

**Pharmacognostical studies on  
*Cissus quadrangularis* L. variant I & II**

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**ABSTRACT :** The aerial parts of *Cissus quadrangularis* L. Variant I and II are being used therapeutically for various ailments in indigenous system of medicine. Detailed pharmacognostical studies on the aerial parts were made. Variant I and II were analysed for their physiochemical, microscopical, fluorescent, qualitative and quantitative phytochemical, TLC and HPTLC characteristics. Quantitative variations were noted among seasonal samples and between variants and the results are presented.

**KEYWORDS :** *Cissus quadrangularis*, variants, pharmacognostical studies, seasonal changes.

## INTRODUCTION

*Cissus quadrangularis* Linn (Vitaceae) is a medicinal shrub widely distributed in the hotter parts of India. It is widely used for indigestion, dyspepsia, bone fractures, diarrhoea, fistula etc<sup>1</sup>. It is a climber with stout fleshy quadrangular stem. Three variants on morphological characters occur; square - stemmed, round-stemmed and flat-stemmed termed as variant I, II and III respectively. The square-stemmed and round-stemmed variety is the most commonly available. It is a rich source of ascorbic acid, carotene A, anabolic steroid substance and calcium. The round-stemmed variety termed as variant II is characterised by the presence of wingless stem. It is found rarely in selected areas like Seithur hills near Rajapalayam of Viruthunagar district, Tamilnadu, India and in some medicinal plant gardens<sup>2</sup>. The internode is four sided and depressed

slightly on four sides. Variant III is rare. Occurrence of variants had been reported earlier <sup>3,4,5</sup>. Pharmacological, toxicological and helicobactericidal activities of variant I and II has been carried out earlier <sup>6,7,8,9,10,11</sup>. Pharmacognostical difference between these two variants and seasonal changes in phytochemical constituents of these variants were not made. Hence variants were collected during two growth phases and their pharmacognostical variations were noted.

## MATERIALS AND METHODS

Variant I was collected from Vallanad, Tirunelveli district and variant II was collected from the Tamil University Herbal garden, Thanjavur, Tamilnadu, India and identity verified at Botanical Survey of India, Coimbatore. A voucher specimen is being deposited in the department herbarium

for future reference (Variant I - TUH No.189; Variant II - TUH No.190). Plant samples were collected during two different seasons i.e., flowering (November-2000) and vegetative season (May-2000)<sup>12,13</sup>. The plant materials were cleaned, shade-dried and powdered (sieve 40) and taken for pharmacognostical studies.

The aerial parts were fixed in formalin, acetic acid and alcohol mixture. Customary microtoming was done and sections stained in safranin-fast green<sup>14</sup>. Physicochemical tests<sup>15</sup>, fluorescent characters<sup>16</sup>, qualitative phytochemical<sup>17</sup>, quantitative phytochemical estimations for calcium<sup>18</sup>, ascorbic acid<sup>19</sup>,  $\beta$ -sitosterol<sup>20</sup> and total free amino acids<sup>21</sup> of the drugs were also analysed out in the extracts by standard methods.

Aqueous ethanolic extracts were subjected to paper chromatography for amino acids and enriched ethanolic (50%) extract was subjected to TLC for identification of alkaloids, saponins and terpenoids<sup>22</sup>. 24 standard amino acids for comparison were procured from Loba-Chemie (Mumbai) and used. Enriched alcoholic (50%) extracts of alkaloids, saponins and terpenoids were spotted on precoated silica gel plates (60F<sub>254</sub>) and precoated aluminium foil silica gel plates (GF254). A mixture of acetone and ethanol in the ratio 1:1 was used as mobile phase. The spots were detected under Iodine chamber and in UV rays at 254 and 365nm.

HPTLC study was carried out in alcoholic (50%) extract. 10  $\mu$ l of extract was spotted on precoated aluminium foil silica gel plates (GF<sub>254</sub> E. Merck). The plates were developed in acetone and ethanol (1:1) and scanned at a wavelength of 254 and 365nm.

## RESULTS AND DISCUSSION

The morphological characters especially of stem, leaf and tendril and season of flowering of *C.quadrangularis* are noted differently in different floras and treatises on medicinal plants. The existence of variants in this species is recorded in many botanical literature. Earlier works indicated that stem of the species is nearly leafless or leafless when old<sup>23</sup>. Kannan observed that new leaves and branches appear during rainy season<sup>2</sup>. Leaves being caducous, the stem appears almost leafless in other seasons. Kumbhojkar *et al.* noted two varieties, but he regarded them as 'cultivars'<sup>3</sup>. Another study revealed the formation of three sided branches from the four-sided variety<sup>4</sup>. The variants has been reported as chimeric variations<sup>2</sup>.

