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Original Article

Phyto-Pharmacognostical Standardization of Three Morpho-Variants of *Cissus quadrangularis* L.

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ABSTRACT

Background: *Cissus quadrangularis* is a perennial climber belonging to the Vitaceae family. The plant is characterized by its succulent stems, which are square in cross-section, hence the specific epithet "*quadrangularis*." It is used exhaustively in treating osteoporosis and healing bone injury/fracture due to its rich calcium, phosphorous and phytoestrogen content. The morphological variants *C. quadrangularis* viz. quadrangular, flat and round stemmed varieties are reported and used for the same purpose. **Objectives:** The current study aims to establish a comparative assessment of the morpho-anatomical and phytochemical characteristics of *C. quadrangularis* stem variants, specifically the round, flat and quadrangular varieties. **Materials and Methods:** The present study involved a comparative macroscopic and microscopic characterization, preliminary phytochemical screening and Chromatographic evaluation (HPTLC) of the stem samples of variants of *C. quadrangularis.* **Results:** The present study revealed a lot of similarities in anatomical characteristics, physicochemical parameters, phytochemical analyses and chromatographic observations. **Conclusion:** Phyto-pharmacognostic standards were established for three morpho-variants of *C. quadrangularis*.

Keywords: *Cissus quadrangularis*, *Asthisamhara*, Hadjod, SEM, Microscopy, Stereomicroscopy, HPTLC.

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INTRODUCTION

Cissus quadrangularis is characterized by its thick, fleshy, quadrangular (four-sided) stems, which are greenish, often reddish or purplish in color. These stems are succulent and store water, allowing the plant to survive in arid conditions. *Cissus quadrangularis* is a well-known plant named "Hadjod" in the Unani system of medicine. The word "Hadjod" is a combination of two words: "Haddee" means bone; "Jodana" means attaching. Due to its healing property of broken bones and its resemblance with bones and joints of the human body, it has been named as Hadjod.[1]

Several regions with humid climates, including those in India, Sri Lanka, Bangladesh, Malaya, West Africa[2] and Thailand, are native to *C. quadrangularis.*[3] Morphologically, different variants of *C. quadrangularis* has been identified from various regions of Chittoor district of Andhra Pradesh. The difference is mainly noticed in the stem characters. One is with quadrangular stems having abundant vegetation in dry deciduous forests. The second morpho-variant is identified in the hilly regions of Madanapalle, Chittoor district of Andhra Pradesh with limited distribution. The third morpho-variant has a round or cylindrical stemmed variety maintained in the gardens.

Traditional applications of *C. quadrangularis* include treating bone fractures, scurvy, tumours, haemorrhoids, peptic ulcers, menstrual disorders, gout, syphilis, venereal disease, piles and treating diarrhoea, dysentery, leucorrhoea and diarrhoea in animals.[4-11] To endorse medicinal plants acceptability in the contemporary era, the quality evaluation of medicinal plants is crucial. A major issue confronting the herbal industry is the requirement for stricter quality control profiles for herbal materials. The literature search revealed that three morpho-variants are used in the name of Hadjod and treated as the same species (*C. quadrangularis*). But morphologically, they are different. They adapted these changes as per their habitat. So, it is necessary to evaluate the difference in between these

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three varieties. A pharmacognostic evaluation of stems of three varieties of *C. quadrangularis* has been undertaken for this study.

MATERIALS AND METHODS

Chemicals

All chemicals used in the study were of analytical grade.

Plant materials

The healthy plant materials of *C. quadrangularis*, three morpho-variants were collected from the herbal garden of the Botany department, Sri Venkateswara University (SVU), Tirupati, Andhra Pradesh and the herbal garden of Regional Ayurveda Institute for Fundamental Research, Pune, Maharashtra. These specimens were identified and authenticated by Prof. G. Sudarsanam, Dept. of Botany, S.V. University, Tirupati, with the help of local and regional floras[12-21] and the voucher specimens (Field Book No.: G.S. 2023, 2024, 2025) were submitted to Botany Department, S.V. University for future reference.

Macroscopic and Organoleptic analysis

Macroscopic and organoleptic characteristics of *C. quadrangularis* stem were observed and reported.[22-24]

Microscopic characterization

Stem parts were subjected to freehand sections (T.S.). The obtained thin sections were stained by safranin dye (0.1% w/v solution in distilled water). The Image of T.S. was taken using Olympus B.X. 43 and L.C. 30 camera.[22-24]

Scanning electron microscopic studies

The scanning electron microscope employed for the study was JSM-IT 500 (Model number), Company JEOL, made in Japan. Small stem cuttings of three morpho-variants of *C. quadrangularis* were subjected to surface analysis using SEM.[25]

Powder microscopy

The sample of powder (1 mg) was stained with an Iodine solution (composed of 2 g iodine and 3 g potassium iodide in 100 mL of water) and then mounted in 50% glycerine on one slide. On another slide, the sample of 1 mg was stained with phloroglucinol (0.1% w/v) and dilute HCl (10% solution), then washed in water and mounted in 50% glycerine. The prepared slides were observed for microscopical characteristics using Olympus BX 43 and LC_{30} camera.[26-29]

Histochemistry

The thin sections that were produced were treated with different reagents for histochemical evaluation as per the standard procedures.[28]

Physiochemical analysis

Physicochemical analyses such as Loss on Drying (LOD), alcohol soluble extractive values, water-soluble extractive values, total ash value, acid-insoluble ash and pH were carried out as per the method described in Ayurvedic Pharmacopoeia of India.[25-30]

Preliminary phytochemical screening

Alcoholic and aqueous extracts of *C. quadrangularis* (stems) were prepared by cold maceration method. Coarsely powdered air-dried material 5 g was accurately weighed and placed in a glass stoppered conical flask. It was macerated with 100 mL of the specified solvent for 6 hr, shaken frequently and then allowed to stand for 18 hr. It was filtered rapidly without losing any solvent; the filtrate was transferred to a tared flat-bottomed dish and evaporated to dryness on a digital water bath at 60°C. Both extracts were subjected to phytochemical screening for qualitative analysis for the presence and absence of secondary metabolites.[26-29,31]

HPTLC

2 g of powdered sample was taken and refluxed with 200 ml of chloroform using a soxhlet apparatus on a water bath for 30 min. The mixture was filtered and evaporated to 5 ml of final volume. The extracts (concentrated residue) were applied with Linomat V applicator of CAMAG on TLC silica gel 60 F254 aluminium coated plates (Merck, KgaA, Germany) and developed with Toluene: Ethyl Acetate: (8:2) as mobile phase and was saturated for 45 min in CAMAG® Flat Bottom Chamber of 10x10 cm. These plates were further subjected to the HPTLC instrument Desaga Sarstedt Gruppe (Germany). The plates were observed under Ultraviolet (UV) light at 366 nm and 254 nm. UV cabinet was arranged with imaging and camera set up with remote shooting. The developed bands' retention factor (R_f), area and peak height were recorded.[22,26,28,29]

RESULTS

Macroscopic and organoleptic evaluation

It involves characterization based on external features of crude drug, colour, odour, taste, shape, size, touch and texture (Table 1). The morphography of different variants of *C. quadrangularis* is shown in Figure 1.

