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RESEARCH ARTICLE

Foliar Micromorphological Evaluation of *Cardiospermum halicacabum* L. – An Important Medicinal Climber

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Abstract:

Background:

Due to non-availability or short supply of original medicinal plants the crude drugs from the plants are adulterated by similarly resembling plants. Micromorphological studies of leaves could help in the identification and authentication of the original medicinal plant from the crude drug materials.

Objective:

To investigate the qualitative and quantitative micromorphological parameters of the leaves of *Cardiospermum halicacabum*.

Method:

The foliar micromorphological parameters such as orientation, stomatal types and morphology; density, distribution of trichomes and stomata; stomatal index and trichome types, paradermal sections were obtained manually by the standard method. The leaves were excised and fixed primarily in formalin, acetic acid and ethyl alcohol solution; cleared in 70% ethanol, bleached with 5% NaOH and rinsed in distilled water. The cleared leaves were used for the study of venation pattern, vein-islets, veinlet terminations and arrangement of crystals.

Results:

The microscopic examinations revealed the presence of anomocytic and anisocytic types of stomata with the abaxial epidermis of leaves. The stomatal density was 72 and stomatal index reported as 33.1. Glandular trichomes were few with uniseriate stalk, capitate multiseriate and multicellular head. Non-glandular trichomes frequent which were unicellular, uniseriate and bristle in nature. The density of vein islets and veinlet termination was observed as 18 and 13 respectively.

Conclusion:

The foliar micromorphological (stomata, vein clearing etc.) findings could be used in the proper identification and authentication of *C. halicacabum* from the crude plant materials which is essential in quality control measures.

Keywords: *Cardiospermum halicacabum*, Micromorphology, Stomatal indices, Vein islets.

INTRODUCTION

Cardiospermum halicacabum L. belongs to the family Sapindaceae and is universally recognized as the Balloon vine, Heart pea and Love in a puff. It is a dioecious, woody perennial climber, native to the tropical America, distributed in the tropical and subtropical Africa, Asia and throughout India. *C. corumdum*, *C. glabrum* and *C. inflatum*

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are the synonyms of Balloon vine [1]. The plant grows up to 2 to 4 m in length; leaves are deltoid with two highly lobed leaflets. Each leaflet possesses 2-ternate, lanceolate segment with inciso-serrate margins. Stem is long and wiry. Flowers are white, small and hermaphrodite, arranged in axillary, umbellate cymes. Two circinate tendrils situated at the top on a slender peduncles. The fruits are inflated, green and thin-shelled with triangular capsule. The seed coat is black, opaque, smooth and thick with a white finely porous, chordate spot at the micropyle [2].

The chemical profile of *C. halicacabum* reveals the presence of saponins, alkaloids, glycosides, apigenin, quebrachitol, luteolin, chrysoeriol, flavonoids, proanthocyanidin, stigmasterol, tannins, fatty acids and vital minerals [3, 4]. This plant has been exploited enormously for the commercial production of Allergy Relief Liquid™ and Bioforce Pollinosan® Tablets (marketed by Bioforce, USA) in treating hay fever, allergies, sneezing and watery eyes. Florasone *Cardiospermum* Cream (Boericke and Tafel, USA) is used for skin ailments such as swelling, scaling, blisters/vesicles, burning and pain. It is an active ingredient in lotions for dermatitis, eczema, and psoriasis [5]. Traditionally, the plant is reported to be useful in treating stiffness of limbs, chronic bronchitis, snakebites, urinary and stomach disorders [6]. The leaves are used to cure malaria, diarrhea, dysentery and haemorrhoids, asthma, pertusis, oedema, nephritis and oliguria, earaches, ophthalmias and muscular pains [7]. The whole plant possesses various biological activities including anthelmintic, anti-blenorrhagic, antidiarrhoeal, antifertility, anti-inflammatory [8], antipyretic, antiparasitic, analgesic, antibacterial, antioxidant, antiulcer, larvicidal, ovicidal [9], antihyperglycemic and nephroprotective properties [10].

The natural population of this plant is depleting day-by-day due to the uncontrolled harvest of the plants from the wild by the traditional drug manufactures and pharmaceutical companies. The plant materials are adulterated by the cheaper and similar type of plants due to non-availability of the original plant in the forest areas.

