Pharmacognostical evaluation of Cardiospermum halicacabum Linn. leaf and stem

Article · March 2013
DOI: 10.4103/0257-7941.134561 · Source: PubMed

CITATIONS
8

READS
195

4 authors, including:

Ashish Suresh Zalke
Eris Lifesciences
5 PUBLICATIONS 27 CITATIONS

Upendra B Gandagule
College of Pharmacy - Sakegaon, Bhusawal
20 PUBLICATIONS 20 CITATIONS

Some of the authors of this publication are also working on these related projects:

Pharmacognostical and phytochemical evaluation of the leaves of Ziziphus xylopyrus (Retz) Willd View project

All content following this page was uploaded by Upendra B Gandagule on 05 December 2018.

The user has requested enhancement of the downloaded file.
Pharmacognostical evaluation of *Cardiospermum halicacabum* Linn. leaf and stem

Ashish S. Zalke, B. Duraiswamy, Upendra B. Gandagule, Nidhi Singh

Department of Pharmacognosy, JSS College of Pharmacy, Rocklands, Ootacamund, Tamil Nadu, India

**ABSTRACT**

**Background:** *Cardiospermum halicacabum* Linn (Sapindaceae) is an important medicinal plant in the traditional system of medicine, known as *karnaphota*. The root of it is officially included in Ayurvedic Pharmacopoeia for its therapeutic uses such as *jvara*, *kṣuṭha*, *pāṇu*, *kṣaya* and *sandhīvāta* etc. As no detailed analysis of macroscopy, microscopy characters of the plant, except root, have been carried out till date, it was thought worth to carry out the detailed macroscopic and microscopic study of leaves and stem, following standard pharmacognostical procedures.

**Materials and Methods:** Pharmacognostic studies of *C. halicacabum* were carried out, and in this, the macroscopic, microscopic, physicochemical, fluorescence and phytochemical analyses were done. Physicochemical parameters such as total ash, moisture content, extractive values were determined by World Health Organization guidelines. The microscopic features of leaf and stem components were observed.

**Results:** Macroscopically the leaves are bi-ternate, ovate-lanceolate in shape with dentate margin. Microscopically, leaf shows prominent midrib and thin dorsiventral lamina. The midrib shows the presence of epidermal layers, angular collenchyma, palisade cells and vascular strands comprised of thin walled xylem and thick walled phloem elements. The lamina shows prominent, narrow and cylindrical upper epidermis. The upper epidermal cells are large and contain mucilage, whereas lower epidermis possesses thin, small and elliptical epidermal cells. The mesophyll was differentiated into two zones upper and lower. The upper zones show narrow cylindrical palisade cells and lower zone shows 2-3 layers of loosely arranged spongy parenchyma cells. In the Paradermal section of the lamina we observe anomocytic stomata. The transverse section of stem shows a pentagonal appearance with five short blunt ridges and prominent cuticle. Parenchymatous cells, cortical sclerenchyma, lignified xylem fibers, phloem and pit were also found. In the powder microscopy of whole plant, glandular trichomes, non-glandular trichomes, fragments of lamina, xylem elements, parenchyma cells and fibers are observed. Phytochemical screening reveals that the *C. halicacabum* extract contains glycosides, carbohydrates, flavonoids, phytosterols, phenolic compounds and saponin.

**Conclusion:** Various pharmacognostic characters observed in this study help in identification, quality, purity and standardization of *C. halicacabum*.

**KEY WORDS:** *Cardiospermum halicacabum*, fluorescence analysis, macroscopy, microscopy, physicochemical, phytochemical