Scanning electron Microscoping

Surface analysis of different variants of *C. quadrangularis* using SEM shows stephanocytic stomata and epidermis. However, fracture surfaces show specific acicular crystals. The stomata, epidermis and acicular crystal types are the same for three varieties of *C. quadrangularis* (Figure 2).

The study of stomatal index revealed that stomata frequency in the form of stomatal indexing is 17.5% for the quadrangular stem, 16.9% for the round stem and 15.6 for the flat stem.

Microscopic evaluation

The Transverse Section (T.S.) allows a detailed examination based on histological characteristics. Microscope, by its property magnification, permits the minute structure understudy to be magnified to confirm the structural details of the drugs from plant origin. T.S. of the stem for three species revealed that the significant characteristics are similar. It was divided into five zones; the outermost zone is the epidermis traversed by stephanocytic stomata and sclerenchymatous zones are noticed below the epidermis at ridges. The cortex is the next zone to the epidermis between the ridges. The upper region of the cortex is chlorenchymatous, followed by the parenchymatous area. A massive number of mucilage cells traverse the cortex. The prominent endodermis is not noticed. The vascular bundles 3

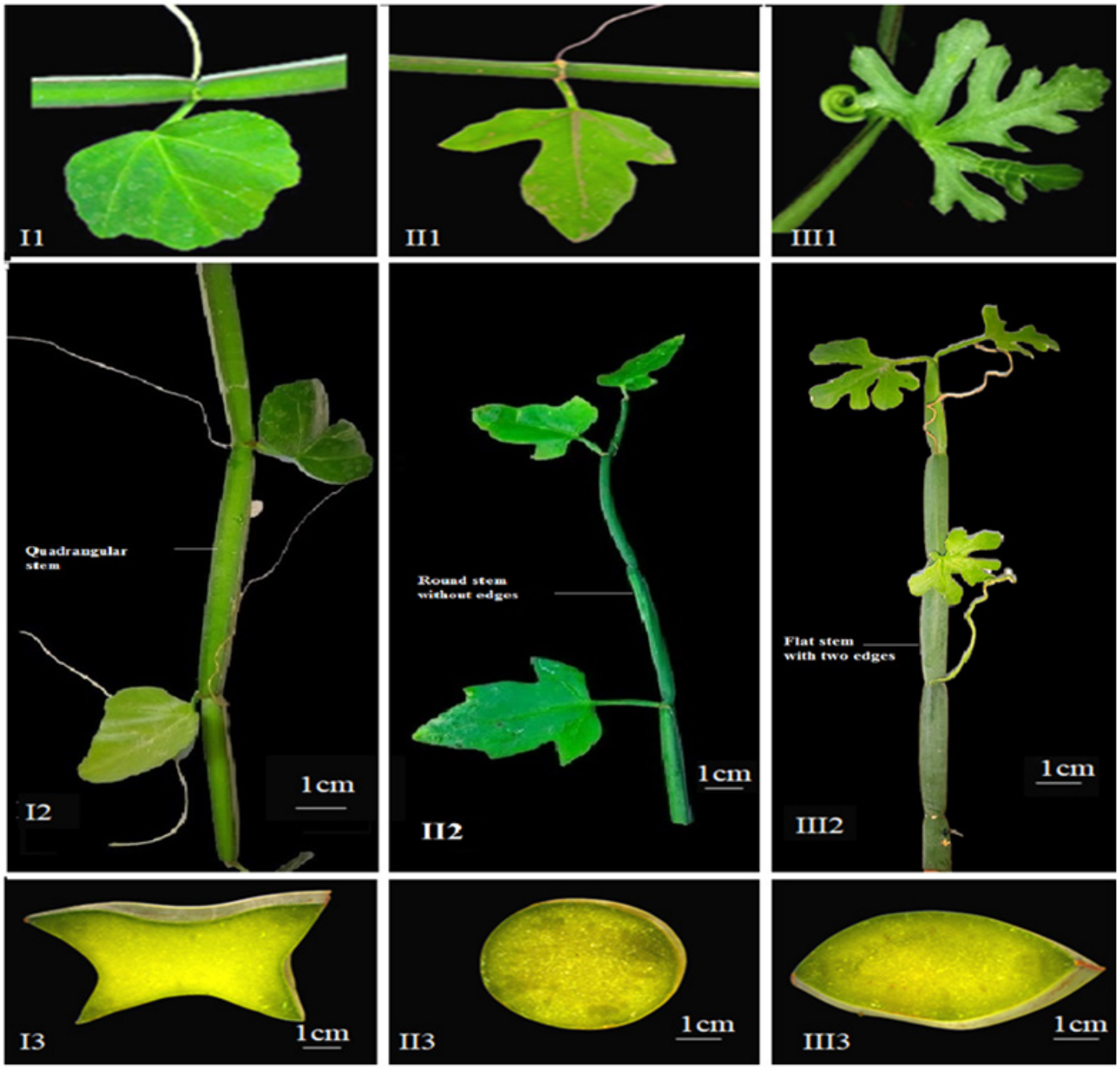

Figure 1: Morphology of three different variants of *C. quadrangularis:* I represent morphology of *C. quadrangularis* with quadrangular stem: I1. Twig with leaf, I2. Stem with four angles, I3-transverse view of the stem. II represents the morphology of *C. quadrangularis* with a rounded stem: II1-twig with leaf, II2. Stem with no angles, II3. Transverse view of the stem. III showing the morphology of *C. quadrangularis* with flat stem: III1. Twig with leaf, III2. Stem with two angles, III3. Transverse view of stem.

to 5 are seen below the ridges. The vascular bundles are open, collateral and endarch. Sclerenchymatous caps are identified on each vascular bundle upon the phloem region. In between each vascular bundle, medullary rays were noticed. Pith is parenchymatous, along with calcium oxalate crystals and mucilage cells. Histological characters observed are shown in Figure 3 and micrography is reported in Table 2.