### Anatomical characters

TS of stem of the two variants have three zones; cortex, vascular bundles and broad pith. The outline of the stem varies from gibbose to dumbbell depending upon the variants. Epidermis is single layered with cuticle and stomatal openings (Fig.2,10). The epidermal cells are small, radially elongated, varied in shape (4-7 x 1-15 $\mu$ ). Cortex is differentiated into outer chlorenchyma of 2-4 layers and inner parenchyma of 3-6 layers. chlorenchyma cells are round to polygonal in shape with intercellular spaces, compactly arranged (10-15 $\mu$ ). Small stomatal cavities are observed. Parenchyma cells are round to polygonal in shape, ranging from 10-15  $\mu$  in diameter and have intercellular spaces. Large size mucilage cells (25-48 $\mu$ ) are frequently observed in the cortex. Patches of collenchyma are present at the corners of the stem. Primary vascular bundles are present in groups of 3-5 at the corner. Endodermis is not distinct. Smaller secondary vascular bundles appear along the interfascicular cambium forming a ring.

Vascular bundle is collateral, open and endarch. Sclerenchyma cap (3-5 $\mu$ ) is present above the phloem of each bundle. Vessel elements are large and 7-25  $\mu$  in diameter. Ground tissue present between corner vascular bundles consist of smaller and compact cells varying in size and shape (4--8 $\mu$ ). Pith is parenchymatous, present at the centre and occupying most of the area. Pith parenchyma cells are round to irregular and polygonal in shape (15-25 $\mu$ ). A number of large mucilage cells (25- $\mu$  48 $\mu$ ) are present in the pith. Raphides of calcium oxalate crystals (30 x 75  $\mu$ ) are also present (Fig.12). On observing the development of secondary growth, it is noticed that the cambial ring formed is connecting the vascular bundles. Secondary vascular bundles are formed one on each side of the stem and four in four-sided stem. Second set of secondary vascular bundles are formed in between the primary bundles and first formed secondary vascular bundles and thereby giving the appearance of beads. The following distinguishing stem anatomical features are noted among the variants.

In variant I, the stem has a dumbbell shaped outline with slight modifications. The four corners are extended forming the wing of the stem (Fig.1,2). It has three cellular zones as in the general pattern. However, the mature stem is distinguished by the presence of a patch of sclerenchymatous cells at the four corners below the epidermis. In variant II, the outline of the stem is more or less rectangular in shape with blunt corners (Fig.9,10). Sclerenchyma is absent in the cortex.

Petiole in T.S.is more or less circular or pentagonal in outline. A notch is present on the adaxial side with two flanks and these features are absent in the apical region. TS of petiole shows four distinct zones;

epidermis, cortex, collenchyma and vascular zone embedded in parenchymatous ground tissue (Fig.3.) The epidermis is single layered with cuticle. The epidermal cells are barrel-shaped (20-28 x 12-20 $\mu$ ). Cortex is made up of 4-8 layers of parenchyma cells (20-45 $\mu$ ). 2-3 layers of cells are chlorenchymatous in nature. Next to cortex is the collenchyma zone. It is 3-5 layers thick, continuous along the petiole in the basal end, but it is restricted as a patch below the vascular bundles in the middle and in the tip of the petiole. Vascular bundle is endarch open, collateral with a collenchymatous cap. Vessel elements are large upto 40 $\mu$  in diameter. If the numbers of bundles are five, they are restricted to five corners of the petiole. Inner to this, is the ground tissue, which is made up of parenchyma cells (25-58  $\mu$ ) with clear intercellular spaces. Large sized mucilage cells and raphides of calcium oxalate crystals are frequently observed in the pith and cortex.