---

**INTRODUCTION**

*C. halicacabum* Linn. (Sapindaceae), English name: Balloon Vine, is an annual or sometimes perennial climber, commonly found as a weed throughout India. The tender, young shoots are used as a vegetable, fodder, diuretic, stomachic and rubefacient. It is used in conditions like rheumatism, lumbago, nervous diseases, and as a demulcent in orchitis and in dropsy. In Sri Lanka, it is used for the treatment of skeletal fractures. The juice of the herb is used to cure ear ache and to reduce hardened tumors. It exhibits significant analgesic, anti-inflammatory activity and vasodepressant activity, which is transient in nature. *In vitro* studies have revealed its antispasmodic and curate-like actions. It is for this reason perhaps that the plant finds its use in Ayurvedic medicine.[1] The leaf of this plant mixed with castor oil is administered internally to treat rheumatism and to check lumbago.[2] Two glasses of a 12 h maceration of aerial parts of the plant are consumed or used for bathing in the treatment of hyperthermia in a few places and in some others, water extracts of the seed are used.[3]

Previous studies which have reported phytochemical investigations of the plant revealed the presence of flavones,
Microscopic evaluation

In microscopic evaluation, studies were conducted qualitatively and quantitatively.

Qualitative microscopy

Care was taken to select healthy and normal leaf and stem material. The required samples of stem and leaves were cut and removed from the plant and fixed in a solution consisting of formalin 5 ml + acetic acid 5 ml + 70% ethyl alcohol 90 ml. After 24 h of fixing, the specimens of leaves and stem were dehydrated with graded series of tertiary-Butyl alcohol as per the schedule given by Sass, 1940. Infiltration of the specimens of leaves and stems were carried by gradual addition of paraffin wax (mp 58-60°C) until tertiary Butyl alcohol solution attained super saturation. The specimens of leaves and stems were cast into paraffin blocks.

The paraffin embedded specimens of leaves and stems were sectioned with the help of rotary microtome. The thickness of the sections was 10-12 µm. Dewaxing of the sections was done using the customary procedure.
The sections were stained with toluidine blue as per the method published by O’Brien et al. Toluidine blue is a polychromatic stain. The staining results were remarkably good and some cytochemical reactions were also obtained. The dye rendered the cellulose walls pink, lignified cells blue, suberin dark green, mucilage violet and protein bodies blue etc., Wherever necessary, sections of leaf and stem were also stained with safranin and fast green and potassium iodate (for starch).

To study the stomatal morphology, venation pattern and trichome distribution, paradermal sections (sections taken parallel to the surface of the leaf) as well as clearing of leaf with 5% of sodium hydroxide or epidermal peeling by partial maceration employing Jeffery’s maceration fluid were prepared. Glycerine mounted temporary preparations were made for macerated/cleared leaf.

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon lab photo-2 microscopic unit. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against the dark background. Magnifications of the figures are indicated by the scale bars. Descriptive terms of the anatomical features are as given in the standard anatomy books.

Materials and methods

Plant material

C. halicacabum was collected from Tirupati, Andhra Pradesh, India in the month of October 2009. The plant was identified and authenticated by Professor K. Madhava Chetty, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India under the reference letter number SVU/SC/12/55/09-10.

Macroscopic evaluation

The macroscopic characters of fresh leaves and stem of C. halicacabum such as size and shape, color, surface, venation, apex, margin, lamina, texture, odor and taste were recorded.

Physicochemical and phytochemical analysis

Physicochemical parameters such as ash values and extractive values were determined according to the well-established procedures. Preliminary phytochemical screening was carried out using the standard procedure.

Fluorescence analysis

Powdered material was treated with various chemical reagents and exposed to visible, ultraviolet light (Short UV) and long UV to study their behaviour under fluorescence. The changes in appearance and color were observed and recorded.
Powder microscopy
For powder microscopy, the dried aerial part material was powdered. Powdered material was cleared with sodium hydroxide and mounted in glycerine medium after staining. Different staining reagents such as toluidine blue, safranin, fast green and iodine were used. Different cell components were studied and measured using photomicrography.

