

Comparative Pharmacognostic Study on *Cardiospermum halicacabum* L. var. *Microcarpum* (Kunth) Blume and var. *Luridum* (Blume) Adelb Seeds

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Abstract

Cardiospermum halicacabum is one of the most important medicinal plants of sapindaceae. The present work initiate comparative pharmacognostic study on *Cardiospermum halicacabum* L. var. *microcarpum* (Kunth) Blume and var. *luridum* (Blume) Adelb, through macroscopic and microscopic characters of seeds. Both the varieties of *C. halicacabum* are called by one vernacular name 'Mudakkatran'. These two varieties have distinctive features both in morphology and anatomy. The two varieties of *C. halicacabum* are used medicinally one for the other. Most of the characters are similar but the variations occur in the leaves and seeds. The results of the present study showed diagnostic character and differences among the two varieties. Hence, plant anatomy is one of the alternative steps to differentiate the plant species.

Keywords: *Cardiospermum halicacabum*, Microscopic, Mudakkatran, Pharmacognosy, Sapindaceae

1. Introduction

Cardiospermum halicacabum L. under the family Sapindaceae is a deciduous, branching herbaceous climber, which is distributed throughout the tropical and subtropical regions of the world^{1,2}. The whole plant is used for several diseases like rheumatism, stiffness of limbs, snake bite for centuries³. The roots of these plants are diaphoretic, diuretic, emetic, emmenagogue, laxative, refrigerant, stomachic and sudorife⁴. Leaves and stalks consumed for the treatment of diarrhea and dysentery⁵. Phytochemical constituents such as flavones, glycones, triterpenoids, glycosides and a range of fatty acids and volatile ester are extracted from of this plant⁶⁻⁸. The seeds have been used for anticancerous⁹ and the seed oil has been found to have insect repellent¹⁰. However, the seed has not been studied so far. Hence, an effort was made to study anatomical characters of two varieties namely *Cardiospermum halicacabum*

L. var. *microcarpum* (Kunth) Blume and var. *luridum* (Blume) Adelb

2. Materials and Methods

2.1 Plant Material

Cardiospermum halicacabum L. is a medicinal plant (Balloon vine) belonging to the family Sapindaceae has been selected for the present investigation.

2.2 Collection of Plant Sample

The seeds of *Cardiospermum halicacabum* L. var. *microcarpum* (Kunth) Blume and var. *luridum* (Blume) Adelb. were collected from Thirumalairayan Pattinam, Karaikal, Pondicherry, Union Territory of South India during January – July 2015.

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2.3 Plant Identification

The identity of the plant specimens was confirmed by using the Floras¹¹⁻¹³ and other treatises^{2,14,15}. The botanical identity was also authenticated by Dr. M. Jegadeesan, Professor and Head, Dept. of Environmental and Herbal Sciences, Tamil University, Thanjavur, Tamil Nadu, India. Identity of the plant was confirmed with the help of type specimens available in the Herbarium of Botanical Survey of India, Southern Circle, TNAU Campus, Coimbatore, Tamil Nadu. The Herbarium specimens were prepared following the method of Jain and Rao¹⁶. The herbaria were deposited in Tamil University Herbarium, Voucher specimen No. TH51A and TH51B.

2.4 Taxonomy

Taxonomical studies on the plants were carried out and its systematic position has been assigned as per the angiosperm taxonomic classification of Bentham and Hooker¹⁷.

2.5 Pharmacognostical Studies

2.5.1 Microscopical Studies

Seeds of two varieties of *C. halicacabum* were fixed in FAA (Formalin 5 ml: Acetic acid 5 ml: 70% Ethyl alcohol – 90 ml). The materials were left in FAA for a few days and then they were dehydrated employing Tertiary Butyl Alcohol (TBA) series as per the procedure given by Sass¹⁸. Paraffin infiltration and embedding in wax blocks were done in the usual method¹⁹. Using Spencer Rotary Microtome, Serial paraffin sections of 10-12 μ m were prepared. These sections were stained with Toluidine blue as per the schedule suggested by O'Brien et al.²⁰. Toluidine blue stain was found to be quite satisfactory for microscopic observation of cells and tissue because of the meta chromatic property of the stain. Sections were also stained with fast green.