TS of midrib are broader on abaxial side and have a small protuberance in the adaxial side. It is broader at the basal portion and narrowing towards the tip of the leaf. The midrib has an outer epidermis, followed by collenchyma zone, parenchymatous ground zone and centrally located vascular zone (Fig.5,12). The epidermal cells in the abaxial surface and adaxial protuberance region are smaller in size (8-15 x 10-20 $\mu$ ), square to rectangular in shape. 2-6 layers of collenchyma cells are present in the abaxial and adaxial surfaces. These are compactly arranged, small, 12-28  $\mu$  and polygonal to round in shape. In between these, is the ground tissue made up of parenchyma cells. These cells are large, thin walled with clear intercellular spaces (30-52 $\mu$ ). Mucilage cells (30-52  $\mu$ ) are frequently observed in the midrib (Fig.12). Vascular bundles are centrally located. The number of vascular

bundles varies from basal to apical regions of the leaf; single in the apical, 3-4 in the middle and 4-6 in the basal regions. A collenchymatous cap is present over each vascular bundle. Bundles are open, collateral and endarch vessel elements are large. Raphides are rarely present. Stomatal openings are observed on the abaxial surface.

TS of leaf lamina is homogenous with epidermis on both surfaces and mesophyll cells (Fig.4,13), thickness ranging from 40-120  $\mu$ . The epidermal cells are rectangular to barrel shaped (12-40 x 8-11 $\mu$ ) with a thin cuticle. Stomatal openings are observed on both the surfaces. Mesophyll is made up of round to irregular polygonal cells with clear intercellular spaces. Chlorophyll pigments are observed in the mesophyll of both surfaces. Sometimes they are present in the middle region also. In some instances, mesophyll cells present in the upper surface are columnar shaped. Large sized mucilage cells are frequently observed (110-145 $\mu$ ). Calcium oxalate raphides (30-70 $\mu$ ) are present (Fig.13,14). Surface view of the epidermis show polygonal shaped cell with straight margins (Fig. 7,8,16,17) Striation of epidermal cells are common in variant I (Fig.8), but absent in variant II. Stomata is of anomocytic type.

TS of tendril is circular in outline. The internal structure is differentiated into epidermis, parenchymatous cortex, collenchymatous zone, vascular bundles and pith (Fig.6,15). Epidermis is single layered with cuticle. It encloses 4-7 layers of cortex. It is made up of polygonal and compact parenchyma cells (15-23 $\mu$ ). Medium sized mucilage cells and raphides are also observed. Inner to the cortex is collenchyma zone of 3-5 layers thick. Inner to this is the 2-4 layers of parenchyma cells, which are polygonal in shape. Vascular bundles are

more than 6 in number, 8 being most common. As secondary thickening proceeds, all the bundles are interconnected by interfascicular cambium forming a ring. Vascular bundle is exarch open and collateral. Vessel elements are upto 18 $\mu$  in diameter. Pith is made up of polygonal shaped parenchyma cells without intercellular spaces (18-49 $\mu$ ). Mucilage cells and raphides are occasionally present in pith.

Anatomical characters of stem, petiole, leaf and tendril of the two variants do not show any marked variations. Presence of pearl glands reported in earlier works<sup>24,25</sup> was not observed in the specimens of present work.

### **Quantitative microscopy**

Quantitative microscopical values like stomal index, vein islet number and vein termination number of the two. Variants are given in Table 1. Stomal index value of leaf lower surface is higher than in upper surface in both variants. Higher value in average stomal index is observed in the upper and lower leaf surfaces of variant I.

### **Physicochemical characters**

Analytical values like total ash, water-soluble ash, acid insoluble ash, sulphated ash, loss on drying, solubility in alcohol and water and successive extractive values in acetone, chloroform, alcohol and water are given in Table 2. Total ash value, acid insoluble ash, loss on drying and solubility percentage in alcohol and water are high variant I, when compared to variant II. Successive extractive values in alcohol was higher in variant I, whereas that in chloroform and water was higher in variant II. There was no marked change in the analytical values of samples collected during

vegetative and flowering periods in the two variants. Behaviour of powder of aerial parts of the variants of *C. quadrangularis* on treatment with different chemical reagents and their fluorescent behaviour are given in Table 3 and 4. With Iodine and Sodium hydroxide they reported marked dissimilarity. No difference in the behaviour of powder was observed in the samples collected during different periods in the two variants. Qualitative phytochemical analysis is given in Table 5. Carbohydrate is present in both alcohol and water extracts of variant I. But carbohydrate is absent in water extracts of variant II. Gums and mucilage are present in water extracts of variant I and II. Benzene and chloroform extracts were green and sticky in both variants. But, in alcohol and water it was brown in colour, its consistency was solid in water but semisolid in alcohol. Qualitative phytochemical analysis showed no difference among the plants samples collected during two different seasons in the two variants. Quantitative estimation is given in table 5. Content of calcium, ascorbic acid,  $\mu$ -sitosterol and free amino acids are more in variant I than variant II. In both variants these chemical constituents show slight increase during flowering periods.

