



Research Article

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PHARMACOGNOSTIC EVALUATION OF DRIED LEAF POWDER OF *CARDIOSPERMUM HALICACABUM* LINN. AND DRIED SEED HULL POWDER OF *HYDNOCARPUS PENTANDRA* (BUCH.-HAM.) OKEN

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ABSTRACT

The aim of this study was to establish pharmacognostic profiles of dried leaf powder of *Cardiospermum halicacabum* Linn. and dried seed hull powder of *Hydnocarpus pentandra* (Buch.-Ham.) Oken. The present study investigates various qualitative and quantitative parameters like microscopic evaluation of plant powder, physicochemical constants and preliminary phytochemical analysis. Microscopic evaluation of powders of both the plant materials revealed presence of various cellular structures and components. Powder of dried leaves of *Cardiospermum halicacabum* Linn. showed the presence of calcium oxalate crystals, trichomes, xylem vessels, etc. Powder of dried seed hulls of *Hydnocarpus pentandra* (Buch.-Ham.) Oken. showed the presence of compound starch grains, fragmented oil cells, stone cells etc. Physicochemical constants such as the percentage of total ash, acid insoluble ash, water soluble ash and moisture content, loss on drying, alcohol soluble extractive and water soluble extractive were evaluated for both the powdered plant materials and the values were documented. Preliminary phytochemical analysis for both the powdered plant materials was carried out to check the presence of tannins, alkaloids, saponins, terpenoids, cardiac glycosides, steroids and flavonoids. The qualitative tests revealed the presence of tannins, alkaloids, saponins and flavonoids in both the plant materials. However, steroids were found to be present only in dried leaf powder of *Cardiospermum halicacabum* Linn. Pharmacognosy is a simple and reliable tool, for obtaining complete information about crude drug. The present study establishes the pharmacognostic profiles of the selected plant materials which will help in standardization of crude plant material with respect to quality, purity and identity.

Keywords: *Cardiospermum halicacabum* Linn., *Hydnocarpus pentandra* (Buch.-Ham.) Oken, pharmacognosy, microscopic, preliminary phytochemical analysis, physicochemical constants

INTRODUCTION

According to World Health Organization (WHO), 80 percent of the developing world's rural population depends on traditional medicines for its primary health care needs.¹ Herbal medicinal products may vary in composition and properties, unlike conventional pharmaceutical products, which are prepared from synthetic, chemically pure materials using reproducible manufacturing techniques and procedures. Correct identification and quality assurance of the plant materials is an essential prerequisite to ensure reproducible quality of herbal medicine, which will contribute to its safety and efficacy.² Hence there is an urgent need for developing quality control methods for standardization and development of traditional medicines. The process of standardization can be achieved by stepwise pharmacognostic evaluation and physicochemical studies. Pharmacognosy is an important tool for pharmaceutical industry in providing qualitative information about purity and quality of herbal crude drugs. Pharmacognosy involves macroscopic and microscopic characterization, determination of physico-chemical parameters and preliminary phytochemical analysis of herbal crude drugs. The present research work was carried out to establish pharmacognostic profiles of leaves of *Cardiospermum halicacabum* Linn. and seed hulls of *Hydnocarpus pentandra* (Buch.-Ham.) Oken. The plant materials were selected for the present study owing to the array of medicinal properties they exhibit. *Cardiospermum halicacabum* Linn. is a climber which belongs to the

family Sapindaceae. This plant exhibits a wide range of biological and pharmacological properties. The active constituents contributing to extracts and powders from the leaves, roots and seeds of this plant are used in the preparation of shrubs and infusions in traditional medicine against diabetes and arthritis. The leaves are rubefacient and are good for arthritis and piles.³ Its leaves and stalks are used in the treatment of diarrhea, dysentery, headache and as a poultice for swellings.⁴ *Hydnocarpus pentandra* (Buch.-Ham.) Oken. is a dioecious evergreen tree endemic to Western Ghats, India and it belongs to family Flacourtiaceae. It is commonly known as Chaulmoogra. Its seeds yield chaulmoogra oil which has been proved to be effective in leprosy management.⁵ Acetone extract of seed hulls of *Hydnocarpus pentandra* (Buch.-Ham.) Oken has been reported to possess strong free radicals (DPPH and ABTS) scavenging activity. It has also been reported to possess inhibitory activity against the enzymes like α -glucosidase and N-acetyl- β -D-glucosaminidase which may be responsible for the antidiabetic property as advocated in traditional medicine.⁶ Literature survey reveals that pharmacognostic studies have been carried out on whole plant of *Cardiospermum halicacabum* Linn.⁷ and seeds of *Hydnocarpus pentandra* (Buch.-Ham.) Oken.⁵ However, no such studies are carried out on specific parts such as leaves of *Cardiospermum halicacabum* Linn. and seed hulls of *Hydnocarpus pentandra* (Buch.-Ham.) Oken. Hence, in the present research work, microscopic characterization,