Microscopic observations were made both under normal and polarized lights. Photomicrographs were also taken with NIKON ALPHA PHOTO – 2 microscopic unit using normal and polarized lights. The polarized light was very much useful to detect the lignified elements, crystals and starch grains.

3. Results and Discussion

The taxon *Cardiospermum halicacabum* L. consists of two varieties viz., var. *microcarpum* (Kunth) Blume and var. *luridum* (Blume) Adelb. The var. *microcarpum* could be distinguished from var. *luridum* by the morphological

differences in leaves, fruits and seeds. *C. halicacabum* var. *microcarpum* is an herbaceous vine climber with coiled tendrils of the modified lower flowers of the inflorescence (Figures 1a, b). The leaves are 2- ternate, leaflets ovate lanceolate margins incised, lamina glabrous, petiole upto 3 cm long. Inflorescence: axillary raceme; flowers unisexual, plant monoecious. Male flowers: tetramerous with 4 sepals and 4 petals. Stamens: 8, filaments unequal, pilose, basally connate. Bisexual flowers with 8 stamens, tricarpellary, syncarpous ovary, one ovule in each cell; stigma 3 – fid. capsule sharply three lobed, not bloated; winged along the angles. Seeds – globose black, 3 mm with circular white, thin heart shaped aril (Figure 2a).

C. halicacabum var. *luridum* is a dense vine with hooked tendril of the longer modified flowers of the inflorescence (Figures 1c, d). The leaves are 2- ternate and densely tomentose below; Inflorescence: axillary raceme, male flowers 6 mm long, tetramerous with 4 sepals and 4 petals; stamens: 8, filaments pilose basally connate; Bisexual flowers tetramerous; ovary – 3 celled, one ovule in each cell; capsule bloated 3 lobed but not winged; seeds globose, 5 mm; aril white, larger, deeply heart shaped (Figure 2b).

Cardiospermum halicacabum var. *microcarpum*.

The seed is less than 4 mm in diameter. The surface is smooth and even. The aril is thin and covers only the top portion of the seed. The funiculus is thick to start with and becomes long, thin and whip like at the end. (Figures 3a, b).

Seed Coat: It is 650 μ m thick (Figure 3c). It consists of an outer seed coat which is the epidermal layer and inner seed coat and middle zone of seed coat. The epidermis has a thin cuticle. The epidermal cells are radially elongated and palisade like in shape; the epidermal layer is 50 μ m thick. The middle zone of the seed coat has five or six layers of oblong cells, which are densely filled with tannin content. The inner zone of the seed coat has thin walled, angular compact parenchyma cells, which have spherical mass of tannin (Figure 3d).

Cotyledons: The cotyledons are fleshy, folded and 550 μ m thick. It has thin epidermal layer consisting of isodiametric cells and compact, polygonal parenchymatous ground cells (Figure 3c).

Cardiospermum halicacabum var. *luridum*

The seed is 4.4 mm in diameter. The surface is smooth and even (Figure 4a). The aril covers one third of the seed and form a cap with long thin folded whip like funiculus (Figure 4b). The aril consists of thin epidermal layer 2–4 layers of small darkly stained cells and inner broad zone of soft parenchyma cells.

Seed coat: The seed coat (Figure 4a) is 630 μm thick. It is differentiated into three regions. The outermost region is the epidermis or the outer seed coat. It has prominent cuticle with smooth outer surface. The epidermal cells are radially elongated and palisade like measuring 50 μm in thickness. Their walls are thick and lignified. Inner to the epidermis is a broad zone of darkly stained parenchyma cells. These cells are tanniniferous. Inner to the tannin filled cell-zone is a wide zone of thin walled, compact parenchyma cells (Figure 4b).

The seed coat has abundance of calcium oxalate crystals. The epidermal cells have rhomboidal type of crystals, while the inner tissue has druses or phaeocrystals (Figures 4c, d). The rhomboidal crystals are 40 μm long; the druses are 30 μm in diameter.

Cotyledons: The cotyledons are folded and are thick and fleshy. It is 450 μm thick. It has thin epidermal layer and circular, thin walled, less compact parenchymatous ground tissue. Small less prominent vascular strands are seen in the cotyledons (Figures 5a, b).

