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Research Article

Pharmacognostical Standardization of *Cardiospermum Halicacabum* (L). Leaf

Suganya R[1*], Periyanayagam K[2] and Senniappan P[3]

[1*] *Department of Pharmacognosy, Swamy Vivekananda College of Pharmacy, Elayampalayam,Tiruchencode, Namakkal-637 205, Tamil Nadu, India*
[2] *Department of Pharmacognosy,College of Pharmacy, Madurai Medical College, Madurai-625020, Tamilnadu, India.*
[3] *Department of Pharmacognosy, Vinayaka Mission's College of Pharmacy, Vinayaka Mission's Research Foundation (Deemed to be University), Kondappanaickenpatty, Salem-636008, Tamilnadu, India.*

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ABSTRACT
The present study deals with a widely available plant leaf, *Cardiospermum halicacabum* L. It is popularly known as "balloon vine", tamil name "Mudakkathan". It belongs to the family sapindaceae. Leaves are pubescent or nearly glabrous annular perennial with slender branches climbing by means of tendriller. 2-4cm in length, pale or light green, dentate irregularly deeply incised margin, acute apex, obtuse-truncate beneath base. Microscopic evaluation revealed that the presence of anamocytic stomata in both upper and lower epidermis, uniseriate or multicellular trichomes, xylem vessels, phloem, and fibers. SEM showed the uniseriate multicellular trichomes. Vein islet numbers, vein termination numbers, Stomatal number, stomatal index and other physicochemical tests like ash values, loss on drying, extractive values were determined. Preliminary phytochemical screening showed the presence of alkaloids, carbohydrates, sterols, saponins, tannins, proteins and free amino acids, terpenoids, flavonoids and absence of volatile oil, fixed oil, and glycosides. Histological identification, microscopic constants and other physicochemical examinations of the leaves of *Cardiospermum halicacabum L.* can be used as rapid, inexpensive botanical identification technique and is useful in standardization, hence it would be of immense value in authentication of the leaf.
Keywords: *Cardiospermum halicacabum (L.), Pharmacognostical Standardization of Leaves.*

INTRODUCTION

Plant materials are used throughout the world as home remedies, over the counter drug products and raw materials for the pharmaceutical industry and represent a substantial proportion of the world drug market. It is therefore important to establish their quality. Present study deals with a widely available plant leaf, *Cardiospermum halicacabum* L. It is popularly known as "balloon vine" in Tamil it's called as "Mudakkathan" belonging to the family sapindaceae.[1,2,3]

It was reported that the leaves contain saponins, alkaloids, (+) pinitol, apigenin, leutolin, sterols, terpenoids, flavonoids. *C.halicacabum* frequently useful in Ayurveda, Sidha, Homeopathy, Unani and other Indian system of medicine to treat Rheumatoid Arthritis, GI disease, Respiratory disease, Inflammatory disease in India and China.[4] The leaves used to treat fever associated with cough, ear ache, hemorrhoids, diarrhea.[3] The seeds of *C.halicacabum* is used as oral pain relievers or applied to aching joints as a paste.[5]

The root is mucilaginous and considered emetic,laxative a nd anti rheumatic.[6] It has screened pharmacological activity such as antimicrobial[7], antiarthritic[8], antidiabetic[9], CNS activity[10], radical scavenging effect[11,] antihyperlipidimic[12], anti-inflammatory & antipyretic [13], increase sperm motility [14], antiulcer[15] , antioxidant[16], anxiolytic & memory enhancing effect.[17] In short, there is good level of traditional and experimental evidence to support various claims and advantages of this widely available plant. As mentioned earlier several reports have been published on the beneficial effects of the plant extracts and chemical constituents on different biological activities *in vivo*. An investigation to explore its pharmacognostic examination is inevitable. Hence, in this work we report an attempt on microscopic evaluation including scanning microscopy, physicochemical determination and phytochemical screening for the standardization and quality assurance purpose of this plant.

MATERIAL AND METHODS

Chemicals:

Formalin, acetic acid, ethyl alcohol, blue, phloroglucinol, glycerin, hydrochloric acid and all other chemicals used in this study were analytical grade.

Plant collection and authentication:

The leaves of the healthy plant Cardiospermum halicacabum L. selected for the study was collected from in and around Allampattu, karaikudi, Sivagangai District, Tamilnadu, India and identified by Dr. G. Stephen Asst. professor, botany, The American College, Madurai.