Light microscopy has long been used to study the internal and exomorphic features of vegetative and reproductive characteristics of plants [11]. Micromorphological characterization provides information for the investigation of taxonomic/systematic relationship between varieties of species, resolving problems in evolutionary relationships [12], and to understand the environmental parameters, which support the survival of species under particular climate etc. Developments in herbal medicine researches require good quality of raw material, standard methodology of drugs formulations and quality control parameters [13]. Microscopic examination of plant species or powdered materials is easy and cost effective method of identification and authentication [14].

Therefore, the objective of the present investigation is to establish foliar micromorphological and architectural parameters of traditional medicinal vine *C. halicacabum*, which could be useful in plant identification and authentication from the crude drugs material, monograph preparation and to understand the adaptation mechanism of plants developed under various environments.

MATERIALS AND METHODS

Collection and Identification of Plant Materials

Healthy vines of *C. halicacabum* were collected during the months of January to August, 2016 from the coastal areas of Puducherry (India) and identified with the help of Gamble flora [15]. Plants were randomly selected and the required leaf samples were collected at third to seventh leaves (4-5 leaves per plant) from the base with sterilized scissor. Fresh leaves were used for qualitative as well as quantitative micromorphological and leaf architectural studies. Ten sites were marked and ten plants per site were collected.

Foliar Micromorphological Studies

Experiments were conducted to study the micromorphological features of *C. halicacabum* leaves under normal environment. The foliar micromorphological parameters such as orientation, stomatal types and morphology; density, distribution of trichomes and stomata, stomatal index and trichomes types, the paradermal sections were obtained manually by standard method [16].

Venation Pattern and Evaluation of Vein Density

The leaves were excised and fixed primarily in formalin, acetic acid and ethyl alcohol (FAA in the ratio of 1:1:3, v/v) solution. The fixed leaves were cleared in 70% ethanol (v/v) until chlorophyll was completely removed (12-24 h), bleached with 5% (w/v) NaOH for 24-48 h, rinsed three times in distilled water and allowed to remain in saturated Chloral hydrate solution for 24-48 h [17]. The cleared leaves were used for the study of venation pattern, vein-islets, veinlet terminations and arrangement and morphology crystals.

The materials were stained with 1% (v/v) safranine (Loba chemie, India) aqueous solution for 3-5 min, mounted in water, examined under photomicroscope (Labomed iVu 3100, USA) and analyzed using software Pixelpro. These micrographs with different magnifications were used for identification of micromorphological and leaf architectural parameters. Magnifications of the figures were indicated by the scale-bars. The data for various parameters like stomatal density, frequency and stomatal index were calculated by the methods suggested by Salisbury [18]. Types of stomata have been described by following the classification and terminology as suggested latest by Prabhakar [19]. The terminology adopted for venation pattern was of Hickey and Wolfe [20].

Statistical Analyses

The statistical analyses were performed by ANOVA using SPSS version 16 (SPSS Inc., Chicago, USA). The significance of differences among mean values was calculated by Duncan's multiple range tests at $P < 0.05$ and results were presented as mean ± standard deviation (SD) of three experiments.

RESULTS AND DISCUSSION

The study of micromorphological and architectural parameters of *C. halicacabum* leaves using light microscopy revealed a number of important features, which were intrinsic to its habitat. The leaves were ovate, biternate with dentate margins. The leaves have prominent, thick crescent midrib towards abaxial epidermis, which was less prominent and conical on the adaxial epidermis.

The epidermal cells were compactly arranged and interrupted by stomatal apparatus. Almost all the cells were amoeboid in shape and visibly larger than the stomatal guard cells. The paradermal sections of the abaxial epidermis illustrate the presence of large, distinct and undulated regular anticlinal walls. In addition to these, glandular and non-glandular trichomes were also observed.

Stomatal Analysis

The guard cells of the stomata were equal and interpreted with some intercellular spaces. Stomata positioned at the same level of epidermal layers. Subsidiaries were not distinguished except in developing stomata. Stomata were observed on both the epidermal surfaces (amphistomatic leaves), but abaxial epidermis presented higher stomatal density than the adaxial epidermis. Predominant anomocytic stomata with indistinct subsidiaries and anisocytic stomata were observed on the abaxial surface (Fig. **1A**). Stomata were distributed randomly, facing all directions over the epidermis on the intercoastals regions and lying close to each other (Fig. **1B**). Stomatal density and index of the abaxial epidermis were 72 and 33.1 respectively (Table **1**).