In *Cardiospermum halicacabum*, two varieties are identified, var. *microcarpum* and var. *luridum*. Both the varieties of *C. halicacabum* are called by one vernacular name 'Mudakkatran'. These two varieties have distinctive features both in morphology and anatomy. The two varieties of *C. halicacabum* are used medicinally one for the other. Most of the characters are similar but the variations occur in the leaves and seeds. Var. *luridum* possess a larger leaf (5 – 12 cm), flower and fruit than var. *microcarpum*. Another important distinct difference is noted on the fruits and seeds. Large and pink coloured fruits are noted in the var. *luridum* but small, green colour fruits are seen in var. *microcarpum*. The size of the seeds showed marked difference, seeds are 4 mm and 5 mm respectively. Both the seeds are black in colour; while var. *microcarpum* but which showed thin heart shaped aril and var. *luridum* have deeply heart shaped aril.

In most of the flora, *Cardiospermum canescens* Wallich and *Cardiospermum halicacabum* are treated as two independent species^{11,13}. Mathew¹² however cites the opinion of P. Leench of Leiden and writes that *C. canescens* is synonymous with *C. halicacabum* var. *luridum*. The similarities (both morphological and anatomical) between the two varieties viz., var. *microcarpum* and var. *luridum* warrant their inclusion within a single species.

The seeds of *C. halicacabum* var. *microcarpum* and var. *luridum* could be distinguished by their anatomical characters. T.S. of seeds of both the varieties is more or less circular in outline had studied anatomy of seeds of *C. halicacabum*. They illustrated that the embryo is pachychalazal in con-

struction and due to concentric shape of embryo in the seed, transverse section of the seed has single cavity representing the embryo sac. In both the varieties the vascular bundles are arranged circularly and the integument is bitegmic²¹.

Calcium oxalate crystals constitute one of the major ergastic storage products in plant tissues. Their physiological role in the nutritive system of the plant or its economic value in pharmacological applications remains ambiguous. Extensive survey of crystals in plants by Solereder²², Chattaway²³, Metcalfe and Chalk²¹, Arnott and Pautard²⁴ is mostly related to the taxonomic studies of plants. Franceschi and Horner²⁵ have thrown light on the crystal morphology and their biosynthesis in plants. The relevance of crystallography in pharmacognostic studies has been well recognized by Wallis²⁶ and Evans²⁷. Generally, frequency, distribution and size of crystals are used as diagnostic characters in microscopical and powder studies of herbal drugs²⁸.

In var. *luridum*, the seed coat has abundance of calcium oxalate crystals. The epidermal cells have rhomboidal type of crystals, while the inner tissues have druses or phaeocrystals, which may be a used as diagnostic marker of this variety. No crystals are found in var. *microcarpum*.

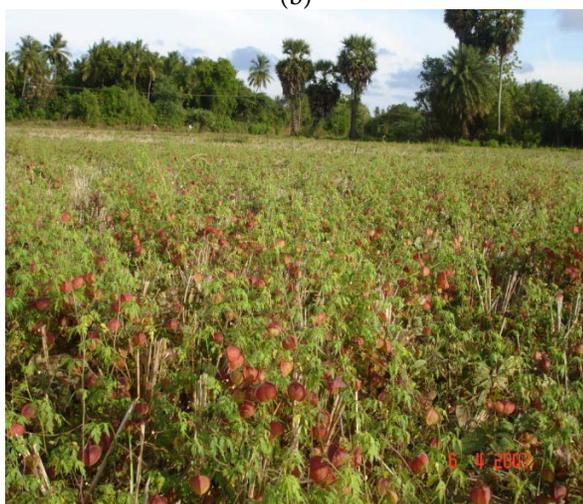
Results of the present investigation have brought to light the anatomical standards of certain specific features of both the varieties. This plant having a wide spectrum of pharmacological claims has not been given due importance in recent pharmacopoeias. The present study has provided for the first time, detailed botanical standards for the taxon as per the norms prescribed by WHO. Anatomical characters are always used in taxonomy for identification, and for grouping of plants in classification and for indicating patterns of relationship that may have been observed by superficial convergence in morphological features²⁹.



