ANATOMICAL STANDARDIZATION OF *CISSUS QUADRANGULARIS* L. STEM

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Abstracts
*The whole plant of **Cissus Quadrangularis L.** is used for urinary schistosomiasis and it is antibacterial and antioxidant, analgesic anti-inflammatory, anti-malarial, gastro-protective and anti-ostroporic. Climbing shrubs, perennial, stems or branches thick, quadrangular, the angles of the young branches winged, tendrils long, opposite to leaf, slender, simple. Leaves, broadly ovate or reniform. Flowers in shortly peduncled cymes with spreading umbellate branches. Berry obovoid or globose, scarcely 6mm long, apiculate, red when ripe, 1 or (very rarely 2) seeded, obovoid. The transverse section of the stem showed a quadrangular outline, interrupted by sunken stomata. The outer cortex is made up of 7-15-layered, thin-walled, parenchymatous cells, filled with druses of calcium oxalate. Some cells give rise as idioblasts of rhaphaide crystals. The inner cortex is multilayered with 10-15 layers with patches of sclerenchymatous tissue. Medullary rays 3-4 cells wide or multiseriate, storied with idioblasts, tannin or phenolic substances and druses. Pith very large, parenchymatous, filled with idioblasts. Stem maceration shows thick-walled parenchyma pits, large, random, paired, opposite or alternate, horizontally oval. Boarded vessels have pits horizontally broader, perforations plates simple, beaks having 4-15 bars, squared and horizontal. Annular vessels have perforations simple, circular, or oblique, and beaks very short or absent. The measured dimensions are 150 x 15 – 730 x 30 µm. Tracheids showing ends blunt or sharply pointed, slightly forked, pits minute. Bordered tracheids pits bordered. Fibers tapering at the ends and sharply pointed, minute pits; the internal lumen is wider than tracheids.*
Keywords: *Stem, Maceration, Anatomy, Standardization*

Introduction:

Literature Review:

Emeje *et al;* (2010) elucidated the use of gum from the stem of *Cissus refescens* L. in intestinal drug delivery. Swamy *et al;* (2010) evaluated the hepatoprotective activity of *C. quadrangularis* L. stem extracts against isoniazid-induced liver damage in rats. Mate *et al;* (2010) evaluated the anti-nociceptive activity of *C. quadrangularis* L. on albino mice. Swamy *et al;* (2006) studied the neuropharmacological effects of methanol root extract of *C. quadrangularis* L. in mice. Mishra *et al;* (2010), undertook studies on pharmacological and therapeutic uses of *C. quadrangularis* L. and showed its potential in regulating gastroprotective activity in conjunction with NSAID therapy and in Lipid metabolism and oxidative stress. Potu *et al;* (2010) studied the anti-osteoporotic activity of the petroleum ether extract of *C. quadrangularis* L. in ovariectomized Wistar Rats. The extract significantly increased the thickness of both cortical ($p < 0.001$) and trabecular bone ($p < 0.001$). Deka *et al;* (1994) studied the effect of *C. quadrangularis* L. in accelerating the healing process of experimentally fractured radius-ulna of the dog and revealed faster initiation of the healing process. Balasubramaniana *et al;* (2010) studied *in vitro* antiviral activity of purified methanol extract of *C. quadrangularis* L. against HSV1 and HSV2, and Vero cells, which were found to be highly sensitive against the partially purified compound. Its phytochemical analysis showed the presence of Steroids and Terpenoids.

Parts Used: Stems.

Chemical Constituents: Quadrangularins A-C, δ-amyrin, δ-amyrone, resveratrol, piceatannol, pallidol, parthenocissine A. The plant contains hytogenic steroid, ketosteroids, sitosterol, alphaamyrin, alpha-ampyrone and tetracyclic triterpenoids. Phytogenic steroids showed bone healing properties. Coloside-A possesses a smooth muscle relaxant effect. The total alcoholic extract of the plant neutralizes the anti-anabolic effect of the cortisone in the healing of fractures. The aqueous extract of the plant hastens fracture-healing by reducing the total convalescent period by 33% in experimental rats and dogs; it aids in the recovery of the strength of the bones up to 90% in 6 weeks (Ling et al; 2009).