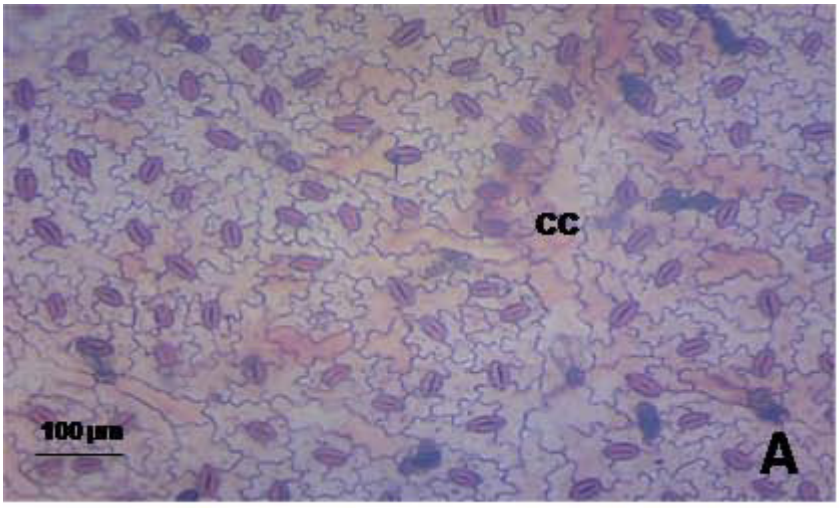

Fig. (1A). Paradermal section of abaxial epidermis showing epidermal cells and stomatal density (CC – Costal Cells).

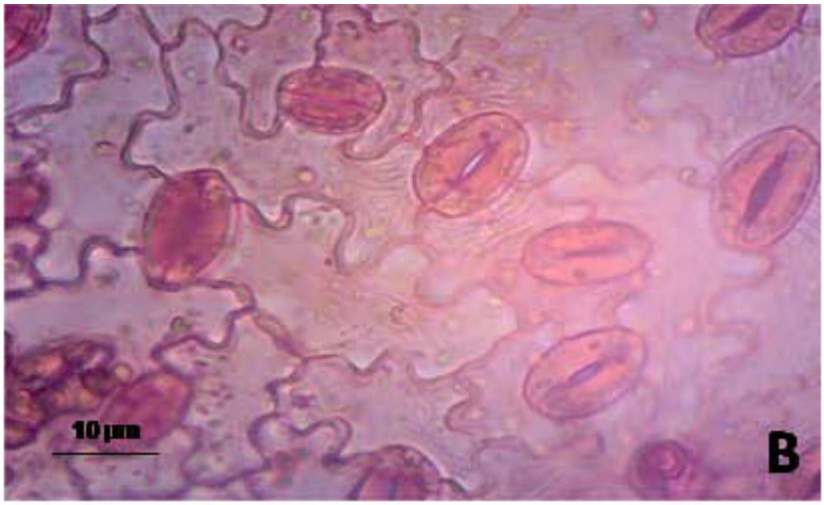

Fig. (1B). Magnified view of stomatal apparatus on abaxial epidermis.

Table 1. Stomatal density and stomatal index of abaxial surface of leaves of *C. halicacabum*.

Field No.	Stomatal Density (Mean±SD)	Stomatal Index (SI) (Mean±SD)
1	75.0±0.23^{g}	33.0±0.11^{c}
2	69.0±0.16^{b}	29.9±0.19^{a}
3	70.0±0.10^{c}	30.5±0.14^{b}
4	68.0±0.18^{a}	34.7±0.10^{d}
5	71.0±0.12^{d}	35.6±0.22^{e}
6	73.0±0.20^{e}	33.8±0.15^{c}
7	69.0±0.11^{b}	33.1±0.20^{c}
8	74.0±0.29^{f}	33.5±0.17^{c}
9	70.0±0.24^{c}	33.9±0.21^{c}
10	73.0±0.18^{e}	33.0±0.13^{c}
Mean	72.0±0.31	33.1±0.17

Note: Mean separation was analyzed using SPSS software (ver. 16.0); the values represented in corresponding column followed by same letters are not significantly different according to DMRT at $P < 0.05$.

Abaxial surface of the midrib is triangular in shape and made up of thick walled, angular cells with a prominent vascular strand. The values of stomatal frequency and index are directly proportional to the rate of transpiration [21]. Orientation of stomata on epidermis and their spacing are the indicative parameters of the nature of physiological plasticity of the guard cells. The leaves are liable to change towards climatic changes with great variations within the species and same individuals to some extent. Foliar micromorphological studies could be used as a constructive key in taxonomy between the families [22]. Stem surface possesses flattened epidermal cells with anomocytic stomata, and glandular and non glandular covering trichomes in this plant species as reported by Patil [23].