Quantitative microscopy
In quantitative microscopy determination of stomatal number, stomatal index, vein islet, vein termination number and palisade ratio were carried out.[26]

RESULTS
Macroscopy of stem and leaves
The plant is a herbaceous vine. The leaves are bi-ternate. Leaflets are ovate-lanceolate, having glabrous texture, smooth surface and dentate margins. Length of the leaf is 3-5 cm and breadth 1.5-2 cm. Stems having width 0.2-0.3 cm and green in color. Petiole is 1.5-2.5 cm. Leaf has a bitter taste and characteristic leafy odor. Flowers tetramerous irregular, 2 + 2 sepals, 2 + 2 unequal petals. Stamens-8, unequal. Ovary is tricarpellary with one ovule in each carpel. Stigma is trifid. Fruits are globose, winged bloated capsule.

Physicochemical and phytochemical analysis
Physicochemical analysis of aerial part plant powder viz. ash values, extractive values and moisture content are presented in Table 1. The preliminary phytochemical analysis of the extract of the aerial part shows the presence of glycoside, carbohydrate, flavonoid, phytosterols, phenolic compounds and saponin.

Fluorescence analysis
The fluorescence analysis of aerial part plant powder material under day and UV (short 254 nm) light is recorded in Table 2.

Microscopy evaluation
Microscopy of leaf
The leaf has prominent, adaxially and abaxially projecting midrib and thin dorsiventral lamina [Figure 1a]. The adaxial part of the midrib is thick and pyramid like and the abaxial part is semicircular with undulate outline. The midrib is 350 µm thick. The adaxial cone is 150 µm wide at the base and the abaxial part is 300 µm wide. The midrib possesses thick, distinct epidermal layers of fairly large squarish, thick walled cells. The inner part of the adaxial cone includes a cluster of angular collenchyma cells. The palisade cells extend up to the shoulders of the adaxial cone. The lower part of the midrib consists of fairly thick walled, angular cells. The vascular strand is fairly prominent. It is triangular and comprises a cluster of wide circular thin walled xylem elements and a thick band of phloem elements [Figure 1a].

Table 1: Physicochemical evaluation C. halicacabum powder

| Physicochemical constant (%w/w) | Powder of whole plant |
|---------------------------------|------------------------|
| Total ash                       | 7.3±0.08               |
| Water soluble ash               | 6.29±1.45              |
| Acid insoluble ash              | 1.26±0.19              |
| Water soluble                   | 8.96±1.89              |
| Alcohol soluble                 | 7.06±2.95              |
| Moisture content                | 11.40±0.36             |

Table 2: Fluorescence analysis of whole plant powder of C. halicacabum

| Reagents (%) | Short UV (254 nm) | Long UV (366 nm) | Visible         |
|--------------|-------------------|------------------|-----------------|
| Powder as such | Green            | Brownish green   | Light green     |
| Powder +1N NaOH         | Black            | Greenish black   | Green           |
| Powder +picric acid     | Black green      | Black            | Green+yellow    |
| Powder +acetic acid     | Black green      | Green or grey    | Green+brown     |
| Powder +1N HCl          | Black green      | Black            | Brownish green  |
| Powder +1N HNO₃         | Black green      | Black            | Green+brown     |
| Powder +5 iodon        | Dark brown       | Black            | Brown+green     |
| UV: Ultraviolet light  |                  |                  |                 |
The lamina is 60-70 µm thick. The adaxial (upper) epidermis is prominent, narrow and cylindrical. Some of the upper epidermal cells are dilated and possess dense mucilage. The abaxial epidermal cells are thin, the cells being small and elliptical. The mesophyll is differentiated into upper band of narrow cylindrical palisade cells and lower zone of two or three layers of lobed loosely arranged spongy parenchyma cells [Figure 1b].

As seen in paradermal section of the lamina, the epidermal cells are wide and possess thin highly wavy anticlinal walls [Figure 2a]. The cells assume amoeboid outline. The surfaces of the cells are smooth and no cuticular markings are evident. The stomata are dense and diffuse in distribution. They were of anomocytic type. No specific subsidiary cells are seen. The guard cells are elliptical and the stomatal pores are slit like. The guard cells are 50 × 80 µm in size [Figure 2b].

The lateral veins are conspicuous. They are straight and uniformly thin. The vein-islets are distinct and they had distinct thin straight vein boundaries. The islets are fairly wide and their shape may be rectangular, squarish or polygonal [Figure 3a].