Powder microscopical observations

Powder microscopy of all three variants shows uniform microscopic characters (Figure 4), but they differ in the size of characters as well as the distribution. Powder microscopic observations of three variants exhibit the presence of epidermal fragments with a surface view along with stephanocytic stomata (Figure 4, Ia, IIa, IIIa). Epidermal cells are quadra- penta-hexangular, straight and thin-walled. The subsidiary epidermal cells of stephanocytic stomata are arranged in a rosette manner (Figure 4, Ia, IIa, IIIa). Variant I has massive epidermal cells and a stomatal complex, followed by variant II and, finally, variant III. Chlorenchyma cells are polygonal, deposited with abundant chlorophyll and rosette calcium oxalate crystals; variants I and II have more giant cells, while variant III has smaller cells (Figure 4, Ib, IIb, IIIb). Parenchymatous cells are polygonal, deposited with acicular crystals, rosette crystals and mucilage cells (Figure 4, Ic, IIc, IIIc). An abundant amount of free acicular crystals of calcium oxalate and aleurone grains are present within ground tissues (Figure 4, Id, IId, IIId). Lignified and non-lignified fibres, which are filiform and long, are noticed (Figure 4, Ie, IIe, IIIe, If, IIf, IIIf). Spiral (Figure 4, Ig, IIg, IIIg), reticulate (Figure 4, Ih, IIh, IIIh) annular (Figure 4, Ii, IIi, IIIi) and scalariform lignified (Figure 4, Ij, IIj, IIIj) vessels are identified.

Table 1: Morphological description of different variants of *C. quadrangularis*

Characters	Quadrangular stem	Flat stem	Rounded stem
Habitat	It is common in dry deciduous and scrub forests throughout India.	It has a rare occurrence in the foothills of scrub jungles with xeric conditions. In its habitat as xeric conditions, the plant has adopted its flattened stem and leaves parted or dissected to reduce the transpiration effect and exposure towards the Sun.	It is maintained in the houses and gardens and does not have wild occurrences.
Habit	Succulent climbing shrub with leaf-opposed tendrils.	Succulent climbing shrub with a thick rootstock and leaf opposed tendrils.	Succulent climbing shrub with leaf-opposed tendrils.
Stem shape	Quadrangular externally. Concave quadrangular in transverse view.	Externally flat. Lanceolate in transverse view.	Rounded externally. Convex quadrangular in transverse view.
Stem size	1 to 1.4 cm in diameter.	1 to 1.6 cm in diameter.	1 to 1.6 cm in diameter.
Leaf morphology	Leaves alternate, broadly ovate-rhomboid to reniform, crenate, rounded at base, dentate at the margin, subacute-obtuse at apex, glabrous.	Leaves are simple, leathery, alternate, parted or dissected; lobes mucronate, leaf base truncate and parted.	Leaves simple, alternate, fleshy, glabrous, hastate-lobed, lobes acute-mucronulate; leaf base truncate.
Type	Aerial		
Interior	Solid, fleshy, mucilaginous		
Branching	Branched		
Nodes and internodes.	Constricted at the nodal region. Leaves and tendrils arise at nodal region.		
Surface	Glabrous or slightly downy	Glabrous	Glabrous
Tendrils	Long, slender and simple	Spring-like tendrils.	Wiry tendrils.
Edges	Four winged edges	Two winged edges.	No distinct edges seen.
Colour	Dull green	Dull green	Dull green
Odour	Less aromatic	Less aromatic.	Less aromatic.
Taste	Tasteless	Tasteless	Tasteless
Fracture	Fibrous	Fibrous	Fibrous

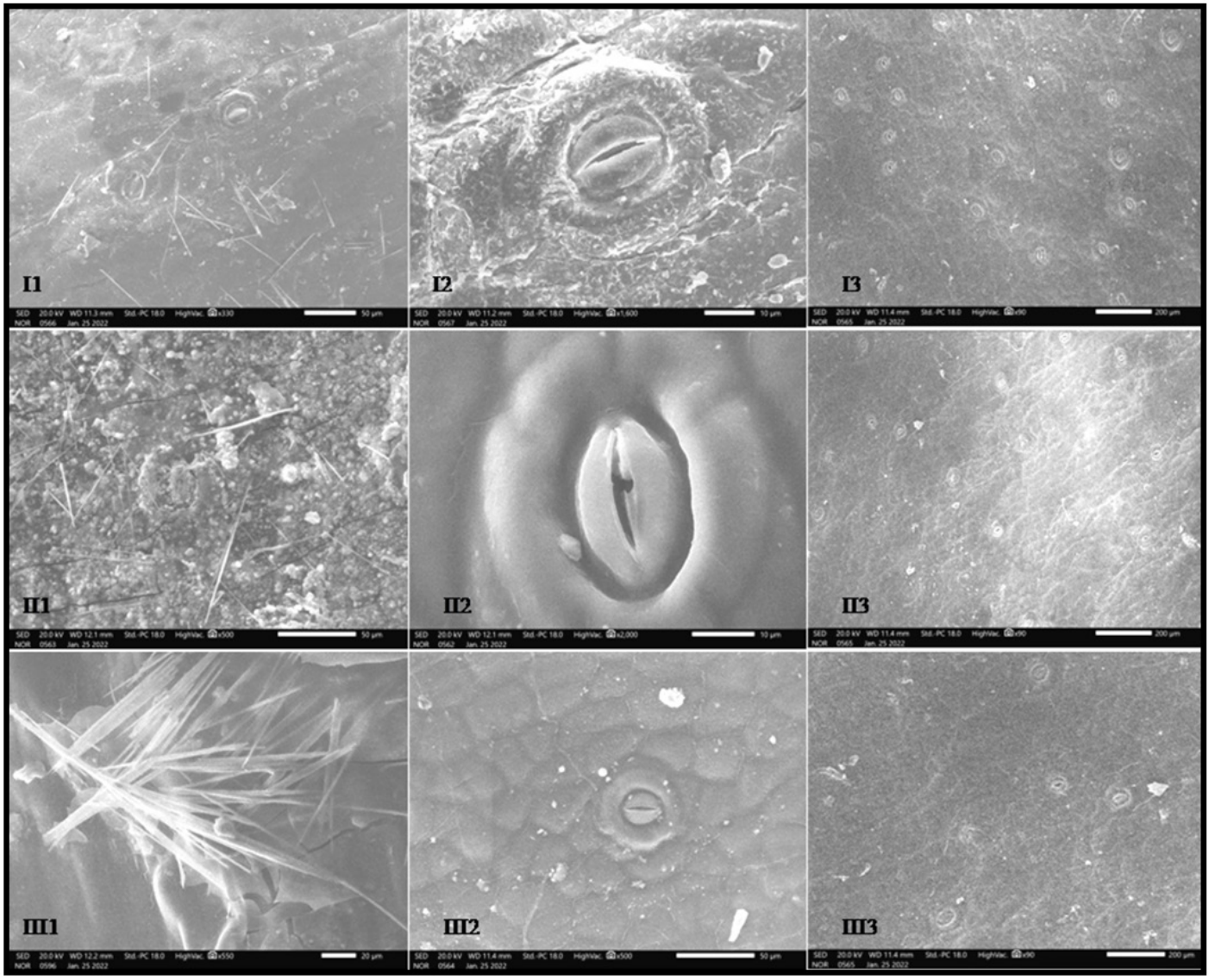

Figure 2: *C. quadrangularis* with quadrangular stem (I); rounded stem (II) and flat stem (III), 1. Acicular crystals, 2. Stomatal complex, 3. Distribution of stomata.