Trichomes Studies

Two types of foliar trichomes were observed in *C. halicacabum*, glandular capitate trichome and bristle like non-glandular hairy trichomes. Glandular trichomes possessed multicellular head (4 cells arranged in two rows and two columns), one celled stalk (subsessile), uniseriate and covered by a thick wall (Fig. **1C**). The non glandular hairy trichomes were unicellular with fine curved tip and broad base (Figs. **1E** and **1F**). The density of non glandular trichomes was more than capitate glandular trichomes (data not shown). Zalke *et al.* [24] measured the size of the trichomes (glandular trichomes 50 × 80 µm with dense cytoplasm, non-glandular trichomes 15 × 50 µm). Mucilagenous cells were also observed with both the epidermal cells (Fig. **1D**). Distribution of trichomes and their types are considered as the structural defense of plants and these are prejudiced by various external factors like, to reduce herbivore attack, tolerance to wind and frost, and to check water loss due to transpiration [25]. Glandular trichomes are known to secrete certain exudates such as resin and mucilage and prone to reduce the attack of herbivores [26].

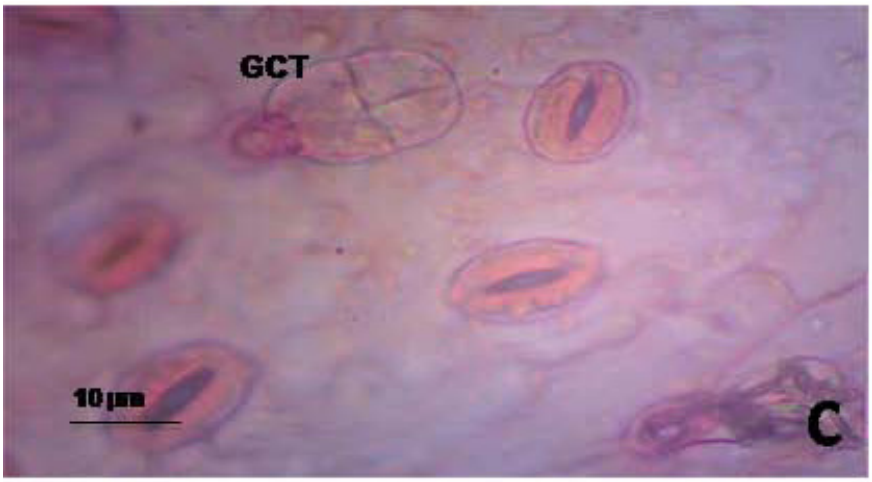

Fig. (1C). Glandular capitate trichome (GTC) on abaxial epidermis.

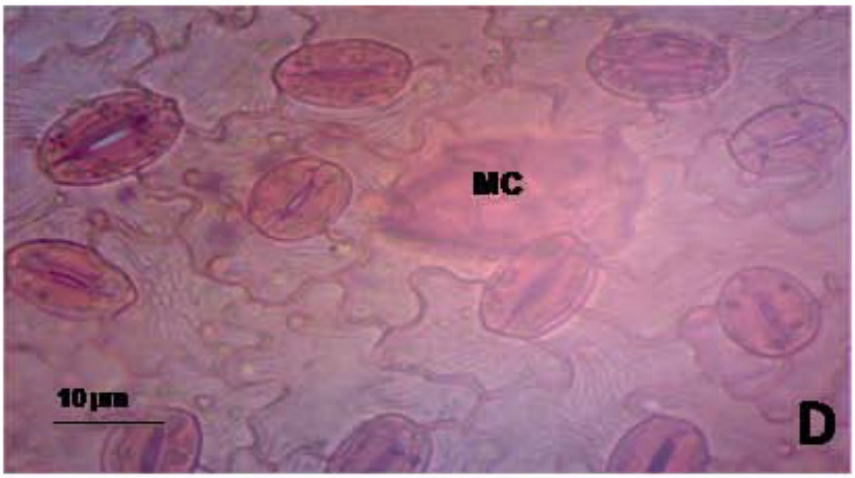

Fig. (1D). Mucilaginous cell (MC) on the abaxial surface.