Vein-terminations are present in all islets. They are unbranched or branched once/twice and spread within the islets [Figure 3b].

Microscopy of stem
The stem is pentagonal in sectional view, having five short blunt ridges [Figure 4a]. It consists of a continuous layer of epidermis which possesses small spindle shape cells with prominent cuticle [Figure 4b]. Inner to the epidermis is a layer of small darkly strained cells followed by one or two layers of hyaline parenchyma cells. Within the ridges, the parenchyma tissue is wider and the cells are enlarged [Figure 5c].

There as a fairly thick continuous cylinder of cortical sclerenchyma, running all around the stem. The cylinder is 4-cells thick. In the region of the ridges, the sclerenchyma cylinder becomes much thicker having several layers of small cells [Figures 4b and 5c].

The vascular cylinder is angular in outline. It possesses outer wide cylinder of phloem enclosing inner xylem cylinder. Xylem includes a circle of solitary wide and narrow vessels associated with xylem fibers [Figure 5a and b]. The fibers are thick walled, lignified and have wide lumen. The vessels are circular and thin walled; the smallest vessel is 20 µm wide; the largest vessel is 80 µm wide. The pith consists of angular compact thin walled parenchyma cells.

Powder microscopy
Whole plant powder material exhibited the following inclusions when examined under the microscope.

Glandular trichomes
Capitate type glandular trichomes are common in the powder [Figure 6a]. They have thick, one celled stalk and a glandular body comprising two upper cells and two lower cells. The body cells have dense cytoplasm and prominent nucleus [Figure 6b]. The gland is 50 × 80 µm in size.
Non glandular trichomes
Non glandular trichomes are more abundant than the glandular type. The non-glandular trichomes are unicellular, unbranched, and straight or curved, broad at the base, pointed at the tip. They are 15 × 50 µm in size [Figure 6c and d].

Lamina in powder
Fragmentary lamina is frequently seen in the powder, wherein vein-terminations are visible. The veins are thick and straight. The vein terminations are either unbranched or branched once or twice [Figure 6d].

Xylem elements
Different types of xylem elements are seen spread in the powder. Xylem parenchyma, xylem fibers and vessel elements are common inclusions.

Parenchyma cells
Long, scale-like, thin-walled cells are seen mixed with other xylem elements. These parenchyma cells seem to serve the function of storage. The cells are up to 260 µm long and 30 µm wide [Figure 6e].

Fibers
Two types of fibers are seen in the powder [Figure 6e-g]. Some of the fibers have wide lumen and thin walls. Their ends taper abruptly and the middle part is spindle shaped. The wide fibers are 480 µm long and 30 µm wide in the middle.

The second type of fibers include narrow fibers. They have thick walls and narrow lumen [Figure 6g]. The cells are gradually tapering at the ends into pointed tips. The narrow fibers are 630 µm long and 15 µm wide.

Vessel elements
Vessel elements of primary xylem and secondary xylem are seen. Primary xylem vessels have close spiral lateral wall thickenings [Figure 6g]. The secondary xylem vessel elements have dense, multiseriate lateral wall pits [Figure 6h]. The vessel elements are long and cylindrical or short, wide and drum shaped [Figure 6i and j]. The vessel elements have wide, simple horizontal perforations. The lateral wall pits are either elliptical or circular. The vessel elements are 550-750 µm long.

Quantitative microscopy
Anomocytic type of stomata is present on the upper surface of leaves. The stomatal numbers of upper surface and lower surface were found as 19 and 15 respectively. The stomatal indexes of upper surface and lower surface were found 12.2 and 10.7 respectively. The vein islet and vein termination were calculated as 21 and 12 respectively. The palisade ratio was found to be 7.5.

