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Comparative Anatomical and Biochemical Characterization of Three Morphotypes of Ancient Medicinal Plant *Cissus quadrangularis* L.

Naveen Joshi[1] and Kriti Joshi[2]
Department of Biotechnology and Microbiology[1,2]
SJHS Gujarati Innovative College of Commerce and Science, Indore, Madhya Pradesh, India
naveenjoshi44@gmail.com and kriti_dave07@yahoo.co.in

Abstract: *The emphasis on search of new plant-derived drugs containing medicinally useful chemical constituents has increased in the last two decade. Additionally medicinal herbs are considered to be a chemical factory as it contains multitude of chemical compounds like alkaloids, glycosides, saponins, resins, oleoresin, and oils (essential and fixed). The studies on medicinal plant begin with knowing the anatomy and morphology of the plant followed by biochemical charcetrization and extraction of compounds of interest.Cissus quadrangularis L. is an important medicinal plant with diverse applications in alternative medicines. The extracts obtained from plant were used in treatment from ancient time as documented in Ayurveda.*

Keywords: Anatomy, *Cissus quadrangularis* L., Herb, Biochemical, Ayurveda

I. INTRODUCTION

Mother Nature has gifted divine medicinal plants to humans from her green remedies from plant kingdom to fight death from disease and cure themselves from ailments. It is up to us to explore, seek, search and reap the benefit of this treasure. India is endowed with a rich wealth of medicinal plants. These plants are the major collaborator in the formulation of Indian material medica. *Cissus quadrangularis* L. is an important perennial herb used incessantly as medicine in India (Sundaran *et al.,* 2020). It belongs to family Vitaceae and mostly seen in tropical forest of Asia and Africa (Oben *et al.,* 2006). Commonly named "Harjora" in hindi and "Pirandani" in tamil, the plant is succulent climber and is characterized by quadrangular stem. *Cissus quadrangularis* L. stem is used in healing bone fracture, piles, joint pains, gout, asthma and swelling (Malik and Kaur, 2014). They are medicinally important due to the presence of various phytochemicals like flavonides, resveratrol, calcium etc... The production and accumulation of chemical substance in plant is governed by anatomy and biochemical properties of plant. Due to medicinal importance of *Cissus quadrangularis* L. anatomical and biochemical studies were performed in present study. Leaf and stem morphology of plant was elucidated using microscopic preparation. Biochemical characterization was done by determining chlorophyll content, sugar (reducing & non-reducing) content, and protein content.

II. MATERIALS AND METHODS

2.1 Plant Sample

Three variants of *Cissus quadrangularis* L. were collected and coded as CQV1, CQV2 and CQV3. The cuttings of all the three variants were raised and maintained in the replicated pots. Four months old replicates were used as source of young leaf tissue and stem for anatomical and biochemical study.

A. Anatomical Studies

Hand sections from fresh plant sample were taken and fine section of stem parts from three different variants of *Cissus quadrangularis* L. were selected for anatomical studies. The sections were stained with saffranin for two min. and excess stain was removed using water. The sections were mounted and observed under compound microscope. Images were captured through image analyzer at 10X and 40X.

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B. Biochemical Studies

The fresh samples of leaf and stem of all the three variants were collected at 10.00 to 12.00 hrs. Fine leaf and stem parts were used for estimation of chlorophyll content, reducing and nonreducing sugar and protein content**.**

C. Chlorophyll Estimation

The chlorophyll content from leaves was determined using method described by Arnon *et al.,* (1949). The fresh and matured leaves were collected in morning. 1.0 g of leaf was grinded in mortar pestle and homogenized in 20 ml of chilled 80% acetone. Centrifuged at 5000 rpm in cooling centrifuge for 5 min. supernatant was collected in 250 ml measuring cylinder. The process was repeated with the residue till it gets colourless. The supernatant were collected and final volume was made to 100 ml. Absorbance was recorded at 645nm, 652nm, and 663nm using spectrophotometer. Chlorophyll a, b, and total chlorophyll was calculated using following formula

Chlorophyll a in mg/gm of leaf = $12.7 \times (A^{663}) - 2.69 \times (A^{645}) \times V/ 1000 \times W$

Chlorophyll b in mg/gm of leaf = $22.9 \times (A^{645}) - 4.68 \times (A^{663}) \times V/1000 \times W$

Total Chlorophyll in mg/gm of leaf = $20.2 \times (A^{645}) + 8.02 \times (A^{663}) \times V/1000 \times W$

Where A= Absorbance at specific wavelengths.