**Author for Correspondence: suganyar@gmail.com*

Macroscopic analysis:
Macroscopic observation of the plant was done. The shape, size, surface characters, texture, colour, odour, taste etc were noted.[18]

Microscopic analysis:
Transverse section midrib region of fresh leaf pieces was cut and fixed in FAA and then dehydrated by employing graded series of ethyl alcohol and tertiary butyl alcohol[19] Paraffin embedded sections were taken using microtome. Permanent mount was prepared using toluidine blue staining technique. In order to supplement the descriptive part, the photomicrographs in different magnifications of all necessary cells and tissues were taken with NIKON Coolpix 8400 digital camera and Labphot2 microscopic unit.

SEM sample preparation:
Sample for SEM analysis were mounted on the specimen stub using carbon adhesive sheet. Small samples were mounted with 1sq.cm glass slide and kept in carbon adhesive sheet. Samples were coated with gold to a thickness of 100 AO using Hitachi vacuum evaporator. Coated samples were analyzed in a Hitachi Scanning Electron Microscope 3000 H model.[20]

Powder microscopy:
Coarse powder of the leaf was used to study the microscopical characters of the leaf powder.

Physicochemical analysis:
Total ash, acid insoluble ash, water soluble ash, sulphated ash, loss on drying, extractive values and leaf constants such as vein islets numbers, vein termination number, stomatal number and stomatal index, palisade ratio were determined.[21,22,23]

Preliminary phytochemical screening:
Preliminary phytochemical screening was carried out to find out the presence of various phytoconstituents using standard procedure.[21, 24, 25, 26, 27].

RESULTS

Macroscopy:
Cardiospermum halicacabumis a pubescent or nearly glabrous annular perennial with slender branches climbing by means of tendriller, belonging to the family Sapindaceae. (Fig-1). The shape of the leaves is Ovate–lanceolate, 2-4 cm in length, pale or light green in colour, dentate irregularly deeply incised margin. Apex is acute with obtuse truncate beneath base (Fig2). Stems are green in colour, puberulous, slightly woody, tendrils present, 5 6 sulcate, slender, glabrous, flowers are white with yellowish centre, bears 4 sepals (2 large, 2 small), 4 whitish petals, 4mm long, 3-celled ovary bears one ovule per cell, 8 stamens present. Fruits are Inflated, paperycapsule, 3 chambered, 3 - 4.5 cm in diameter, before ripe, green colour, after ripen slight yellowcolour. 8-10 prominent longitudinal ribes not covered with spines or papillae. Seeds are opaque, finely porous heart shape, black, smooth with white in colour and 5mm diameter in size.

Figure 1 Habit of *Cardiospermum halicacabum* (L**)**

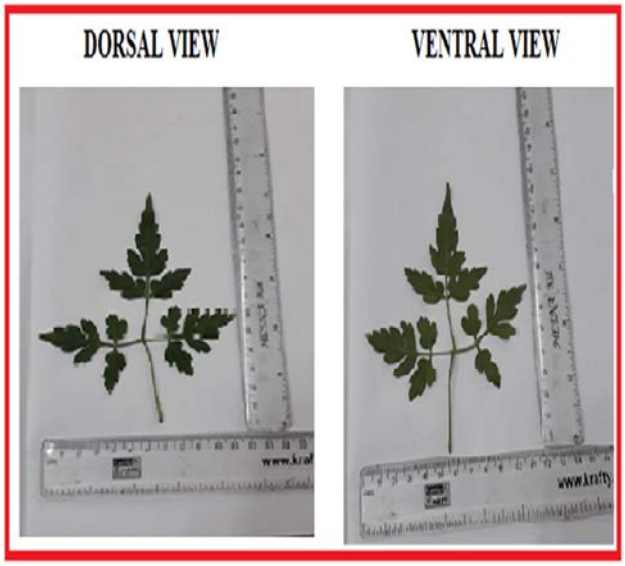

Figure 2 Dorsal and ventral view of *C.halicacabum* (L) leaf

Microscopy of the leaf:
Transverse section (T.S) of the leaves through midrib showed the following tissue systems. Shape: It is project in both adaxial and abaxially. The adaxial part is thick and pyramid like but abaxial part is semicircular with undulate outline. The inner part of the adaxial cone includes a cluster of angular collenchyma cells.