DISCUSSION AND CONCLUSION
To establish the identity, purity, safety and quality of herbal drugs, standardization is an important tool. In order to standardize a drug, various macroscopic, microscopic, fluorescence analyses are done. Microscopic analysis is one of the economical and the simplest methods to begin with establishing the correct identification of the source material.[27]

The macroscopy and microscopy of leaves and stem of Cardiospermum halicacabum have been carried out for the first time in this study. Macroscopic and microscopic studies of the leaf and stem will help identify the crude drug. The quantitative determination of some pharmacognostical parameters
is useful for setting standards for crude drugs. Stomatal number, stomatal index value and palisade ratio, vein islet and vein termination value determination are equally important in the evaluation of crude drugs. These values help in the evaluation of purity of drugs.\[29\]

The information obtained from preliminary phytochemical screening will be useful in finding out the quality of the drug. Fluorescence is an important phenomenon exhibited by various chemical constituents show fluorescence in the visible range in day light. UV light produces fluorescence in many natural products (e.g. alkaloids like berberine) which do not visibly fluoresce in day light. If the substances themselves are not fluorescent, they may often be converted into fluorescent derivatives by applying different reagents. Some crude drugs are often assessed qualitatively in this way and it is an important parameter of pharmacognostical evaluation.\[29\]

Based on the above study it can be concluded that the parameters which are reported here can be considered to be distinct enough to identify and decide the authenticity of this drug in herbal industry/trade and this can be included as microscopic standards in Indian Herbal Pharmacopeia.

ACKNOWLEDGMENTS

The authors are grateful to acknowledge the CSIR (Council of Scientific and Industrial Research), New Delhi, India for providing financial assistance to carry out the study and also thank to J. S. S. University Mysore, (India) for providing facilities to conduct this research work.

REFERENCES

1. The Wealth of India. A Dictionary of Indian Raw Materials and Industrial Products, Raw Materials. Vol. 3. New Delhi: Council of Scientific and Industrial Research; 1992. p. 269-71.
2. Kirthikar KR, Basu BD. Indian Medicinal Plants. 2nd ed., Vol. 1. New Delhi: Periodical Experts, Jayyed Press; 1969. p. 623.
3. Neuwinger HD. African Traditional Medicine: A Dictionary of Plant
Acetaminophen-induced nephrotoxicity in rats: in vitro and in vivo study

Zalke, et al.: Pharmacognostical evaluation of Cardiospermum halicacabum Linn. leaf and stem

Use and Applications. Stuttgart, Germany: Medpharm Gmbh Scientific Publishers; 2000. p. 1-300.

4. Hopkins CY, Ewing DF, Chiosholm MJ. A short chain ester from seed oil of Cardiospermum halicacabum Linn. Phytochemistry 1968;7:619-24.

5. Ferrara I, Schettino O, Motesano D. Triterpenoids from Cardiospermum halicacabum Linn. Phytother Res 1996;10 Suppl 1:S192-94.

6. Srinivas K, Choudhary KA, Rao SS, Sathyanarayana T, Rao RV. Phytochemical investigation of Cardiospermum halicacabum Linn. Indian J Nat Prod 1998;14:24-7.

7. Rao NV, Prakash KC, Kumar SM. Pharmacological investigation of Cardiospermum halicacabum Linn in different animal models of diarrhoea. Indian J Pharmacol 2006;38:346-9.

8. Waako PJ, Gumede B, Smith P, Folb PI. The in vitro and in vivo antimalarial activity of extracts of the medicinal plant Cardiospermum halicacabum against Brugia malayi. J Helminthol 2000;74:241-6.

9. Khunkitti W, Fujimaki Y, Aoki Y. In vitro antifilarial activity of extracts of the medicinal plant Cardiospermum halicacabum against Brugia malayi. J Ethnopharmacol 2000;74:241-6.

10. Boonmars T, Khunkitti W, Sithithaworn P, Fujimaki Y. In vitro antifilarial activity of extracts of Cardiospermum halicacabum against Brugia malayi. J Ethnopharmacol 2000;74:241-6.

11. Asha VV, Pushpangadan P. Antipyretic activity of methanolic stem extract of Cardiospermum halicabum Linn. J Ethnopharmacol 2001;74:293-5.