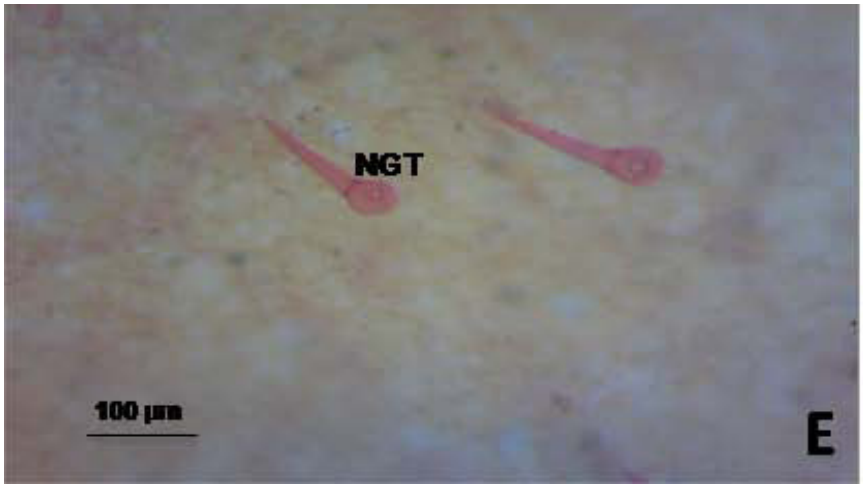

Fig. (1E). Non-glandular trichome (NGT) on abaxial epidermis.

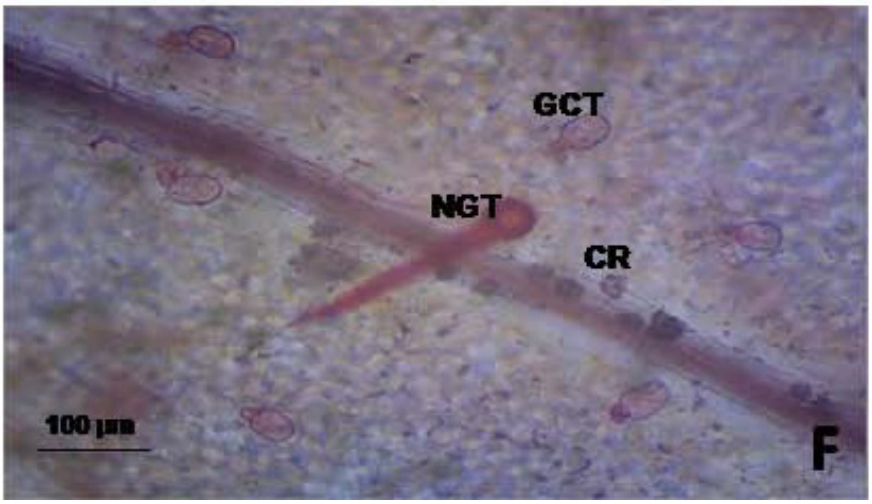

Fig. (1F). Glandular, Non-glandular trichomes and crystals on major vein (GTC– Glandular Capitate Trichome, NGT – Non-glandular Trichome, CR – Crystals).

Venation Pattern and Vein Density

Vein clearing study showed the presence of thick primary veins and the noticeable uniform lateral veins arose from the major vein. The major and minor veins were straight and exhibited clear reticulate venation pattern in *C. halicacabum*. The lateral veins ran towards the margins, joined with one another and encircled some discrete space. Vein-islets were distinct, mostly polygonal and rectangular in shape (Fig. **2A**). Each vein islet presented with simple, straight veinlet terminations (vein endings), mostly unbranched but rarely branched dichotomously. The density of vein islets and veinlet termination observed as 18 and 13 respectively (Table **2**). The developments of vein density in a species are indicative of proper transport of minerals and mechanical reinforcement of leaves which results in the formation of characteristic venation pattern [27]. Growth and increased vein system of species also implies the fitness of the plants towards environmental factors [28]