### Chromatography

Chromatographic bands of the two variants show different amino acid composition. Variant I developed 7 bands in both the seasons. The purple spots at R<sub>f</sub> values of 0.33, 0.50, 0.62, 0.69, 0.81, 0.86 and 0.92 were identified as arginine, threonine, phenylalanine, cysteine, amino-n-butyric acid, lysine and histidine respectively. Variant II developed 5 bands in both the seasons. The purple colored bands were

identified as cysteine, arginine, threonine, leucine and cystine at R<sub>f</sub> values of 0.24, 0.33, 0.50, 0.61 and 0.69 respectively. Arginine, threonine and cystine were identical among both variants. No seasonal variation in amino acid composition was observed in both the variants.

In TLC preparation for alkaloids, 2 bands were developed in both variants. Variant I developed two bands corresponding to R<sub>f</sub> values of 0.45 and 0.68, whereas variant II developed two bands corresponding to R<sub>f</sub> values of 0.42 and 0.51 for alkaloids. For saponins two bands were developed corresponding to R<sub>f</sub> of 0.39 and 0.84 for variant I and at R<sub>f</sub> 0.39 and 0.52 for variant II. For terpenoids a band corresponding to R<sub>f</sub> 0.80 for variant I and two bands corresponding to R<sub>f</sub> 0.66 and 0.78 were developed. Hence, there is a difference in composition of alkaloid, saponin and terpenoids in variant I and II. No additional bands were observed in TLC for plant samples collected during two different seasons in the two variants.

HPTLC finger printing of aqueous ethanolic extracts of *C. quadrangularis* revealed a slight variation in seasonal samples in variant I and no seasonal change in variant II. Variant I developed 4 spots corresponding to R<sub>f</sub> 0.39, 0.52, 0.74 and 0.82 for samples collected during vegetative period (Fig.7) and samples collected during flowering period (Fig.8) showed 4 spots corresponding to R<sub>f</sub> 0.45, 0.53, 0.74 and 0.82. Variant II developed identical 4 spots corresponding to R<sub>f</sub> 0.43, 0.51, 0.66 and 0.78 for samples collected during vegetative and flowering periods (Fig.9).

This study clearly demonstrates difference among variants and variation in the chemical constituents.

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**Table 1 : Quantitative microscopical values of leaves of *Cissus quadrangularis***

Parameters Studies	Variant I	Variant II
Stomal Index		
Upper Surface	3.05–3.79–5.08	2.08-3.04-4.34
Lower Surface	3.75-5.61-6.61	2.66-3.76-6.00
Vein islet number	2.56-2.65-3.50	2.00-2.48-3.70
Vein termination number	5.16-7.71-10.9	5.0-7.18-11.70

**Table 2 : Analytical values for variants of *C. quadrangularis***

Parameters Studies	Variant I	Variant II
Total ash value (%)	11.88 ± 0.006	10.00 ± 0.20
Water-soluble ash (%)	6.52 ± 0.04	6.62 ± 0.11

Acid-insoluble ash (%)	5.70 ± 0.004	3.50 ± 0.04
Sulphated ash (%)	16.89 ± 0.04	18.33 ± 0.004
Loss on drying (%)	91.00 ± 0.20	87.00 ± 0.20
Solubility (%)		
Alcohol	6.00 ± 0.20	5.00 ± 0.16
Water	36.60 ± 0.16	26.80 ± 0.008
Successive extract values		
Petroleum ether	2.86 ± 0.002	3.30 ± 0.12
Benzene	1.00 ± 0.001	1.24 ± 0.001
Chloroform	0.37 ± 0.001	0.48 ± 0.0008
Alcohol	18.64 ± 0.001	13.02 ± 0.007
Water	11.80 ± 0.008	24.92 ± 0.002

Results are mean of 4 values expressed in g%

**Table 3 : Behaviour of the powder for variations of *C. quadrangularis* with various chemical reagents**

Parameters Studies	Variant I	Variant II
Powder as such	Yellowish green	Green
Powder + Conc. H <sub>2</sub> SO <sub>4</sub>	Dark brown	Blackish green
Powder + Conc. HCl	Yellowish red	Reddish brown
Powder + Conc. HNO <sub>3</sub>	Yellowish red	Reddish brown
Powder + Acetic acid	Brownish green	Dark green
Powder + 10% NaOH	Yellow	Grey
Powder + 1N HCl	Yellow	Green
Powder + Iodine	Brown	Grey
Powder + 5% FeCl <sub>2</sub>	Brownish black	Green