Uses: Whole plant is used for urinary schistosomiasis and it is antibacterial and antioxidant, analgesic anti-inflammatory, anti-malarial, gastro-protective and anti-ostroporic (Ling et al; 2009). A plaster of stem is applied to a bone fracture as a plaster. Decoction of the stem is given for swelling. Juice of stem is prescribed in scurvy and ear complaints, dysentery, swelling (Raval et al; 2010). The anabolic and steroidal principles of the aerial part showed a marked influence on the rate of fracture-healing. The drug exerts influence on both the organic and mineral phases of fracture-healing. Stem alterative in scurvy, the plant is rich in vitamin C and irregular menstruation (Khare, 2007).

Material and Methods:

Anatomy:

The plant materials were separately collected in sterile polyethylene bags and immediately brought to the laboratory. For anatomical studies, free-hand transverse sections of stem were taken with the help of razor blades. These sections were dehydrated with alcohol and stained with safranine and light green (Khandelwal, 2005). The slides were observed under a trinocular microscope (Cutler, 1978). Measurements were recorded by using an ocular micrometer.

Maceration:

Maceration involves the separation of tissue cells by disintegration of the middle lamella (Esau 2006) into vascular elements such as vessels, tracheids, xylem fibers, xylem parenchyma and etc. For this purpose, fresh, preserved, or dried stems, roots, leaves or bark were macerated in Jeffrey's maceration fluid (Khandelwal, 2005) The types of vascular elements were observed under a microscope, and measurements were taken.

Research Work:

Enumeration:

Family: Vitaceae

***Cissus quadrangularis* L.** Mant. Pl. 39. 1767. Naik, Fl. Marathwada 1:221.1998; Kulkarni in Singh et.al. Fl. Maharashtra St. Dicot.. 1: 557. 2000. *Vitis quadrangular* Wall. ex Wight & Arn. Prodr. 125. 1834; Laws. in Hook.f. Fl. Brit. India 1: 645. 1875; Cooke, Fl. Pers. Bombay 1: 266. 1958 (Repr.).

Climbing shrubs, perennial, stems or branches thick, leafless when old, very long fleshy, glabrous, much contracted at the nodes, quadrangular, the angles of the young branches winged; tendrils long opposite to leaf, slender, simple. Leaves 2.5-5x3-6 cm long, broadly ovate or reniform, sometimes 3-7 lobed, denticulate, glabrous, cordate, rounded, truncate or cuneate at the base; petiole 6-12 mm long; stipules small, broadly ovate, obtuse. Flowers in shortly peduncled cymes with spreading umbellate branches, greenish white, Calyx cup-shaped, truncate or very obscurely lobed. Petals 4, ovate-oblong, acute 3 mm long, hooded at the apex. Disc erect, 4-lobed. Style short. Berry obovoid or globoes, scarcely 6mm long, apiculate, red when ripe, 1 or (very rarely 2) seeded, obovoid.

Fls. and Frts: January-June.

Distrib: Common in deciduous forest, sometimes cultivated in gardens Aurangabad.

Exsiccata: *Milind College Botanical Garden*, Aurangabad. *APB* 115.