Epidermis: Fairly large squarish thick cells. The abaxial epidermal cells are thin, small and elliptical. Paradermal section shows epidermal cells wide with highly wavy anticlinal walls amoeboid outline.
The stomata are dense and diffuse in distribution and are anomocytic type. The guard cells are elliptical and the stomatal pores are slit like (Fig-4)

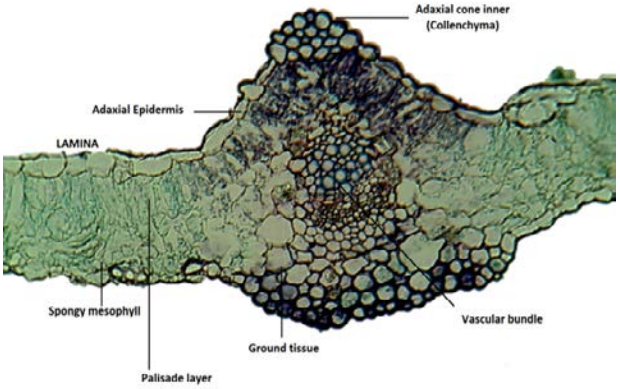

Figure 3 T.S of *C.halicacabum (L)* leaf through the midrib

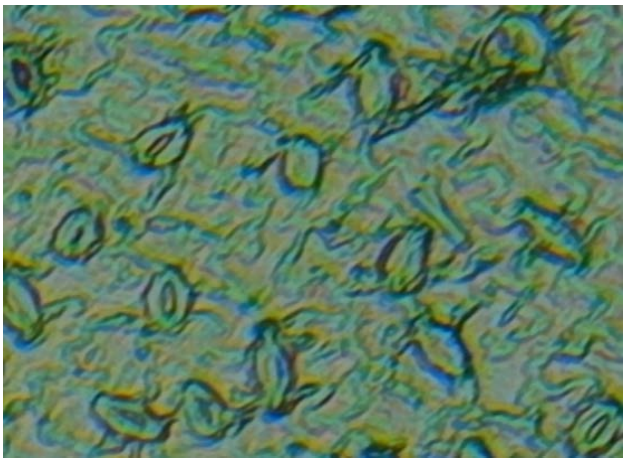

Figure 4 Epidermis surface view showing anamocytic stomata

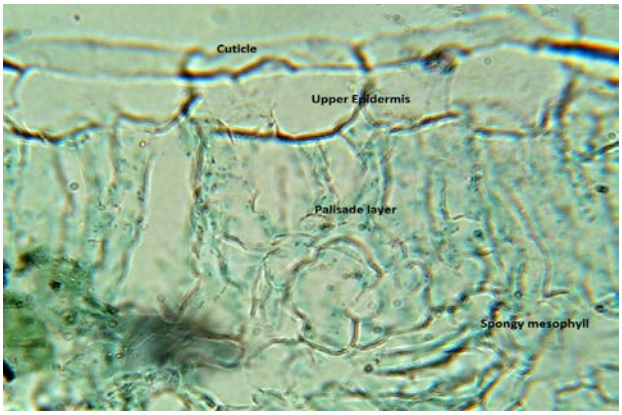

Figure 5 T.S of Lamina

Lamina:

The Mesophyll is differentiated into upper band of narro w cylindrical palisade cells and lower

zone of two or three layer so flobed loosely arranged spon gy parenchyma cells. The lateral veins are conspicuous, straight and uniformly thin. The veinislets are distinct having straight veinboundarys. Vein termination are present in the islets some places branched once or twice and spread within the islets.(Fig 5).

Powder microscopy

The analysis of the dried powder of the leaf showed Ranunculaceous stomata (Anamocytic stomata), parenchyma, collenchyma cells, fibers, uniseriate multicellular trichomes. (Fig -6).

Physicochemical analysis

Physicochemical parameters were found as follows: Total ash 6.8%w/w, Acid insoluble ash 1% w/w, water soluble ash 4.08% w/w, Losson drying 6.72% w/w, ethanol soluble extractive value 13.5 %w/w, water soluble extractive value10.3% w/w. Leaf constants were as follows stomatal index (Lower epidermis:10.7, upper epidermis:12.5), stomatal number (Lower epidermis:22, Upper epidermis:23) vein islet number, veinlet termination numbers 15 and 10 respectively.

Preliminary phytochemical screening:

Preliminary phytochemical screening showed the presence of alkaloids, carbohydrates, sterols, saponins, tannins, protein and amino acids, terpenoids and flavonoids and absence of glycosides, volatile oil, and fixed oil.

DISCUSSION

Adulteration and misidentification of medicinal plants can cause serious health problems to consumers and legal problems for the pharmaceutical industries. The past decade has witnessed the introduction and implementation of new Good Manufacturing Practices (GMP) in quality control of raw materials, intermediates and finished products of botanical origin. The initial step in quality control of medicinal plants is ensuring the authenticity of the desired species for the intended use. It can be conducted via a variety of techniques, namely macro and microscopic identification and chemical and chemical analysis especially description of microscopic botanical aspects to determine definitively the proper species of plant material while it is still in its non extracted form. The observation of cellular level morphology or anatomy is a major aid for the authentication of drugs.