12. Rao NV, Prakash KC, Kumar SM. Pharmacological investigation of Cardiospermum halicacabum Linn in different animal models of diarrhoea. Indian J Pharmacol 2006;38:346-9.

13. Malaviya S, Nandakumar K, Vaghasiya JD, Vaghasiya JD, Bhalodiya YS, Jivani NP. Anxiolytic activity of roots extracts of Cardiospermum halicacabum in mice. Int J Pharmacol 2009;7:1-6.

14. Parmeshappa B, Ali Basha MS, Sen S, Chakraborty R, Kumar GV, Sagar GV, et al. Acetaminophen-induced nephrotoxicity in rats: Protective role of Cardiospermum halicabum. Pharm Biol 2012;50:247-53.

15. Sheeba MS, Asha VV. Effect of Cardiospermum halicabum on ethanol-induced alcoholic cirrhosis in rats. J Ethnopharmacol 2006;106:105-10.

16. Ministry of Health and welfare. Indian Pharmacopeia. 4th ed. New Delhi: Government of India, Ministry of Health and Welfare, Controller of Publications; 1996. p. A53-4.

17. WHO. Quality Control Methods for Medicinal Plant Material. Geneva: WHO; 1992. p. 22-34.

18. Raaman N. Phytochemical Techniques. 1st ed. New Delhi: New India Publishing Agency; 2006. p. 19-24.

19. Edwin S, Joshi SB, Jain DC. Comparative pharmacognostic studies on root powder of Plumbago rosea. Indian J Nat Prod 2008;2:27-9.

20. Chase CR Jr, Pratt R. Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. J Am Pharm Assoc 1949;38:324-31.

21. Sasmal IE. Elements of Botanical Microtechnique. 1st ed. New York: McGraw Hill Book Co.; 1940. p. 222.

22. Johansen DA. Plant Microtechnique. 1st ed. New York: McGraw Hill Book Co.; 1940. p. 523.

23. O’Brien TP, Feder N, McCull ME. Polychromatic staining of plant cell walls by toluidine blue O. Protoplasma 1964;59:364-73.

24. Esau K. Plant Anatomy. 3rd ed. New York: John Wiley and Sons; 1964. p. 767.

25. Esau K. Anatomy of Seed Plants. 4th ed. New York: John Wiley and Sons; 1979. p. 350.

26. Khandelwal KR. Practical Pharmacognosy. 18th ed. Pune: Nirali Publication; 2007. p. 146-8.

27. Singh S, Manchaval L, Chauhan MG. Pharmacognostic study of male leaves of Trichosanthes dioica Roxb. with special emphasis on microscopic technique. J Pharmacogn Phytother 2010;2:71-5.

28. Kumar S, Kumar V, Prakash O. Microscopic evaluation and physicochemical analysis of Dillenia indica leaf. Asian Pac J Trop Biomed 2011;1:537-40.

29. Kumar D, Kumar K, Kumar S, Kumar T, Kumar A, Prakash O. Pharmacognostic evaluation of leaf and root bark of Holoptelea integrifolia Roxb. Asian Pac J Trop Biomed 2012;2:169-75.

Address for correspondence:
Mr. Ashish S. Zalke,
Department of Pharmacognosy, JSS College of Pharmacy, Rocklands, Ootacamund - 643 001, Tami Nadu, India.
E-mail: ashishzalke@gmail.com

How to cite this article: Zalke AS, Duraiswamy B, Gandagule UB, Singh N. Pharmacognostical evaluation of Cardiospermum halicacabum Linn. leaf and stem. Ancient Sci Life 2013;33:15-21.

Source of Support: There is financial support and funding for this work from Council of Scientific and Industrial Research, New Delhi, India. Conflict of Interest: None declared.

Announcement

A free application to browse and search the journal’s content is now available for Android based mobiles and devices. The application provides “Table of Contents” of the latest issues, which are stored on the device for future offline browsing. Internet connection is required to access the back issues and search facility. The application is compatible with all the versions of Android. The application can be downloaded from https://market.android.com/details?id=comm.app.medknow. For suggestions and comments do write back to us.