**Table 4 : Fluorescent behaviour of powdered roots of two variants of *Cissus quadrangularis***

Chemical reagent	Visible Light		UV Light	
	Visible I	Visible II	Visible I	Visible II
Powder as such	Dark grey	Grey	Yellowish green	Green
Powder + 1N HCL	Yellowish green	Green	Brown	Grey
Powder + 50% HCL	Dark green	Grey	Light brown	Grey
Powder + 50% H <sub>2</sub> SO <sub>4</sub>	Brown	Grey	Brown	Grey
Powder + 50% HNO <sub>3</sub>	Dark yellowish green	Green	Dark brown	Yellowish brown

Powder + 1N NaOH in water	Green	Yellowish Green	Yellowish green	Light green
Powder + 1N NaOH in ethanol	Green	Dark green	Yellowish green	Green

Colour reactions are viewed in natural light and ultraviolet light at 256 nm

**Table 5 : Preliminary phytochemical estimation on the two variants of *Cissus quadrangularis***

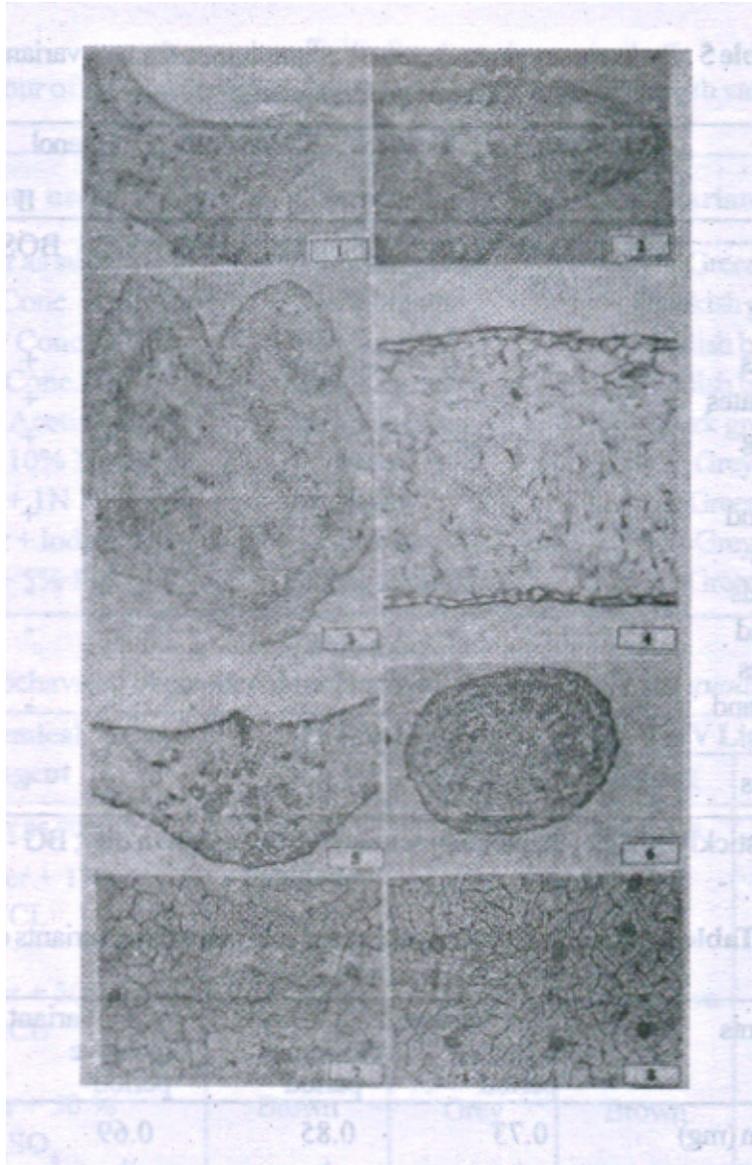
Test for variant	Petroleum ether		Benzene		Chloroform		Ethanol		Water	
	I	II	I	II	I	II	I	II	I	II
Colour and physical consistency	GS	Dark GS	GS	GS	GS	GS	BOS	BOS	BO	BO
Alkaloids	-	-	-	-	+	+	+	+	-	+
Carbohydrates	-	-	-	-	-	-	+	+	+	-
Reducing sugars	-	-	-	-	-	-	+	+	+	-
Tannins and phenols	-	-	-	-	-	-	+	+	+	+
Flavonoids	-	-	-	-	-	-	-	-	-	-
Gums and mucilages	-	-	-	-	-	-	-	-	+	+
Fixed oils and fats	+	+	+	+	-	-	-	-	-	-
Saponins	-	-	-	-	-	-	+	+	+	+