V= Final volume of chlorophyll extract in 80% acetone.

W= Fresh weight of tissue extracted.

D. Determination of Reducing Sugar

The reducing sugar content was determined using method explained by Miller *et al.,* (1972). 100mg of fresh leaf and stem samples were extracted with 10ml of 80% hot ethanol with continuous mixing. Supernatant was collected after centrifugation and evaporated. 10ml of distilled water was added to dissolve sugar. Simultaneously a standard sugar (Glucose) was ruined. 1 ml of sample was pipetted in tubes and final aliquot was made to 3ml using distilled water. 3ml of DNS reagent was added with mixing and placed for 10min. in boiling water bath. 1ml of Rochelle salt were added in tubes and cooled. The absorbance was recorded at 510nm using spectrophotometer. The experiment was ruined in triplicate.

E. Determination of non-reducing Sugar

The supernatant was obtained as obtained earlier in the method. 1ml of sample was taken in tubes and 1ml of 1N H_2SO_4 was added, later mixture was hydrolyzed by heating at 49^0C for 30 min. After cooling one drop of methyl red indicator was added and neutralized by adding 1N NaOH followed by DNS method as mentioned above.

F. Determination of Protein

The protein content was determined by method described by Lowry *et al.,* (1951). Sample extracts were prepared by taking 500mg of sample and mixed with 10ml of 80% alcohol. Extracted sample were evaporated and residues were dissolved in 10ml of 1N NaOH. Place it for 1hr and centrifuge at 5000 rpm for 10min. the supernatant produced were used for protein estimation. Simultaneously standard was ruined. 0.1 ml of each sample were taken in tubes and volume was made to 1 ml using 1N NaOH, 5 ml reagent C was added to all the tubes with mixing and allowed to stand for 10 min. then 0.5 ml of FCR was added and incubated for 30 min in dark. The absorbance was measured at 660 nm using spectrophotometer.

III. RESULT AND DISCUSSION

3.1 Anatomical Studies

The anatomical studies showed variation in morphological pattern between all the three variants. The results showed that CQV1 had much more morphologically difference as compare to other two variants (CQV2 and CQV3). Similarly CQV1 showed well developed vascular bundles in comparison to other two variants. CQV1 has more or less iso diametric mesophyll cells arrangement in leaf tissue. However in CQV2 such type of mesophyll cell showed more elongated pattern in leaf tissue. The transverse section results of stem among the three variants of *C. quadrangularis* stated that in CQV1 epidermal layer has single layer but highly lignified as compared to other variant. In CQV2 well

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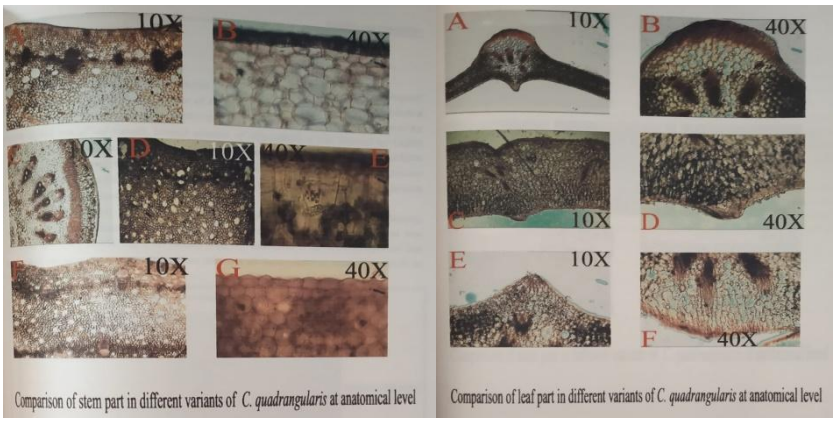
developed phloem fibers were observed in comparison to CQV1 and CQV3. Another remarkable result were observed in section of stems of three variants, observation revealed that CQV3 had more secondary growth as well many number of vessel elements as compared to CQV1 and CQV2. Similar variation was recorded by Nagani *et al.,* (2011) in stem microscopic section among different variants.

Figure 1: Microscopic Images of all the three Variants of *C. quadrangularis* a) Anatomy of Stem b) Anatomy of Leaves.