These characters are especially important for identification of powdered drugs, because in these cases most of the morphological diagnostic features are lost. Microscopic evaluation is one of the simplest and cheapest methods for the identification of the source of the materials. In this present work the selected plant *C.halicacabum*, Muddakkathan (sapindaceae). The macroscopic and organoleptic characters' of the leaf can serve as diagnostic parameters such as presence of anamocytic stomata in epidermis. The inner part of adaxial cone includes a cluster of angular collenchyma cells. Uniseriate and multicellular trichomes are present. The physical constant evaluation of the drug is an important parameter in detecting adulteration or improper handling of drugs. The ash values are particularly

important to find the presence or absence of foreign inorganic matter such as metallic salts and or silica (earthy matter). The extractive values are primarily useful for the determination of exhausted or adulterated drug. Preliminary
phytochemical screening showed the presence of carbohydrates, protiens, aminoacids, alkaloids, saponins, flavonoids, tannins and terpenoids. In conclusion, the present study was undertaken with a view to lay down standards which could be useful to detect adulterants and provide, authenticity of this medicinally useful plant.

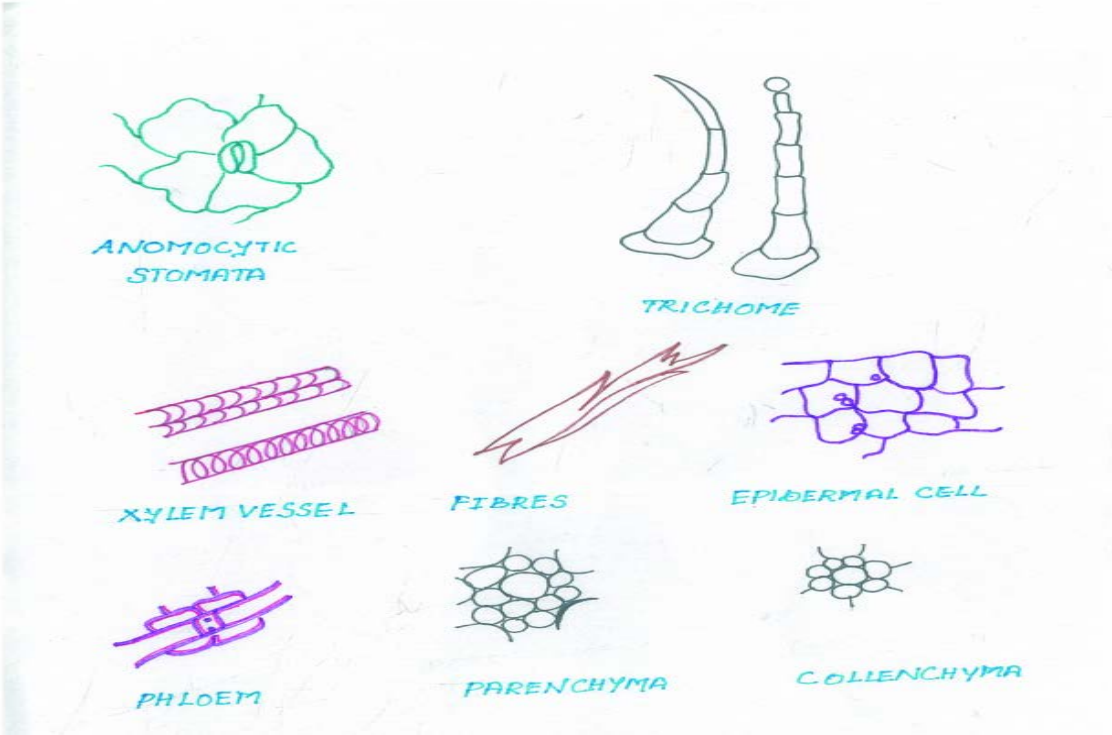

Figure 6 Powder microscopy of the leaf of C.halicacabum *L* (Hand diagram)

CONCLUSION

Standardization of herbal drugs have a great importance and it's subject to variability amongst the species from various geographical region. The microscopic using histological identification, microscopic constants and other physical chemical examinations of the leaves of *Cardiospermum halicacabum* L. can be used as rapid, inexpensive botanical identification technique and is useful in standardization, hence would be of immense value in authentication of the leaf.

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