GS - Green sticky ; BOS - Brown oily semisolid; BO - Brown oily; BG - Brown granular - = Absent; + = Present

**Table 6 : Quantitative photochemical analysis of two variants of *Cissus quadrangularis***

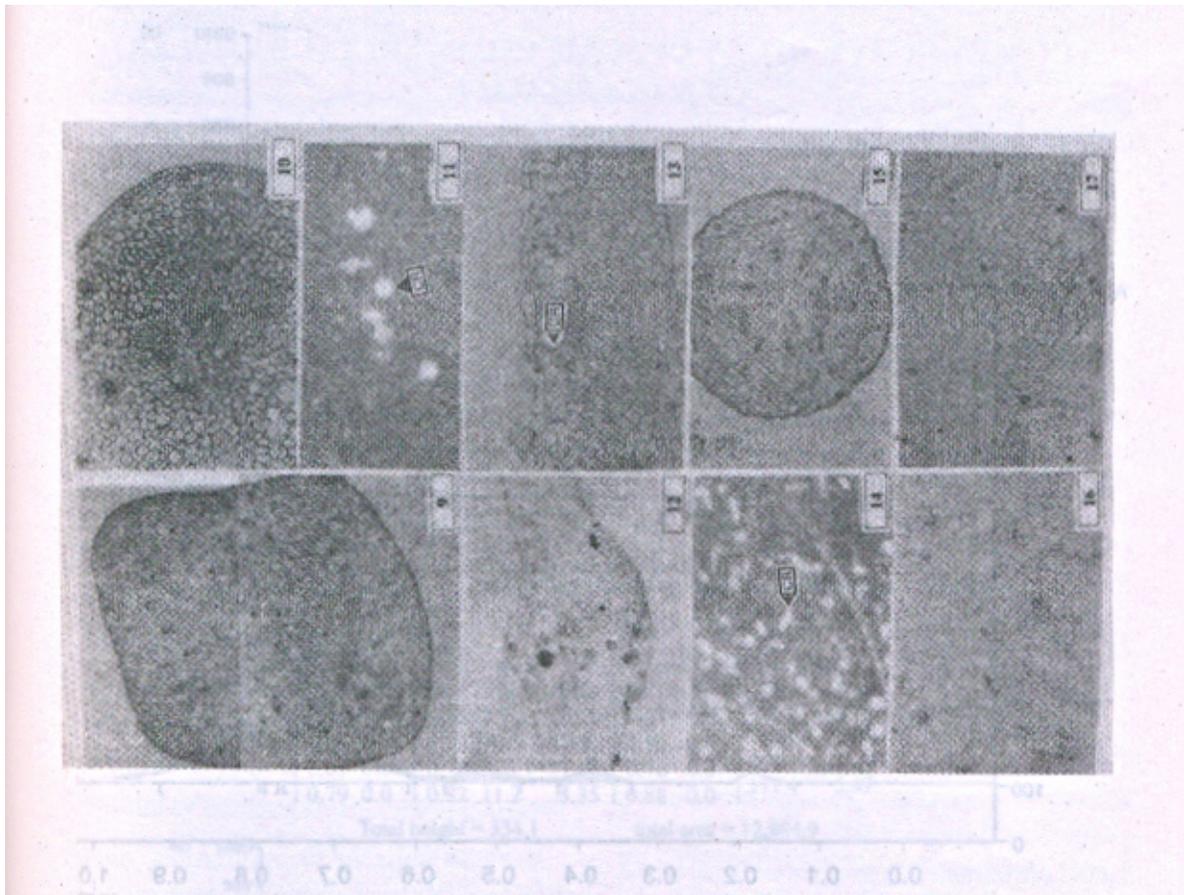
Chemical reagent	Visible Light		UV Light	
	Visible I	Visible II	Visible I	Visible II
Calcium (mg)	0.73 ± 0.001	0.85 ± 0.002	0.69 ± 0.002	0.76 ± 0.002
Ascorbic (mg)	668.55 ± 0.53	773.38 ± 0.50	492.89 ± 0.28	564.17 ± 0.74
β – sitosterol (mg)	3.64 ± 0.002	4.32 ± 0.001	2.57 ± 0.001	3.03 ± 0.002
Free amino acids (%)	8.93 ± 0.001	9.35 ± 0.002	6.27 ± 0.002	7.28 ± 0.001

Results are mean ± SEM of 4 values.