Physicochemical analysis

The quantitative physicochemical analyses of quadrangular (I), rounded (II) and flat (III) stem variety were presented in Table 3. It provides an idea of the earthy matter, inorganic composition, other impurities and the drug.

The observed results characterise the significant variations in-between the three variants.

Chemo-microscopy

It covers the study of plant secondary metabolites using suitable chemicals applied to thin sections or powder samples of crude drugs. The detailed result is reported in Table 4.

Preliminary phytochemical screening of extract

The aqueous and alcoholic extracts of three morpho-variants were subjected to phytochemical screening. The results revealed that all variants show similarity in phytoconstituents, as presented in Table 5.

TLC/HPTLC profiling

Extracts produced from stem parts of three morpho-variants were spotted on the silica gel "G" plate shown in Figure 5. These plates were subjected to the mobile phase as Toluene: Ethyl Acetate: (8:2). Various spots were recognized under UV light at 366 nm and 254 nm Figure 5. Different R_f values are summarized in Table 6. Three separate densitograms for three morpho-variants and a combined dendrogram were developed to establish the similarities between the three morpho-variants Figures 6-9.

TLC studies for round stemmed variety showed Eight major spots under UV 366 nm and their R_f values are 0.02, 0.20, 0.45, 0.60, 0.64, 0.69, 0.80 (all red) and 0.90 (blue) (Figure 5, I,1). Eight major spots were noticed under UV 254 nm and their R_f values

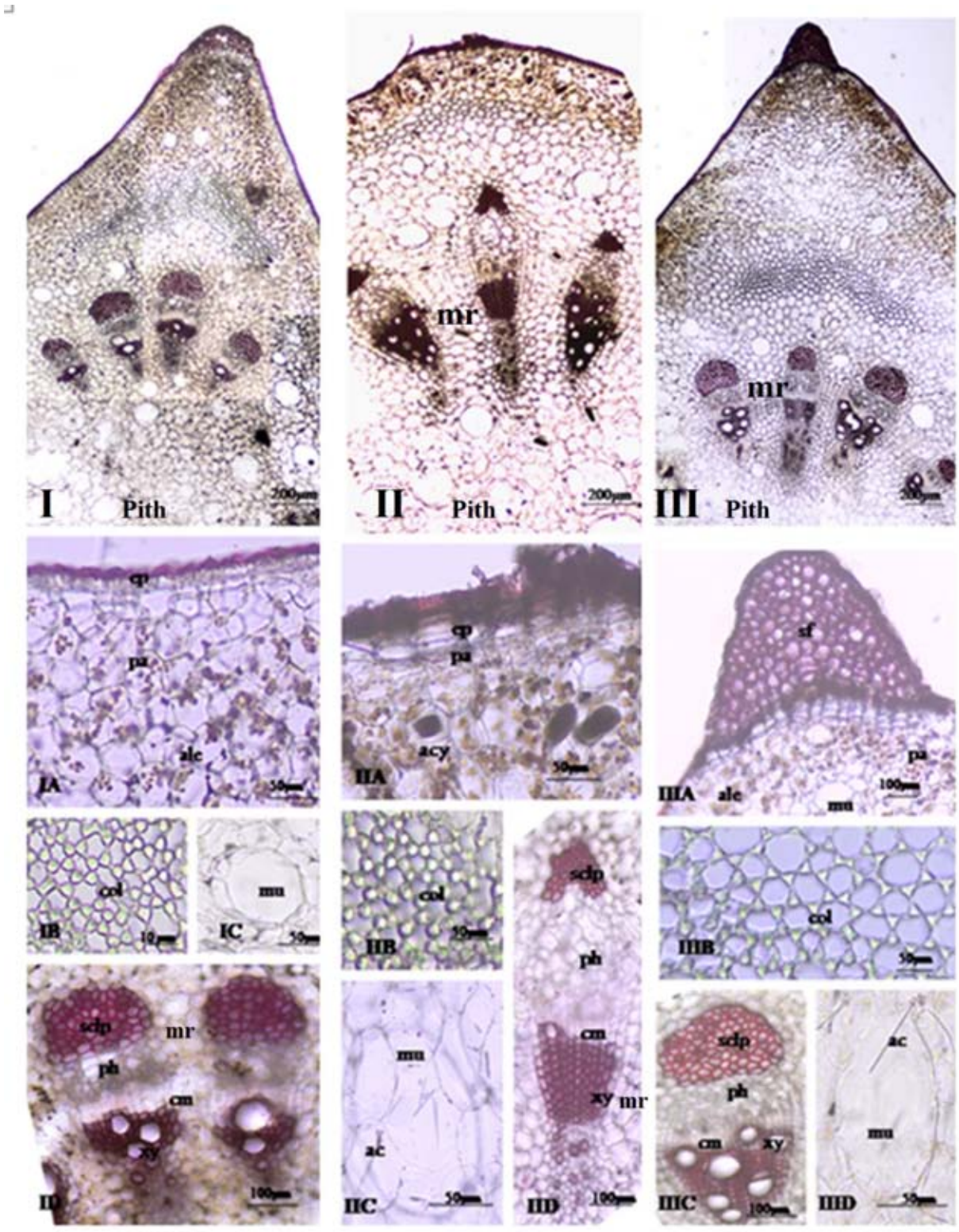

Figure 3: Transverse sections (T.S.) of stems of three different variants of *C. quadrangularis*. IA to ID shows zoom images of the quadrangular stem, IIA to IID shows zoom images of the rounded stem and IIIA to IIID shows zoom images of the flat stem. ac: Acicular crystals, ale: aleurone grains, cm: cambium, col: Collenchyma, ep: Epidermis, mu: Mucilage cell, pa: Parenchyma cells, ph: Phloem, sf: Sclerenchyma fibre, sclp: Sclerenchymatous pericycle, xy: Xylem.