T.S. of Stem:

The transverse section of stem showed quadrangular outline, cuticle thick and continuously covers the epidermis. Epidermis is bi-layered, interrupted by sunken stomata, cell wall thick on outer side and thin on inner side, sometimes highly cutinized, outer layer chlorenchymatoums, cells vertically rectangular, inner layer horizontally rectangular, measured dimensions are 37.5x20-25x22.5µm. Outer cortex 7-15 layered, cells thin-walled, parenchymatous, hexagonal, rhomboidal, 62.5x50-30x25µm, some cells filled with druses of calcium oxalate, some cells give rise as idioblasts of rhaphide crystals 112.5x62.5-87.5x50µm. Inner cortex 10-15 layered with patches of sclerenchymatous cells at each corners, cell wall thickened at corners, aster-shaped, lumen circular, hexagonal to polygonal, dia. 32.5-12.5µm. Perivascular fibers are discontinuous in this zone, or in circular patches of sclerenchymatous tissue, cells thick-walled, triangular to pentagonal, lumen circular to oval, measured dimensions are 25x15-17.5x10µm. Vascular bundles are opened, bicollateral, composed of phloem consisting of sieve cells, companion cells and parenchyma, cells thin thin-walled, pentagonal to rectangular, measured dimensions are 25x20-12.5x10µm. Fascicular cambium is 3-4 layered, cells tangentially rectangular, thin-walled, measured dimensions are 22.5x20-12.5x10µm. Below that, xylem tissues composed of tracheids small, thick-walled, squarish to pentagonal, measured dimensions are 20x12.5-12.5x7.5µm, vessels are very large, thick-walled, polygonal to circular, solitary or few in groups, measured dia. 112.5-25µm and xylem fibers. Medullary rays 3-4 cell wide or multiseriate, cells thin-walled, parenchymatous, vertically squarish to rectangular, measured dimensions are 37.5x20-25x25µm, storied with idioblasts, tannin or phenolic substances and druses. Pith very large, parenchymatous, thin-walled, filled with idioblasts, 62.5-55µm, some cells filled with tannin or phenolic compounds and druses measured dia.37.5-17.5 µm.

Stem maceration: shows thick-walled parenchyma, bordered pit, annular and scalariform vessels, pitted tracheids, bordered pit tracheids and pitted fibers.

Parenchyma:

a) Thick-Walled Parenchyma: These types of parenchyma cells are showing thickened walls dis-continuous due to the presence of pits, irregularly squired to rectangular in shape, pits large, random, paired, opposite or alternate, many and horizontally oval, measured dimensions are 50 x 15 - 287.5 x 25 µm, ca. average length is 158$\pm$85.33µm and ca. average breadth is 20$\pm$5.00µm.

c) Boarded Pit vessels: These types of vessels are oblong and elongated, walls thickened, beaks larger, flattened and blunt, slightly pointed, intern-vascular pitting paired and opposite, pits horizontally broader or scalariform, pits horizontally broader, perforations plates simple, 4-15 in numbers or many, squared and horizontal, rarely vertically oval, measured dimensions are 170 x 30 – 500 x 110 µm, ca. average length is 358±97.24 µm and ca. average breadth is 65.5±31.97µm.

d) Annular Vessels: These types of vessels are long, primary wall thin, secondary wall thick and annular, smaller in breadth as compared to other vessels, perforations simple, circular or oblique, beaks very short or absent, measured dimensions are 150 x 15 – 730 x 30 µm, ca. average length is 436±167.81 µm and ca. average breadth is 22±6.00µm.

e) Scalariform Vessels: These types of vessels are oblong, walls thick and continuous, ends unbaked or slightly beaked, intre-vascular pitting opposite and paired, pits horizontal or scalariform, broader, perforations simple, 4-15 bars present on some beaks or many, squared, oval, vertical or oblique, measured dimensions are 162.4 x 25 – 800 x 40 µm, ca. average length is 439±213.60µm and ca. average breadth is 33±11.00µm.