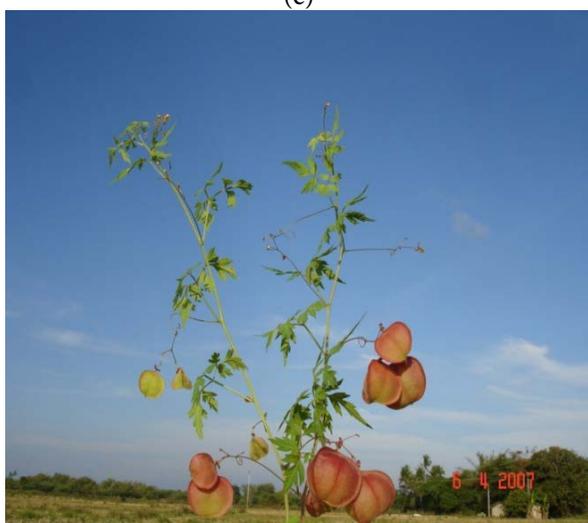
(a)



(b)



(c)



(d)

Figure 1. (a) - (b) Morphology of *Cardiospermum halicacabum* var. *microcarpum*
(c) - (d) Morphology of *Cardiospermum halicacabum* var. *luridum*.

The microscopical studies of *Cardiospermum halicacabum* L. var. *microcarpum* (Kunth) Blume and var. *luridum* (Blume) Adelb. revealed valuable information. The process of standardization of herbal drugs can be achieved by stepwise pharmacognostical studies³⁰. The standardization of crude drug is integral part of initiate its correct identity. Therefore, a proper identification is requiring to exactly identifying herbal ingredients used in remedies sold for home use. Hence, plant anatomy is one of the alternative steps to differentiate the plant species³¹.



(a)



(b)

Figure 2. Morphology of *Cardiospermum halicacabum* seeds
(a) var. *microcarpum*
(b) var. *microcarpum*.



(a)

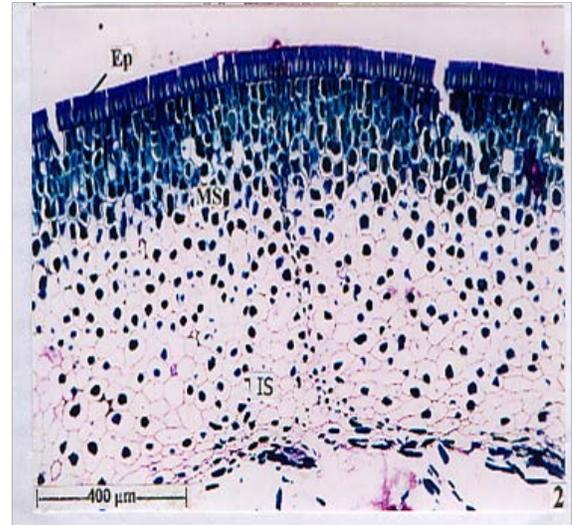


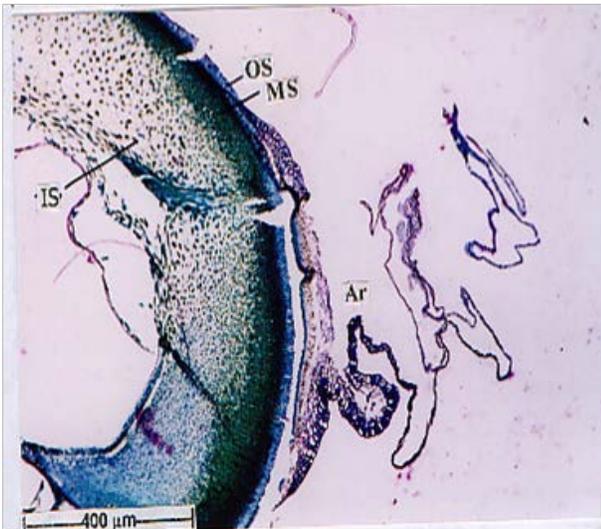
Figure 3. *Cardiospermum halicacabum* var. *microcarpum*.