Table 2: Comparative micrography of quadrangular, rounded and flat stem verity.

Characters	Quadrangular stem	Round stem	Flat stem
The outline shape of transfer section	Concave quadrangular (Figure 1, I3).	Round shaped(Figure 1, II3).	Lanceolate(Figure 1, III3).
Edges	Sharply four-angled (Figure 1, I3).	Prominent edges are not identified (Figure 1, II3).	Sharp two angled (Figure 1, III3).
Epidermis	Cells are sub-rectangular, single-layered and covered with cuticles. The epidermis at corners is slightly different; it is made up of small cells. (Figure 3, IA, IIA, IIIA).		
Sclerenchyma	Bellow the ridges 4-5 layered sclerenchymatous cells are noticed; cells are lignified, polygonal and thick-walled (Figure 3, I).	In the mature stems, 2-3 layered patches of sclerenchymatous cells are noticed; cells are lignified, polygonal and thick-walled. (Figure 3, II).	Bellow the ridges 4-5 layered sclerenchymatous cells are noticed; cells are lignified, polygonal and thick-walled. (Figure 3, III).
Ground tissues	It is divided into chlorenchyma and parenchyma. Chlorenchyma cells are thin-walled, rounded, deposited with chlorophyll and bundles of acicular crystals (Figure 3, IB, IIB, IIIB). Parenchyma cells are rounded, thin-walled and deposited with aleurone grains, prism crystals, acicular crystals, druse crystals of calcium oxalate and mucilage cells (Figure 3, IA, IIA, IA).		
Collenchyma cells	10-12 layered; cells beaded and hexangular (Figure 3, IB).	3-4 layered; cells beaded and hexangular (Figure 3, IIB).	10-12 layered; cells beaded and hexangular (Figure 3, IIIB).
Mucilage cells	Abundant in corners and central ground tissues (Figure 3, I, II, III, IC, IIC, IIID).		
Vascular bundle	5 in number at the corner; collateral, open and endarch type. The single vascular bundle is made up of the sclerenchymatous pericycle, phloem, cambium and xylem (Figure 3, I, ID).	3 in number at the corner; collateral, open and endarch type. The single vascular bundle is made up of the sclerenchymatous pericycle, phloem, cambium and xylem (Figure 3, II, IIC).	5 in number at the corner; collateral, open and endarch type. The single vascular bundle is made up of the sclerenchymatou s pericycle, phloem, cambium and xylem (Figure 3, III, IIID).
Sclerenchymatous pericycle cells	Cells are lignified, polygonal and thick-walled (Figure 3, ID, IID, IIIC).		
Phloem	Polygonal, thin-walled cells deposited with crystals (Figure 3, ID, IID, IIIC).		
Cambium	Cells are thin-walled and isodiametric (Figure 3, ID, IID, IIIC).		
Xylem	Lignified, thick-walled, more or less angular (Figure 3, ID, IID, IIIC).		
Medullary rays	Cells are present between two vascular bundles, uniseriate, elongated and sub-rectangular (Figure 3, II, III, ID, IID).		
Pith	Cells are parenchymatous deposited with aleurone, acicular crystals (Figure 3, I, II, III).		

are 0.02, 0.21, 0.22, 0.59, 0.64, 0.70, 0.76 and 0.99 (all brown) (Figure 5, II, 1).

The results obtained by TLC studies for flat stemmed variety delivered eight major spots under UV 366 nm and their R_f values are 0.02, 0.29, 0.45, 0.60, 0.66, 0.70, 0.77 (all red) and 0.90 (blue) (Figure 5, I, 2). Six major spots were detected under UV 254 nm and their R_f values are 0.02, 0.21, 0.29, 0.69, 0.76 and 0.98 (all brown) (Figure 5, II, 2).

TLC studies for quadrangular stem variety showed nine major spots under UV 366 nm and their Rf values are 0.02,0.20, 0.34, 0.45, 0.60, 0.64, 0.69, 0.80 (all red), 0.90 (blue)(Figure 5, I, 3). Nine major spots were detected under UV 254 nm and their Rf values were 0.02, 0.09, 0.21, 0.23, 0.31, 0.36, 0.65, 0.70, 0.77 and 0.98 (all brown) (Figure 5, II, 3).

DISCUSSION

C. quadrangularis is a well-known traditional plant for the treatment of broken bones. Three morpho-variants were noticed for *C. quadrangularis* from different regions of Chittoor district of Andhra Pradesh. These variants predominantly have differences in stem structure. The commonly available species has a quadrangular stem. The second variety with flat stems is confined to hilly regions with constricted water availability. The third variety is maintained in houses and gardens. The three species were claimed with the same medicinal properties for healing

