



Received on 26 March, 2012; received in revised form 23 April, 2012; accepted 19 June, 2012

PHARMACOGNOSTIC EVALUATION OF *CISSUS QUADRANGULARIS* L. STEM

R. Anitha* and P. Suji

Department of Plant Biology and Plant Biotechnology, Ethiraj College for Women, Ethiraj salai, Egmore, Chennai-600 008, Tamil Nadu, India

ABSTRACT

Keywords:

Cissus quadrangularis,
stephanocytic stomata,
Raphides,
calcium oxalate,
Fiber sclerides

Correspondence to Author:

Dr. R. Anitha

Assistant Professor, Department of Plant
Biology and Plant Biotechnology, Ethiraj
College for Women, Ethiraj salai, Egmore,
Chennai-600 008, Tamil Nadu, India

Cissus quadrangularis L. (Vitaceae) is a common perennial climber also called Asthisamhara meaning bone setter is prescribed both in Ayurveda as well as Unani systems for its medicinal use. Pharmacognostic evaluation of *Cissus quadrangularis* stem revealed a stomatal index of 151 with Stephanocytic stomata in the epidermal peeling. The anatomical section of the stem showed deposits of characteristic acicular or needle shaped calcium oxalate crystals, raphides and intrafascicular cambium. The histochemical studies showed the presence of alkaloids, tannins, phenols and suberin. The phytochemical analysis of aqueous, petroleum ether and dichloromethane extracts were positive for alkaloids, phenol, steroids, flavanoids in all the extracts. The presence of sclerides, fiber sclerides epidermal cells, phloem with companion cells, ray fibers with pits, raphides, acicular and sand crystals of calcium oxalate were evident in powder analysis. Under 365nm UV green fluorescent emission was observed in acetic acid and methanol treated stem powder.

INTRODUCTION: Plants are known to be the source of many chemical compounds. Medicinal plants were used by people of ancient cultures without knowledge of their active ingredients. The common practice of taking crude extract orally is laden with hazards as the extracts may contain some toxic constituents¹.

Plants produce a diverse range of bioactive molecules making them a rich source of different types of medicines. Higher plants as sources of medicinal compounds have continued to play a dominant role in the maintenance of human health care since ancient times.

Over 50% of all modern clinical drugs are of natural origin and natural products play a vital role in modern drug development in the pharmaceutical industry².

Cissus quadrangularis L. (Vitaceae) is a common perennial climber, which is distributed throughout India particularly in tropical regions. It is one of the most frequently used indigenous medicinal plants in India. It is commonly known as Hadjod, Asthisamhara, Harjora, Vedhari, Hadbhanga, Vajravalli in Sanskrit, Kandvel in Marathi, Haddjor in Punjabi, Hadbhanga in Oriya, Vedhari in Gujrati, Perandai in Tamil, Nalleru in Telugu and Veldgrap in Indian languages and in English it is called as Edible-stemmed Vine. It is also known as *Vitis quadrangularis*.

It is prescribed both in Ayurveda as well as Unani systems for its medicinal use as an alternative, anthelmintic, anti-inflammatory, dyspeptic, digestive, tonic, analgesic in eye and ear diseases, and in the treatment of irregular menstruation. It is very useful in asthma, fractures and back pain.

The plant extracts also exhibit cardiotoxic property³. The plant is reported to have antibacterial and antioxidant activities. The whole plant is used in oral re-hydration, while the leaf, stem, and root extracts of this plant are important in the management of various ailments. The antiosteoporotic activity of *C. quadrangularis* maybe justifiably is attributed to the steroids present which probably act as phytoestrogens to effectively prevent or reduce bone loss⁴.

In addition, ulcer protection was confirmed by histo-architecture, which was comprised of reduced size of ulcer crater and restoration of mucosal epithelium⁵ produced significant immunomodulatory effect, antioxidant activity along with the ability to modulate, synthesis and upregulation of the growth factors⁶. Besides these effects, *C. quadrangularis* also possesses analgesic effect, which can be very useful in painful hemorrhoid.