(a) **(b)**

3.2 Biochemical Studies

A. Chlorophyll Estimation

The present investigation demonstrated that the primary photosynthetic pigments, chlorophyll a and b varied among the three variants of *C. quadrangularis* species. The chlorophyll a content was highest in CQV1 (0.95 mg g^{-1} of tissue) followed by CQV2 (0.814 mg g^{-1} of tissue). However chlorophyll b in leaves showed maximum content in CQV1 (0.651 mg g^{-1} of tissue) followed by CQV2 (0.438 mg g-1 of tissue) lowest content was observed in CQV3 (0.195 mg g^{-1} of tissue). Similar pattern was observed in both chlorophyll a and b content in all the three variants. Total chlorophyll content was observed highest in CQV1 (1.56 mg g^{-1} of tissue) followed by CQV2. The lowest total chlorophyll content was recorded in CQV3 (0.456 mg g^{-1} of tissue).

B. Determination of Reducing Sugar

The reducing sugar content was found highest in CQV1 (0.377 mg g-1 of fresh wt. of tissue) followed by CQV2 (0.369 mg g-1 of fresh wt. of tissue), which is slightly lower. In both variants of *C. quadrangularis* there were no much difference was observed in the amount of reducing sugars. Similarly stem part among the three variants had minor difference in reducing sugar content, highest result showed by CQV2 stem (0.747 mg g-1 of fresh wt.) in comparison to rest of the variants CQV3 (0.455 mg g-1 of fresh wt. of tissue) followed by CQV1 (0.393 mg g-1 of fresh wt. of tissue) the variation in reducing sugar in all the variant is due to the accumulation of photosynthetase as the age progressed as well as plant growth and development is generally affected by genotype, environmental and their interactions.

C. Determination of Non-Reducing Sugars

The content of non-reducing sugar was highest in the leaves of CQV1 (0.254 mg g-1 of fresh wt. of tissue) and lower content was recorded in variant CQV2 (0.039 mg g-1 of fresh wt. of tissue). In both the variants of *C. quadrangularis* there was not much difference found in the amount of non-reducing sugars content. The stem part of all the three variants showed variation in the amount. Maximum content of non-reducing sugar was showed by CQA2 (0.121 mg g-1 of fresh wt. of tissue) followed by CQA3 (0.114 mg g-1 of fresh wt. of tissue) both variants has a minor difference in the content. The variant CQV1 (0.066 mg g-1 of fresh wt. of tissue) showed opposite results in stem as they recorded minimum non-reducing sugar in stem. Variation in non-reducing sugars is the result of accumulation of metabolites in the plant with progression of age and also due to genotypic makeup and environmental interaction.

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D. Determination of Protein

The protein present in the leaf as soluble protein, the soluble protein content in the leaves was highest in CQV1 (15.6 µg g-1 of fresh wt. of tissue) and CQV2 (5.12 µg g-1 of fresh wt. of tissue). Variation in soluble protein content was observed in the stem of all the three variants. Highest soluble protein content was showed by CQV2 (10.3 µg g-1 of fresh wt. of tissue), the variant CQV3 showed less content of protein (9.6 µg g-1 of fresh wt. of tissue) in compare to CQV2, the lowest content was showed by CQV1(7.4 µg g-1 of fresh wt. of tissue) in all the three variants. Difference in the protein content primarily depends on genotype of variants and secondly on environmental conditions this condition governs the accumulation of photosynthatase in the tissue with aging.

Table: Biochemical aspects of stem and leaves of all the three variants of *C. quadrangularis*

S. No.	Biochemical Aspect	Variants of *C. quadrangularis* L.					
		CQV1		CQV2		CQV3	
		Leaf	Stem	Leaf	Stem	Leaf	Stem
1	Chlorophyll (mg g^{-1})	1.566	NA	1.252	NA	0.456	NA
2	Reducing Sugars (mg g^{-1})	0.377	0.455	0.369	0.747	0.298	0.393
3	Non-reducing Sugars(mg g^{-1})	0.254	0.066	0.039	0.121	0.183	0.114
4	Protein (µg g^{-1})	15.6	7.4	5.12	10.3	4.62	9.6

NA=Not applicable, Values are the mean of triplicate.

IV. CONCLUSION

Anatomical study is the key to understand the morphology of plant. The morphology of stem, leaf and root varies in variants of same species. The variants show variation in mesophyll of leaf and different arrangement of vascular bundles. Morphology of stem varies in the presence and thickness of epidermal cell, phloem content and growth pattern. These factors are controlled by environmental condition and genotype of variants and shows variation. The morphological characters have control of biochemical constituents of plant. The change in anatomical parameters governs the variation in biochemical constituents.

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