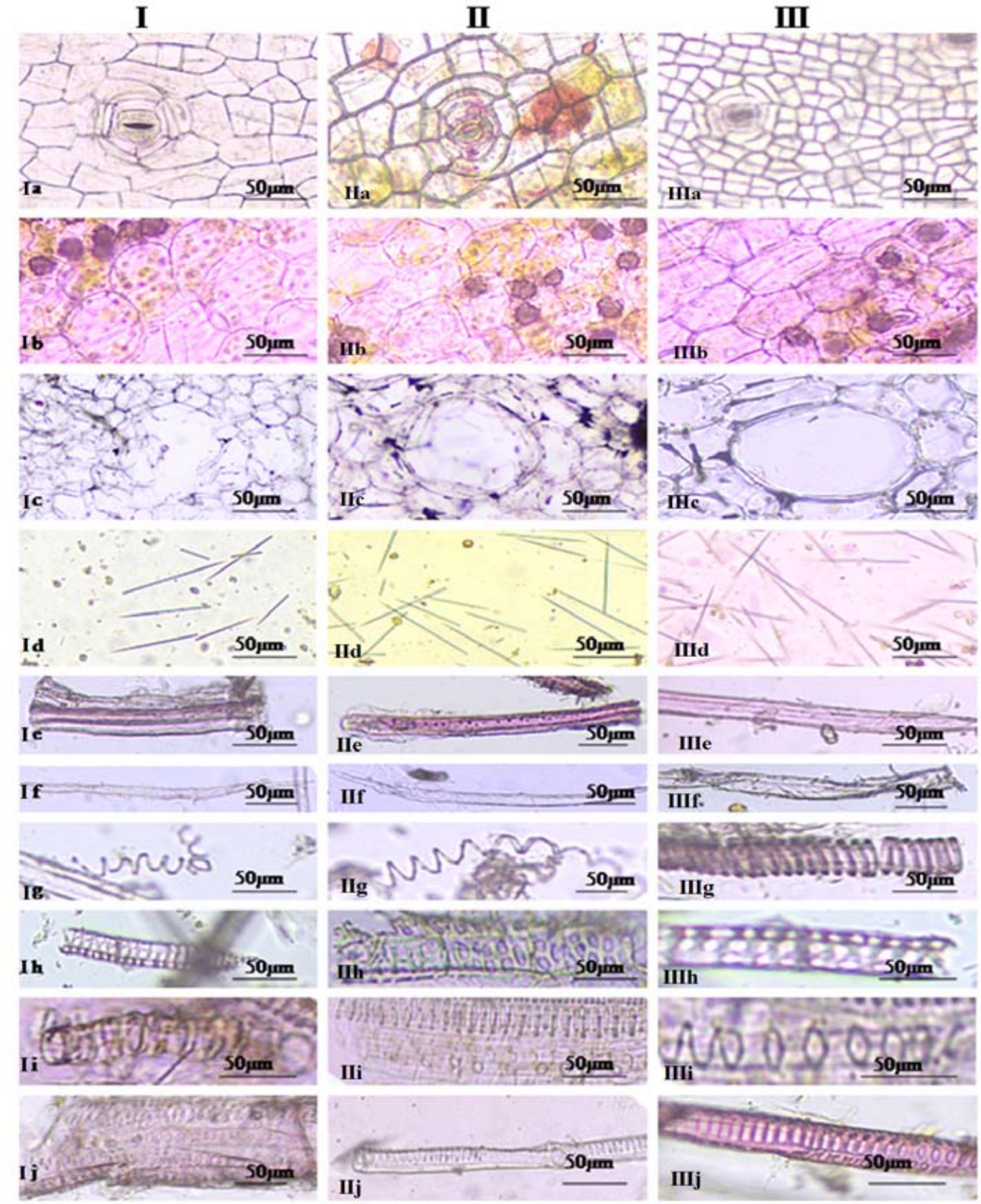

Figure 4: Powder microscopy of three different variants of. *C. quadrangularis* (I. Quadrangular stem; II. Rounded stem; III. Flat stem) (a. Surface view of the epidermis with Stephanocytic stomata; b. chlorenchyma cells with rosette crystals; c. parenchymatous cells with mucilage cells; d. acicular crystals with aleurone grains; e. lignified fibre; f. non-lignified fibre; g. spiral vessel; h. reticulate vessel, i. annular vessel; j scalariform vessel.

Table 3: Physicochemical parameters of flat (I), rounded (II) and quadrangular (III) stem variety.

Sl. No.	Parameter	I	II	III
1	Loss on drying.	80.82	86.46	88.77
2	Water extract (%).	32.22	26.16	21.75
3	Alcohol extract (%).	4.53	4.36	4.74
4	Petroleum ether extract (%).	1.12	1.56	1.74
5	Total ash value.	15.60	19.10	20.34
6	Acid insoluble ash (%).	1.12	1.10	1.10
7	Water soluble ash (%).	1.35	1.44	1.46

Table 4: Histo-chemical evaluation of flat (I), rounded (2) and quadrangular (3) stem variants.

Test	Chemical	Observation	Result		
			1	2	3
Lignified cell.	Phloroglucinol+HCl	Pink to cherry red colour	+	+	+
Cuticular cell.	Sudan red-III	-	+	+	+
Aleurone grains.	Iodine	Yellowish brown to brown	+	+	+
Fats and volatile oils.	Sudan red-III	-	-	-	-
Mucilage fatty oils.	Ruthenium red	Pink	+	+	+
Starch	Iodine	Blue or reddish blue	_	_	_
Calcium oxalate crystals.	Hydrochloric acid	Dissolved	+	+	+
Calcium carbonate crystals.	Hydrochloric acid	-	-		

Table 5: Preliminary Phytochemical screening of flat (I), rounded (2) and quadrangular (3) stem verity.

Sl. No.	Phytoconstituents	Aqueous extract			Alcoholic extract		
		1	2	3	1	2	3
1	Carbohydrate	+	+	+	+	+	+
2	Protein	+	+	+	+	+	+
3	Lipid	-	-	-	-	-	-
4	Alkaloids	-	-	-	-	-	-
5	Glycoside	+	+	+	+	+	+
6	Phenolics	+	+	+	+	+	+
7	Flavonoids	+	+	+	+	+	+
8	Tannins	+	+	+	+	+	+
9	Saponin	+	+	+	+	+	+
10	Volatile oils	-	-	-	-	-	-
11	Triterpenoids	-	-	-	+	+	+
12	Steroids	-	-	-	+	+	+

+ Present, - absent.

broken bones. The present study was aimed to differentiate the three morpho-variants through pharmacognostical studies and to establish pharmacognostical standards. The study was divided into 5 phases 1. Microscopical observation, 2. Physicochemical studies, 3. Histochemical studies, 4. Preliminary phytochemical screening and 5. HPTLC screenings.

Most characteristics observed by microscopical studies were similar. Significant differences were noticed only in the outline of T.S. for the stem structures of the three variants. Slight differences are detected in the observations of physicochemical parameters. The histochemical studies for three variants revealed similar results by the presence of lignified cells, cuticular cells, aleurone grains, fats and volatile oils, mucilage fatty oils, starch and calcium oxalate crystals.

The preliminary phytochemical screening for aqueous and methanolic extracts of the three variants revealed the presence of carbohydrates, proteins, glycosides, phenolics, flavonoids, tannins, saponins, triterpenoids and steroids.

The data obtained through HPTLC studies is a chemical fingerprint profile for three morpho-variants. The number of R_f values calculated at 254 nm are ten for the quadrangular variety, eight for the round variety and six for the flat stemmed variety. Most of the Rf values are similar for quadrangular and rounded

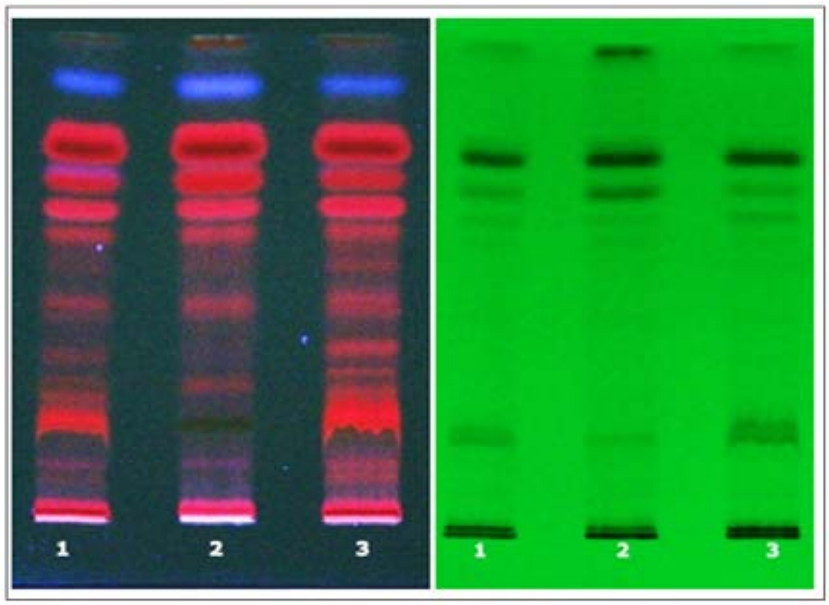

Figure 5: TLC fingerprinting profile of 1. Round, 2. Flatland 3. Quadrangular varieties at 366 (I) and 254 nm (II).