The aqueous extract of *C. quadrangularis* was reported to have anticonvulsant and sedative property⁷. Some other reports on justifies its effectiveness in management of obesity and complications associated with metabolic disorders⁸ its antioxidant and free radical scavenging activity "in vitro"^{9, 10}. In current scenario formulations now contain extracts of *C. quadrangularis* in combination with other active ingredients, used for the purpose of management of overweight and obesity, as well as complications resulting from these conditions, specifically metabolic syndrome (syndrome X).

A key obstacles which has hindered the acceptance of the alternative medicines in the developing countries, is the lack of documentation and stringent quality control. There is a need to standardize and pharmacognostic studies will help in maintaining quality and reduced toxicity. Hence, Pharmacognostic studied on *Cissus quadrangularis* stem is reported in this article.

MATERIALS AND METHODS:

Microscopic Characters: For microscopic studies, the leaves were cut and removed from the plant and fixed in FAA (formalin 5ml + acetic acid 5ml + 7.0% ethanol 90ml). After 24 hours of fixation, the epidermal peels and transverse sections of leaf was taken by free hand.

The section were stained in safrain (1%) and mounted in glycerol.

Quantitative Microscopy: The total number of stomata was calculated by stomatal index = No. of stomata x 100/ total no. of epidermal. The type of the stomata was recorded in the epidermal peeling. The quantitative microscopy was studies as per the procedure given by^{11, 12}.

Organoleptic characters: Organoleptic characterization of dried leaf of *Cissus quadrangularis* was carried out. The texture of the leaf, smell, color, taste was observed.

Anatomical Study: Free hand section of Stem of *Cissus quadrangularis* were taken, stained with Safranin and mounted in glycerol and observed under light microscope and photographed at 40x.

Maceration: The stems of *Cissus quadrangularis* were cut into small piece, boiled repeatedly in water to expel air, till the pieces settled down. Treated pieces of the plants was soaked in jeffery's fluid (equal volume of 10% of nitric acid and 10 % chromic acid) for 24 hours at 30-40c, decanted washed and then stored in 50% alcohol.

Pieces of macerated stem treated with aqueous safranin overnight, dehydrated through alcohol series (50%, 60%, 70%, 80%, 90%,100%) for five minutes and passed through alcohol : xylol (1:1 ration) Series for five minutes. Then each material was macerated and observed.

Histochemical Test: The plant section were treated with various reagent such as Wagners reagent (Potassium iodide and Iodine) for detection of alkaloid, Toludine blue 0 for lignin, ferric chloride in IN Hydrochloric acid for Tannin, Sulphuric acid for calcium oxalate Crystals , methylene blue for phenols.

Phytochemical Screening: The stem of *Cissus quadrangularis* was washed thoroughly, blotted dry and completely air dried. The dried powder was extracted with aqueous, petroleum ether and Dichloromethane. Chemical tests for various extracts were carried out according to the standard procedures described by^{13, 14}.

Powder Analysis: The dried stem was powdered and sieved to obtain coarse powder. The powder thus obtained was placed on to a clean slide and observed under microscope.

Fluorescence Analysis: The dried stem powder was placed on a slide and treating with several drops of specified reagent like Hydrochloric acid, Sodium hydroxide, Nitric acid, Sulphuric acid, Ferric chloride, Iodine Acetic acid, HNO_3 + Ammonia, Methanol, NaOH + Methanol. The slides were observed under UV 265 nm and 365 nm and the emitted fluorescence was observed that helps in identifying the drug in powdered sample. Fluorescence analysis has been carried out according to the method of Kokoshi *et al*¹⁵.

Physical parameters: Determination of total ash, acid Insoluble ash, water soluble ash and moisture content was done according to Indian Pharmacopoeia^{16,17}

RESULTS AND DISCUSSION:

Quantitative microscopy: The total number of stomata (Fig. 1) was counted. *Cissus quadrangularis* had 151 as stomatal index, number of stomata 22 and Stephanocytic type of stomata was recorded.

Organoleptic character: *Cissus quadrangularis* showed dull green appearance, fibrous, less aromatic, taste less, mucilaginous.

