLC profiling. For each extract and fraction, a variety of developing solvent systems were tested. The solvent system that provided the best resolution was deemed to be optimal, legitimate, and useful. In the mobile phase, which is described in Table 6, a good resolution was attained. Photo documentation as depicted in Figure 3 demonstrates this. Additionally, the Rf values of various extracts were determined (Table 7).

Table 5: Total phenolic content and total flavonoid content of stem extracts of *Cissus quadrangularis*

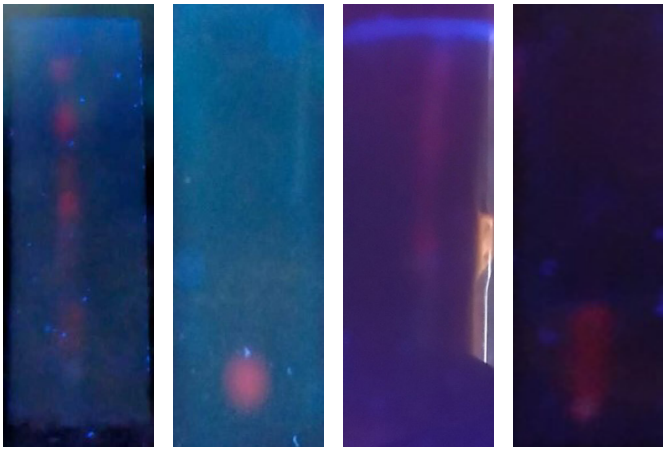
***Cissus quadrangularis* stem extract**	**Total phenolic content**	**Total flavonoid content**
Pet ether	19.867±0.0565 µg/mL	19.2537±0.0632 µg/mL
Chloroform	33.7294±0.0574 µg/mL	33.7294±0.0574 µg/mL
Ethanol	59.8601±0.0363 µg/mL	59.8601±0.0363 µg/mL
Water	25.658±0.0343 µg/mL	45.6305±0.0735 µg/mL

Table 6: Mobile phase for TLC studies of extracts of *Cissus quadrangularis* stem

Test extract	Solvent system	Number of Bands
Ethanol	Benzene:Methanol:Ammonia	01
Chloroform	Benzene:Methanol:Ammonia	01
Pet ether	Benzene:Methanol:Ammonia	04
Water	Chloroform:methanol (95:5)	01

Table 7: The Rf values of different extracts of *Cissus quadrangularis* stem

***Cissus quadrangularis* stem extracts**	**Rf values**
Ethanol	0.815
	0.584
	0.55
	0.46
Water	0.234
Pet ether	0.287
Chloroform	0.55
	0.46



A B C D

Figure 3: TLC photo documentation of stems of *Cissus quadrangularis*

A) Ethanol extract B) Pet ether extract
C) Water extract D) Chloroform extract

Discussion

Microscopy of stems of *Cissus quadrangularis* was four-angled. As it matures, each angle goes deep inside to form a sharp, pointed projection that shows single-layer epidermis followed by hypodermis; narrow cortex and centrally located large pith occupying nearly two-thirds of the section; and band of numerous, small, discontinuous vascular bundles surrounds it. In a detailed section, the epidermis was shown to be rectangular-pentagonal, 1-2 layered, covered by a thin cuticle, followed by a 3-4 layered, circular-polygonal, chlorenchymatous hypodermis that is deposited more near the angle. The cortex was shown to be very narrow, with a cortical parenchymatous, 5-7 layered, pith which was very large, and parenchymatous similar to that. The physiochemical constants of total ash and acid insoluble ash value was found to be 15.12% w/w and 7.46% w/w respectively. The loss of drying of leaves of *Cissus quadrangularis* was found to be 6.1% w/w. Preliminary phytochemical screening of petroleum ether and chloroform extract revealed the presence of steroids, whereas ethanol and aqueous extracts indicated the presence of flavonoids, carbohydrates, saponins, proteins, alkaloids, phenols, steroids, and tannins respectively. TLC of pet ether extract showed constituents having Rf values , chloroform extract showed constituents having Rf values 0.55, 0.46; ethanol extract showed constituents having Rf values 0.815 and 0.584, and water extract showed constituents having Rf values 0.234.

Conclusion

In the current study, *Cissus quadrangularis* plants were examined for pharmacognostic characterization, physiochemical parameter measurement, phytochemical screening, and TLC examinations of the crude extracts. The chosen plants were verified, and in order to prove their authenticity and purity, macroscopic studies were carried out. According to Ayurveda and the Indian Pharmacopoeia (I.P., 1996), physical-chemical analyses such as ash value, acid insoluble ash value, and extractive value were performed. Additionally, fluorescence analysis was performed using various solvents. The significant phytoconstituents, which are well known for their therapeutic potentials and were further identified by TLC, were present as shown by phytochemical screening.

Conflict of interest

None

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