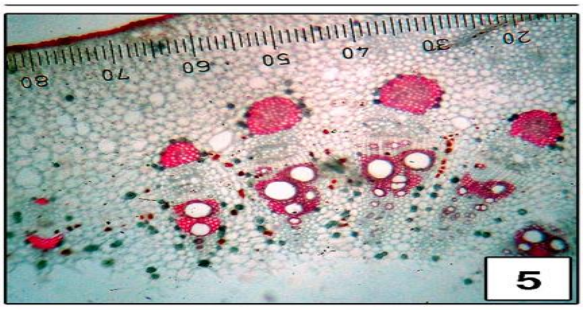
f) Pitted Tracheids: These types of tracheids are slender or tapering, walls thick, continuous, ends blunt or sharply pointed, slightly forked, pits minute, oblique, alternate, throughout wall but few, simple, measured dimensions are 125 x 15 – 520 x 20 µm, ca. average length is 326.5±112.87µm and ca. average breadth is 19.5±1.5µm.

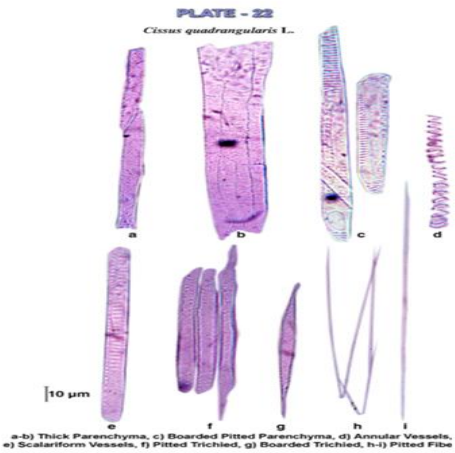
g) Bordered Tracheids: These types of tracheids are elongated, walls thin, continuous or dis-continuous, irregular, ends blunt and pitted, pits many, throughout cell wall, bordered or wide, circular or oval, arranged in straight line, opposite, paired, simple, measured dimensions are 200 x 25 – 337.5 x 35 µm, ca. average length is 265.25±37.85 µm and ca. average breadth is 29±3.74µm.

h) Pitted Fibers: These types of fibers are elongated, long, slender, tapering at the ends and sharply pointed, walls thin and slightly irregular, continuous, pits simple, minute, oblique and alternate, internal lumen is wider than tracheids, measured dimensions are 570 x 15 – 2650 x 30 µm, ca. average length is 2012±849.67 µm and ca. average breadth is 23±6.00µm.

Conclusion

Cissus quadrangularis is a potent drug that has been used in bone healing in rural areas. Present research work findings also show Calcium oxalate crystals and vascular arrangements, macerated elements with peculiarities in shape and size for standardizing this crude drug. Stem anatomy has a quadrangular outline, a thick cuticle and continuously covers the epidermis with a bi-layered, interrupted by sunken stomata, sometimes highly cutinized, outer layer chlorenchymatoums. While maceration shows up thick-walled parenchyma, bordered pit, annular and scalariform vessels, pitted tracheids, bordered pit tracheids and pitted fibers.





***Cissus quadrangularis* L. Maceration Table:**

Sr. No.	Cell Type	Range(µ)	Mean(µ)	S.D.
1)	Thin-wall Parenchyma Length	50-287.5	158	85.33
2)	Thin-wall Parenchyma Breadth	15-25	20	5.00
3)	Boarded Pit Vessels Length	170-500	358	97.24
4)	Boarded Pit Vessels Breadth	30-110	65.5	31.97
5)	Annular Vessels Length	150-730	436	167.81
6)	Annular Vessels Breadth	15-30	22	6.00
7)	Scalariform Vessels Length	162.4-800	439	213.60
8)	Scalariform Vessels Breadth	25-40	33	11.00
9)	Pitted Tracheid Length	125-520	326.5	112.87
10)	Pitted Tracheid Breadth	15-20	19.5	1.5
11)	Boarded Pit Tracheid Length	200-337.5	265.25	37.85
12)	Boarded Pit Tracheid Breadth	25-35	29	3.74
13)	Pitted Fiber Length	570-2650	2012	849.67
14)	Pitted Fiber Breadth	15-30	23	6.00

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