Anatomical character: Transverse section of stem showed single layered epidermis, 6-7 layers of parenchymatous cells. 2-3 Layers of chlorenchyma cells, vascular bundles present at 4 edges. Spherical mass of collenchymas cells of 6-7 layers were present. 4-5 rows of sclerenchyma cells were seen. 4 rows of phloem cells were present around the xylem vessels. Vessels were present. 4 rows of interfascicular cambium cells were present, which gives rise to anomalous growth. Exarch vascular bundle with large central pith was evident.

Maceration: The macerated stem of the plant showed various structures. In *cissus quadrangularis*, bunch of fibres, stomata, vessels were commonly observed (Fig. 1)

Histochemical studies: The histochemical studies showed the presence of alkaloids, tannins, lignin, suberin, phenols (Fig. 1).

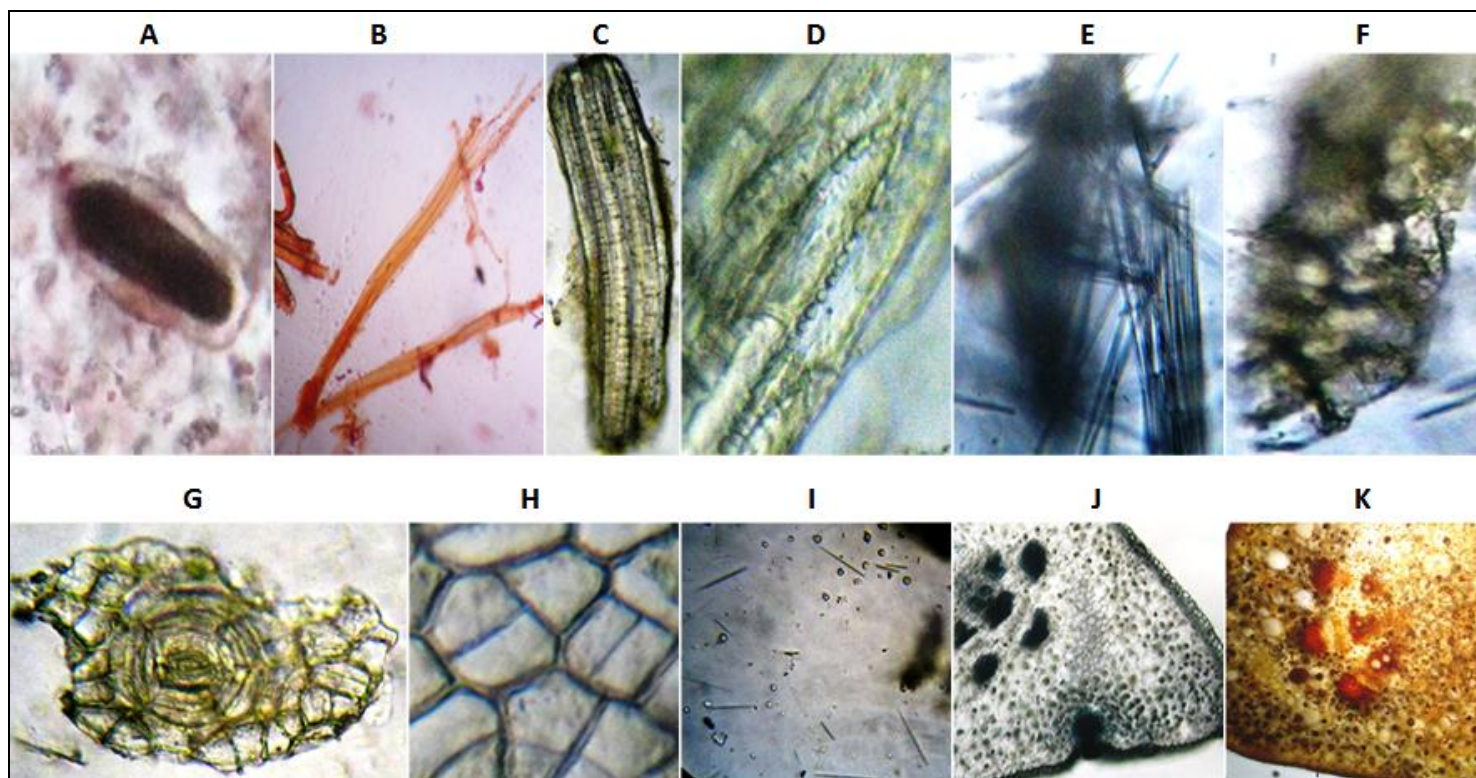


FIGURE 1: A - Raphide in cortical tissue; B- Fibers; C- Fiber scleride; D- Rays with pits; E- Raphides of calcium oxalate crystal; F- Macroscleride; G- Stephanocytic stomata; H- Epidermal cells; I- Acicular and sand crystals (C, D, E, F, G, H, I- Powder analysis) J and K- Histochemical test for Tannin and Alkaloid.

Phytochemical Screening: In *Cissus quadrangularis* alkaloids, phenol, steroids, flavonoids were present and other phytochemicals were absent. The presence of phytosterol, flavonoids, tannin, gum, mucilage, saponin and phenolic compounds were reported, however in the present study, since the material chosen for pharmacognostic analysis was a young stem some of the reported phytochemical were absent, however flavonoids, steroids, alkaloids, mucilage, phenol were present in the young stem as well. The active constituents like phytosterol and flavonoids were responsible for pharmacognostic activity (**Table 1**).

TABLE 1: PHYTOCHEMICAL ANALYSIS OF C. QUADRANGULARIS STEM

Test	Aqueous Extract	Petroleum ether Extract	Dichloromethane Extract
Wagner's (Alkaloids)	+	+	+
Tannin (FeCl ₂)	-	-	-