**Anatomical details of *C. quadrangularis* varient I**

- Fig. 1. : TS of young stem ( x 47)
- Fig. 2. : TS of young stem - A portion enlarged ( x 113)
- Fig. 3. : TS of petiole ( x 32)
- Fig. 4. : TS of leaf lamina ( x 250)
- Fig. 5. : TS of leaf midrib ( x 46)
- Fig. 6. : TS of young tendril ( x 47)
- Fig. 7. : Epidermal peeling of leaf upper surface ( x 200)
- Fig. 8. : Epidermal peeling of leaf lower surface ( x 200)



### **Anatomical details of *C. quadrangularis* varient II**

Fig. 9 : TS of young stem ( x 40)

Fig. 10 : TS of young stem - A portion enlarged ( x 110)

Fig. 11: TS of young stem under polarised light showing the calcium oxalate crystals (x 110) (CR : crystal)

Fig. 12 : TS of leaf midrib (x46)

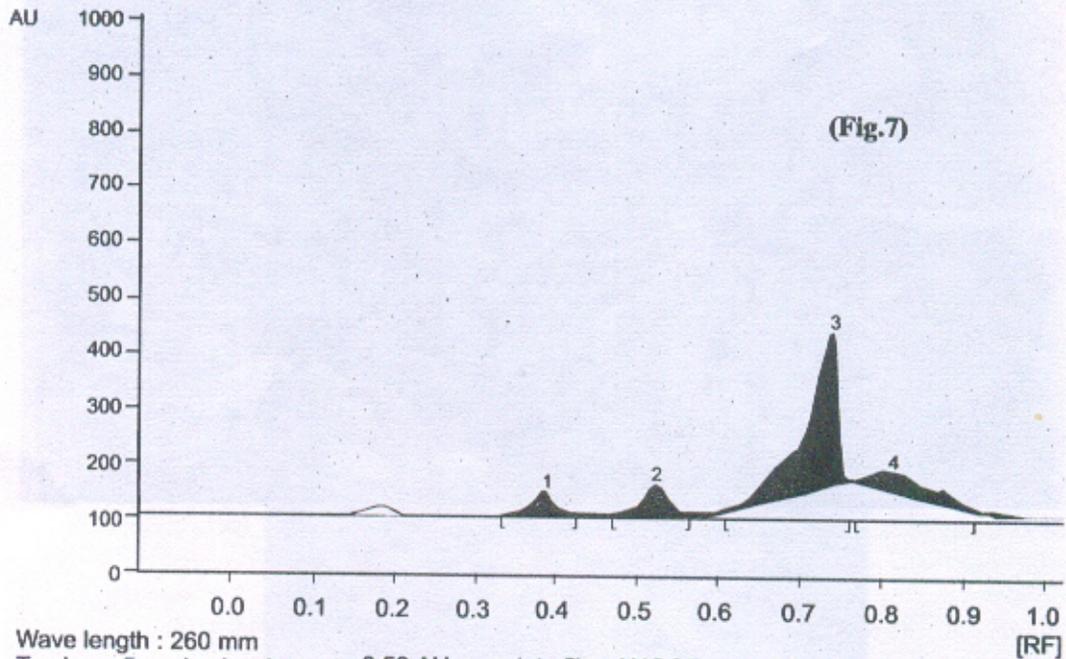
Fig. 13 : TS of leaf lamina (x132) (CR : crystal)

Fig. 14 : Surface view of cleared leaf under polarised light showing the calcium oxalate crystals (x 85) (CR : crystal)

Fig. 15 : TS of young tendril (x 60)

Fig. 16 : Epidermal peeling of leaf upper surface (x 87)