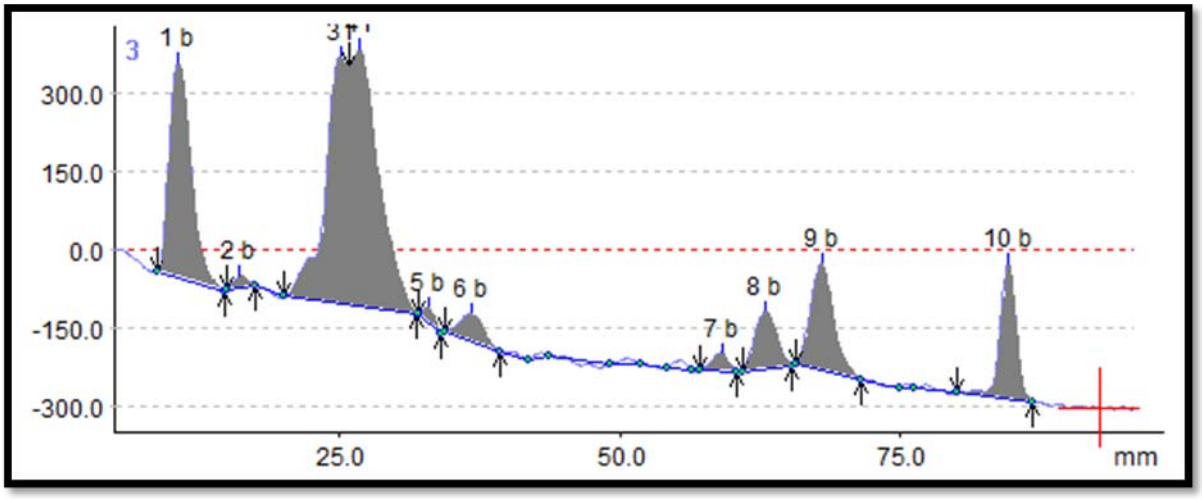

Figure 6: Densitogram at 254 nm for chloroform extract of *C. quadrangularis* (with round stem).

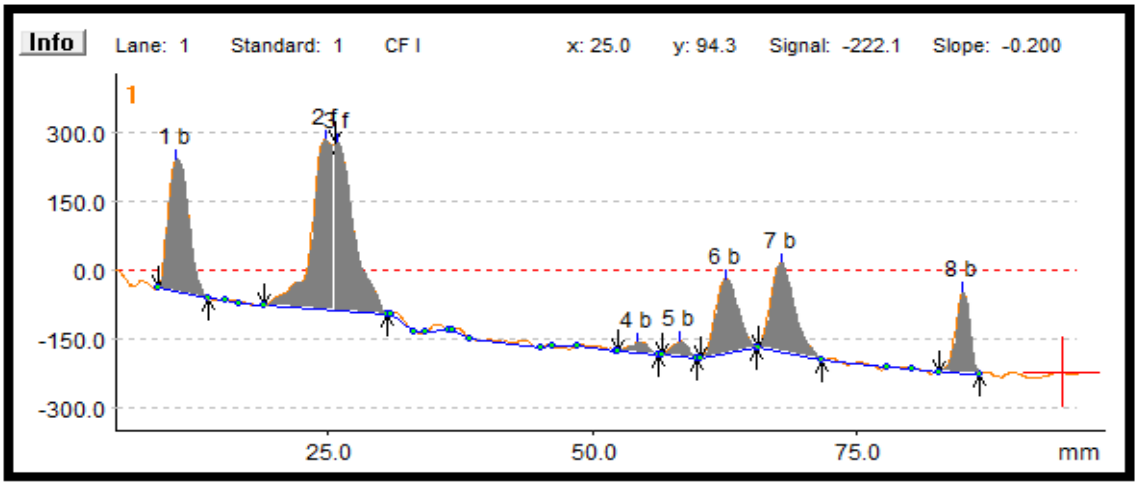

Figure 7: Densitogram at 254 nm for chloroform extract of *C. quadrangularis* (with flat stem).

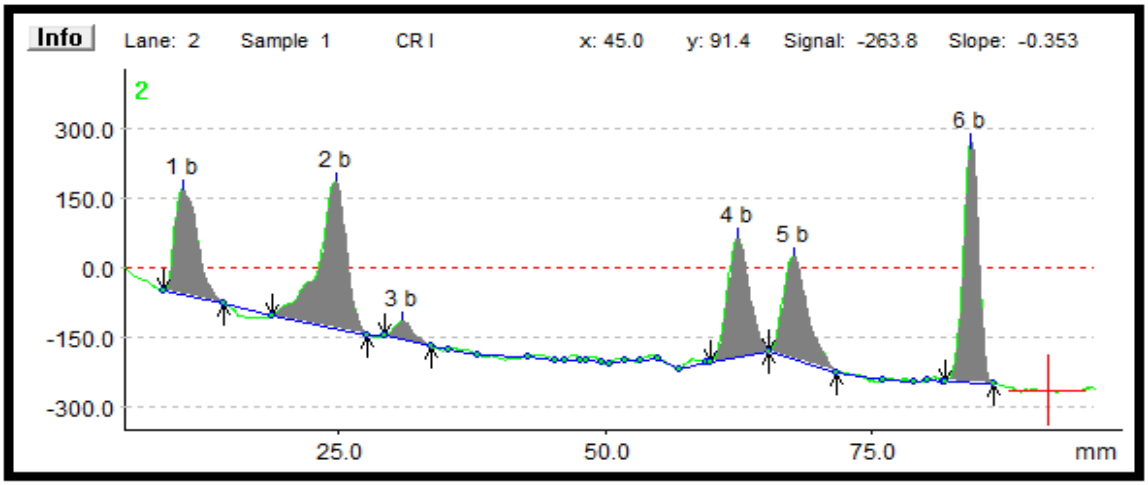

Figure 8: Densitogram at 254 nm for chloroform extract of *C. quadrangularis* (with quadrangular stem).