Flavonoids	-	+	+
Anthroquinone	-	-	-
Saponins	-	-	-
Phenol	+	-	-
Terpenoids	-	-	-
Gum	-	-	-
Resins	-	-	-
Phlabotannins	-	-	-
Steroids	-	+	+
Glycosides	-	-	-
Protein	-	-	-
Carbohydrate	-	-	-

The presence of flavonoids, saponin, phytosterol, saponin and flavonoids in ethyl acetate was reported. In the presence study, flavonoids were present in petroleum ether extract.

Powder Analysis: *Cissus quadrangularis* stem powder showed different types of calcium oxalate crystals such as raphides, acicular and sand crystals. Phloem with companion cell, xylem vessels, fibersclerids, rays with pits, macroscleride, Stephanocytic stomata and epidermal cells were present (**Fig. 1**).

Fluorescence analysis and Qualitative parameters:

Under white light, Hydrochloric acid, Sodium hydroxide, Nitric acid, Sulphuric acid, Ferric chloride, Iodine, Acetic acid, Nitric acid + Ammonia, Methanol, Sodium hydroxide + Methanol showed pale green, brown, light green, fluorescent green, blackish green

and yellowish red respectively. Under 265nm UV, reddish brown in sulphuric acid, yellowish brown in sodium hydroxide + methanol was observed. Under 365nm UV fluorescent green emission was observed in acetic acid and methanol (**Table 2**).

TABLE 2: FLOURESCENCE ANALYSIS OF C. QUADRANGULARIS STEM POWDER

Reagent	UV Light		
	White light	265nm	365nm
FeCl ₂	Pale Green	Black	Pale Green
Iodine	Brown	Black	Black
NaOH	Light Green	Pale Brown	Pale Green
HCl	Light Brown	Deep Brown	Pale Green
Acetic Acid	Pale Green	Pale Brown	Fluorescent green
HNO ₃ + Ammonia	Pale Green	Pale Brown	Pale Green
Methanol	Fluorescent Green	Pale Brown	Fluorescent Green
H ₂ SO ₄	Blackish Green	Reddish Brown	Blackish Green
NaOH + Methanol	Fluorescent Green	Yellowish brown	Pale green
Nitric Acid	Yellowish Red	Reddish brown	Pale Green

The moisture percentage of *Cissis quadrangularis* powder was 4%, the acid in soluble ash and water soluble ash were 53% and 51.06% respectively. The total ash content was 62% (**Table 3**).