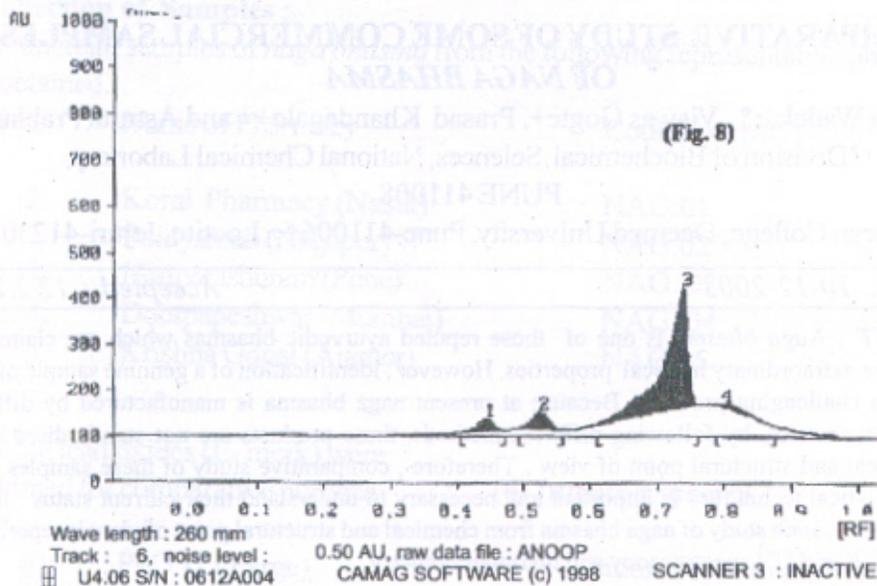
Fig. 17 : Epidermal peeling of leaf lower surface (x 90)



Wave length : 260 mm  
 Track : 5, noise level : 0.50 AU, raw data file : ANOOP  
 U4.06 S/N : 0612A004 CAMAG SOFTWARE (c) 1998 SCANNER 3 : INACTIVE

Peak #	start		max			end		area	
	Rf	H	Rf	H	[%]	Rf	H	F	[%]
1	0.41	0.0	0.45	2.77	8.28	0.47	0.0	570.7	5.96
2	0.49	0.0	0.53	36.5	10.92	0.56	0.0	764.9	7.98
3	0.61	0.0	0.74	258.7	77.45	0.76	0.0	7895.9	82.39
4	0.80	0.0	0.87	13.6	4.15	0.94	0.0	371.4	5.67

Total height = 364.2      total area = 12,884.9

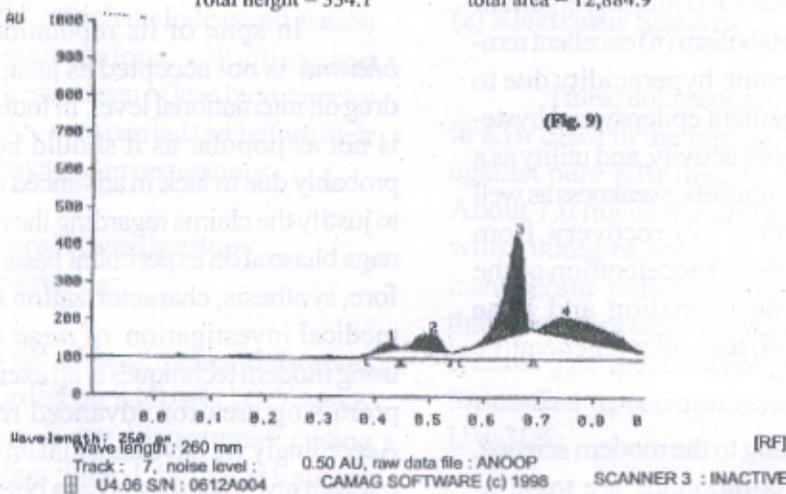


Track 6, Analysis f : A6

Peak #	start		max			end		area	
	Rf	H	Rf	H	[%]	Rf	H	F	[%]
1	0.41	0.0	0.45	2.77	8.28	0.47	0.0	570.7	5.96
2	0.49	0.0	0.53	36.5	10.92	0.56	0.0	764.9	7.98
3	0.61	0.0	0.74	258.7	77.45	0.76	0.0	7895.9	82.39
4	0.79	0.0	0.82	11.2	3.35	0.88	0.0	371.4	5.67

Total height = 334.1

total area = 12,884.9



Track 6, Analysis f : A6

Peak #	start		max			end		area	
	Rf	H	Rf	H	[%]	Rf	H	F	[%]
1	0.38	0.0	0.43	20.9	5.55	0.45	0.0	506.4	3.31
2	0.45	0.0	0.51	43.9	11.65	0.54	0.0	1722.8	11.25
3	0.56	0.0	0.66	262.2	69.56	0.69	0.0	8091.9	52.83
4	0.70	0.0	0.78	49.9	13.24	0.90	0.0	4996.0	32.62

Total height = 377.0

Total area = 15317.1