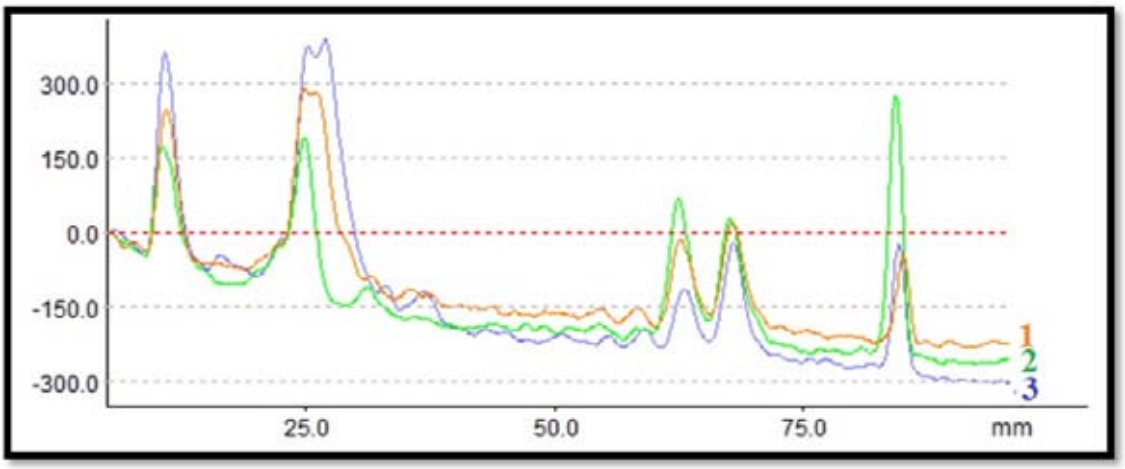

Figure 9: Combined densitogram at 254 nm for three varieties.

Table 6: Peak lists and R_f values of densitograms produced for three varieties at 254 nm of UV light.

Round stem	Peak no	Y-Pos	Area	Area %	Height	R_f values
	1	10.8	620.26	18.57	285.39	0.02
	2	24.8	734.15	21.98	364.56	0.21
	3	25.9	740.09	22.16	359.89	0.22
	4	54.3	50.13	1.50	24.47	0.59
	5	58.3	57.55	1.72	33.05	0.64
	6	62.6	387.65	11.60	158.89	0.70
	7	67.8	486.40	14.56	190.93	0.76
	8	85.0	264.22	7.91	174.16	0.99
Flat stem	Peak no	Y-Pos	Area	Area %	Height	R_f values
	1	10.5	540.58	15.20	223.04	0.02
	2	24.8	950.63	26.73	312.40	0.21
	3	31.1	84.37	2.37	42.30	0.29
	4	62.4	593.13	16.68	253.83	0.69
	5	67.6	568.46	15.98	217.16	0.76
	6	84.2	819.71	23.05	508.08	0.98
Quadrangular stem	Peak no	Y-Pos	Area	Area %	Height	R_f values
	1	10.7	887.41	19.04	401.27	0.02
	2	16.2	32.73	0.70	23.83	0.09
	3	25.2	1033.44	22.17	465.23	0.21
	4	26.9	1367.95	29.35	483.29	0.23
	5	33.1	29.01	0.62	27.85	0.31
	6	36.8	126.11	2.71	51.55	0.36
	7	59.1	57.31	1.23	34.70	0.65
	8	63.0	241.01	5.17	108.51	0.70
	9	68.0	468.76	10.06	201.91	0.77
	10	84.5	417.03	8.95	253.49	0.98

varieties. A small deviation is noticed in the flat-stemmed variety. This result is in corroboration of earlier reported work on DNA barcoding for rbcL gene sequencing, wherein 99% of homology was seen between three species. However, a slight deviation was noticed in the flat-stemmed variety compared to the other two varieties through phylogenetic tree analysis.[32]

CONCLUSION

The present study established pharmacognostic standards for three morpho-variants of *C. quadrangularis.* Macroscopical observations conclude that the three varieties differ in their vegetative morphological characters, especially in the stem structure. Microscopical observations conclude that most characters are similar for the three varieties; significant differences are not noticed. Histochemical and preliminary phytochemical studies concluded that the three morpho-variants are identical for their phytoconstituents. Physicochemical parameters revealed minor variations due to their habitat. HPTLC fingerprinting profiles conclude that the variations in the total count of the R_f values between the three morpho-variants. But most of the R_f values are similar. It concludes that major phytoconstituents in the three morpho-variants are similar, leading to similar pharmacological activities for the three variants and may be used as substitutes for one another.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

ac: Acicular crystals; **ale:** Aleurone grains; **cm:** Cambium; **col:** Collenchyma; **ep:** Epidermis; **HCl:** Hydrogen Chloride; **HPTLC:** High-Performance Thin Layer Chromatography; **mg:** Milligram; **mu:** Mucilage cell; **nm:** Nanometer; **pa:** Parenchyma cells; **pH:** Potential of Hydrogen; **ph:** Phloem; **rbcL:** Ribulose-Bisphosphate Carboxylase Gene; **R_f:** Retention Factor; **S.V.U.:** Sri Venkateswara University; **Sclp:** Sclerenchymatous pericycle; **SEM:** Stands for Scanning Electron Microscope; **sf:** Sclerenchyma fibre; **TLC:** Thin layer chromatography; **UV:** Ultraviolet; **xy:** Xylem.

SUMMARY

C. quadrangularis is a renowned traditional plant utilized in the treatment of fractures. Distinct morphological variations have been observed in *C. quadrangularis* across various regions of Chittoor district of Andhra Pradesh, particularly in the stem structures. This research aims to differentiate the three morpho-variants through pharmacognostical studies. The study encompasses five phases: 1. Microscopical observation, 2. Physicochemical studies, 3. Histochemical studies, 4. Preliminary phytochemical screening and 5. HPTLC screenings.

Microscopic examinations reveal a substantial likeness in the characteristics of the three varieties, with no discernible significant differences. Findings from histochemical and preliminary phytochemical studies affirm the identical presence of phytoconstituents across the three morpho-variants. Physicochemical parameters exhibit minor variations attributed to their respective habitats. HPTLC fingerprinting profiles indicate disparities in the overall count of R_f values among the three morpho-variants, yet a prevalent similarity is observed in most R_f values.

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