TABLE 3: PHYSICAL PARAMETERS OF C. QUADRANGULARIS POWDER

Physical parameters	Percentage
Moisture	4%
Water soluble ash	51.06%
Acid insoluble ash	53%
Total ash	62%

According to WHO 80 % of the population in developing countries are dependent on herbal medicine for health care programs. Standardization and purification of these herbal drugs become inevitable.

Documentation of such herbal plants is vital and an initial step towards authentication of the drug. The present pharmacognostic studies on *Trigonella foenum graecum* will be of great importance in standardization of herbal preparations.

ACKNOWLEDGEMENT: The authors wish to thank the Head of the Department and The Principal, Ethiraj College for Women, for their constant support and encouragement.

REFERENCES:

1. Lown JW: Discovery and Development of Anthracycline, Antitumor, Antibiotic. Royal Society of Chemistry 1993:165
2. Baker JT, Barris RP and Carte B: Natural product drug discovery: New perspective on International collaboration, J Nat Prod 1995; 58: 1325 – 1357.
3. Rostogi RP and Mehrotra BN: *Compendium of Indian Medicinal Plants* Vol. 3, CDRI Lucknow and PID, New Delhi. 1995; 173-174.
4. Shirwaikar A, Khan S, Malini S: Antiosteoporotic effect of ethanol extract of Linn. on variectomized rat. Journal of Ethnopharmacology 2003; (89): 245– 250.
5. Jainu M, Srinivasulu C, and Devi S: Gastroprotective action of extract against NSAID induced gastric ulcer: Role of proinflammatory cytokines and oxidative damage, Chemo-Biological Interactions 2006; 161: 262–270.
6. Jainu M, Mohan KV: Protective role of ascorbic acid isolated from on NSAID induced toxicity through immunomodulating response and growth factors expression, International Immunopharmacology 2008;8:1721–1727.
7. Bum EN, Ngoupaye GT, Talla E, Dimo T, Nkantchoua GCN: The anticonvulsant and sedative properties of stems of in mice. African Journal of Pharmacy and Pharmacology 2008;2: 042-047.
8. Oben J, Kuate D, Agbor G, Momo C, Talla X: The use of a formulation in the management of weight loss and metabolic syndrome. *Lipids in Health and Disease*. 2006; 5: 24.
9. Mallika J, Shyamala CSD: *In vitro* and *In vivo* evaluation of free radical scavenging potential of *Cissus quadrangularis*. *Afri J of Biomed Res* 2005; 8: 95-99.
10. Mehta M, Kaur N, Bhutani K: Determination of marker constituents from Linn and their quantification by HPTLC and HPLC. *Phytochem Anal* 2001; 12:91-105.
11. Wallis TE: *Textbook of Pharmacognosy*, 5th ed, CBS Publishers and Distributors, NewDelhi, India 1958; VI: 139-140.
12. Lala PK: *Practical Pharmacognosy*, 1st ed, Vallabh Prakashan, New Delhi 1981; 86-95.
13. Kokate CK: *Practical Pharmacognosy*, 1st ed, Vallabh Prakashan, New Delhi 1986b, 1:15.
14. Harborne JB: *Methods of extraction and isolation*. In: *Phytochemical methods*, 3rd Ed, Chapman and Hall, London 1998;60-66.
15. Kokoshi CJ, Kokoshi RJ and Sharma FT: Fluorescence of powdered vegetable drugs under Ultraviolet radiation. *J. Pharm. Asses*. 1958; 47: 715-717.
16. *Indian Pharmacopoeia*: 3rd Edn., Vol. 2, Controller of Publication, Govt. of India, New Delhi, 1985; A88-A90.
17. *Indian Pharmacopoeia*: Vol.-II 4th Edition, Controller of Publications, Government of India, New Delhi, 1996; A-47.

How to cite this article:

Anitha R. and Suji P.: Pharmacognostic Evaluation of *Cissus quadrangularis* L. Stem.. *Int J Pharm Sci Res*, 2012; Vol. 3(7): 2296-2300.