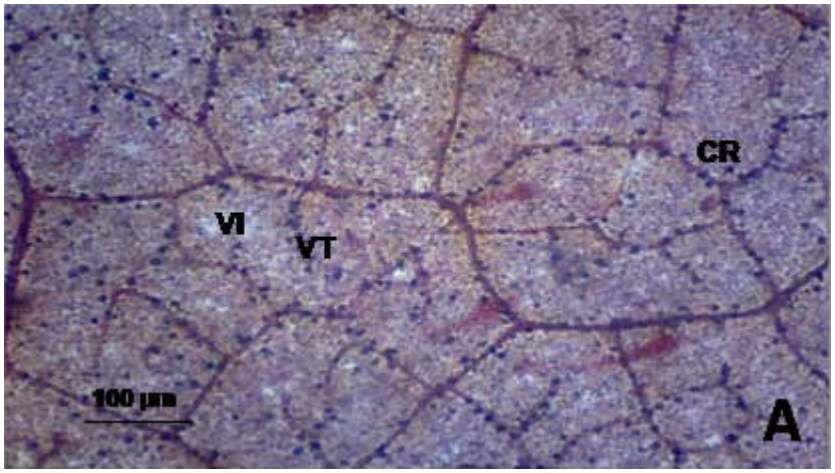

Fig. (2A). Venation pattern and vein density through cleared leaf and distribution of crystals over the veins (VI - Vein Islet, VT - branched Veinlet Termination).

Table 2. Leaf architectural parameters in *C. halicacabum*.

Field No.	Number of Vein Islets/mm^2 (Mean±SD)	Number of Veinlet Terminations/mm^2 (Mean±SD)
1.	16.0±0.15^a	11.0±0.19^a
2.	19.0±0.23^d	15.0±0.16^e
3.	16.0±0.17^a	12.0±0.20^b
4.	20.0±0.11^e	13.0±0.17^c

(Table 2) contd.....

Field No.	Number of Vein Islets/mm^2 (Mean±SD)	Number of Veinlet Terminations/mm^2 (Mean±SD)
5.	18.0±0.20^{c}	12.0±0.26^{b}
6.	22.0±0.27^{f}	14.0±0.15^{d}
7.	17.0±0.18^{b}	13.0±0.22^{c}
8.	20.0±0.13^{e}	15.0±0.10^{e}
9.	16.0±0.20^{a}	13.0±0.16^{c}
10.	16.0±0.24^{a}	12.0±0.11^{b}
Mean	18.0±0.19	13.0±0.10

Note: Mean separation was analyzed using SPSS software (ver. 16.0); the values represented in corresponding column followed by same letters are not significantly different according to DMRT at $P < 0.05$.

Crystals in *C. Halicacabum* Leaves

Oxalates of calcium (sand crystals) were observed all along the primary and secondary veins, arranged solitary as well as clustered on mesophylls (Fig. **2B**). Presence of crystals and their type in a species is determined genetically. The quantitative parameters of crystals/crystal density are influenced by several environmental factors such as intensity of light, existence of herbivory and concentration of calcium in the soil [29].

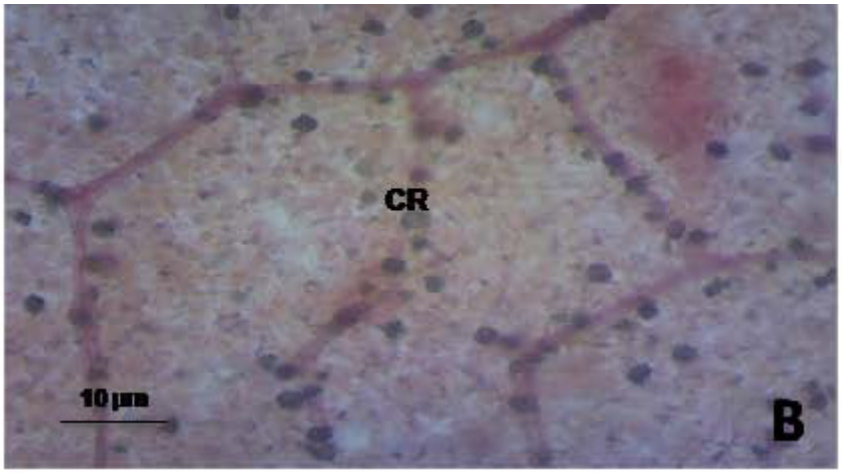

Fig. (2B). Arrangement of calcium oxlate sand crystals over major and minor veins (CR – Crystals).

CONCLUSION

The findings of the present study revealed the peculiar foliar micromorphological features of *C. halicacabum* collected from ten sites of Coromandel coastal areas of Puducherry, India. It could be useful for systematics as well as diagnostic studies of this plant in future. Besides authentication and identification, micromorphological evaluation of leaves could serve as a source of information to ascertain the environmental influence and plant's physiological performances. The present study may open new platform to analyze the response of leaf epidermal micro-parameters under various climatic conditions.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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