(a) L.S of Seed

(b) T.S of Seed

(c) T.S of Seed showing structure of seed coat and cotyledons

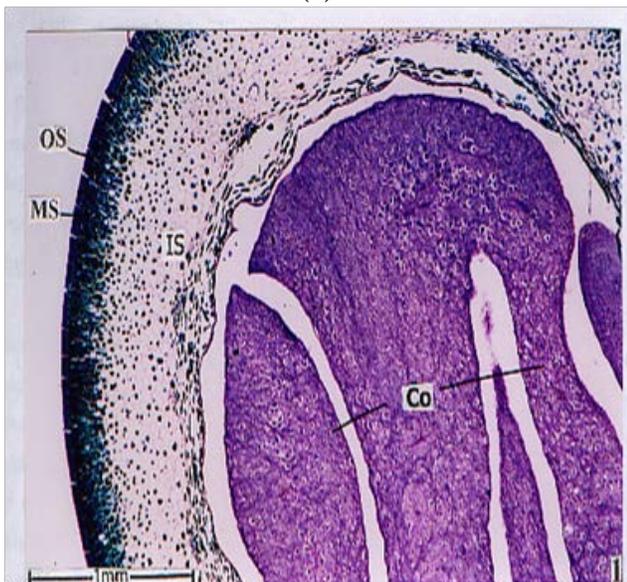
(d) Seed coat enlarged



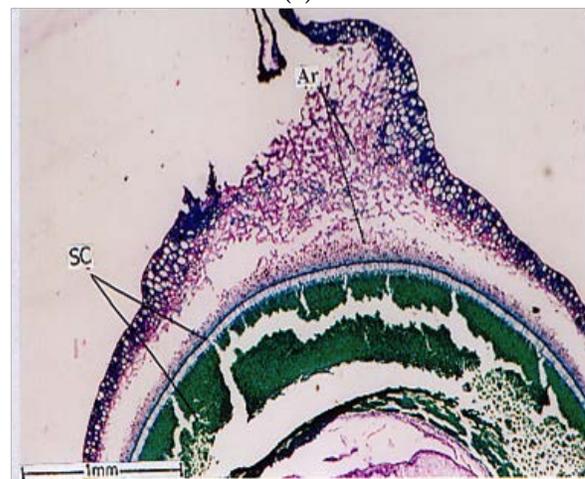
(b)



(a)



(c)



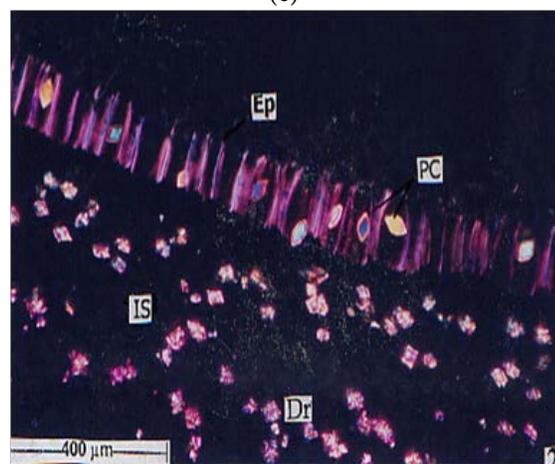
(b)



(c)



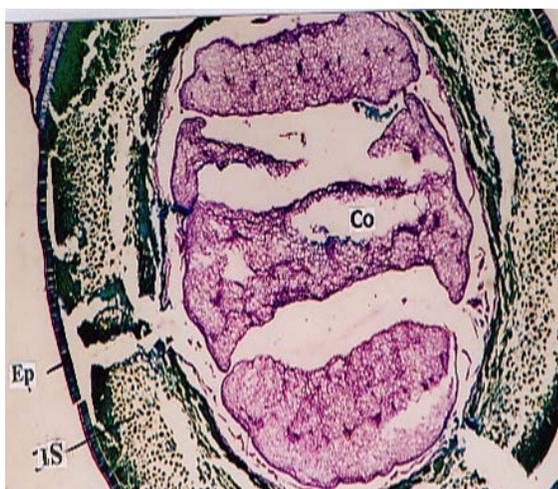
(b)



(d)

Figure 4. *Cardiospermum halicacabum* var. *luridum*.

- (a) T.S of Seed
- (b) L.S of Seed
- (c) Seed coat and cotyledons
- (d) Seed coat under polarized light showing the presence of crystals



(a)

Figure 5. (a) L.S of Seed showing cotyledons.
(b) Cotyledons in enlarged view

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