determination of physico-chemical parameters and preliminary phytochemical analysis of dried leaf powder of *Cardiospermum halicacabum* Linn. and dried seed hull powder of *Hydnocarpus pentandra* (Buch.-Ham.) Oken was carried out to ascertain their purity and identity.

MATERIALS AND METHODS

Plant material

The climber, *Cardiospermum halicacabum* Linn. was procured from Keshav Shrushti, Bhayander, Mumbai, India. In the present research work, powder of dried leaves of *Cardiospermum halicacabum* Linn. were used. Fruits of *Hydnocarpus pentandra* (Buch.-Ham.) Oken. were procured from Castle Rock, Karnataka, India. In the present research work, powder of dried seed hulls of *Hydnocarpus pentandra* (Buch.-Ham.) Oken was used. The herbaria of *Cardiospermum halicacabum* Linn. and *Hydnocarpus pentandra* (Buch.-Ham.) Oken were prepared and authenticated by Botanical Survey of India (BSI), Pune, India and the voucher number of herbaria were assigned as NSCARH2 and NSHYP1 respectively. The plant material was washed with water and shade dried. The dried plant material was finely powdered. The powder was then passed through the BSS mesh no.85 sieve and stored in an airtight container at room temperature ($25^{\circ}\text{C} \pm 2^{\circ}\text{C}$).

Experimental reagents

Iodine, Phloroglucinol, Ruthenium Red and Sudan III stains were procured from Lobachemie. Glycerol (90 % purified), Saffranin stain, Glacial acetic acid (AR Grade), Ethanol (AR Grade), Potassium iodide (AR Grade), Lead acetate (AR Grade) were procured from E. Merck. Hydrochloric acid, sulphuric acid, acetic anhydride, FeCl_3 , Karl Fischer reagent was procured from Sigma-Aldrich. Distilled Water used, was purified with a Sartorius water purification unit. (Arium 61315, made in USA)

Instrumentation

Labomed 2000 microscope was used for the microscopic analysis of the plant material under the magnification of 10x, 40x and 100x lenses of microscope. AV USB 2.0 Capture application software was used for image capturing.

Microscopic evaluation of dried leaf powder of *Cardiospermum halicacabum* Linn. and dried seed hull powder of *Hydnocarpus pentandra* (Buch.-Ham.) Oken

The powders of both plants were mounted on separate clean glass slides. A drop of glycerol was added to the powdered plant material on the slide and a cover slip was placed over it. The slide was then viewed under the microscope and images were captured at desired magnification. For effective results various stains were also used to distinguish cellular structures. Each powder was stained with Iodine S, Phloroglucinol S, Sudan III, Ruthenium Red stain and examined under the microscope⁸⁻¹¹.

Physicochemical parameters of dried leaf powder of *Cardiospermum halicacabum* Linn. and dried seed hull powder of *Hydnocarpus pentandra* (Buch.-Ham.) Oken

Physicochemical constants such as the percentage of total ash, acid insoluble ash, water soluble ash, moisture content, loss on drying, alcohol soluble extractive and water soluble extractive were evaluated.¹⁰⁻¹³.

Total ash

About 2.0 g of powder of dried leaves of *Cardiospermum halicacabum* Linn. and powder of dried seed hulls of *Hydnocarpus pentandra* (Buch.-Ham.) Oken. were accurately weighed and transferred to different pre weighed silica crucibles and were ignited with a flame of Bunsen burner, for about 1 hour. The ignition was completed by keeping in muffle furnace, at $550^{\circ}\text{C} \pm 20^{\circ}\text{C}$, till a white carbon free ash was formed. The silica crucibles were then cooled in desiccators and weighed, and the result obtained is given in Table 1.

Acid insoluble ash

About 2.0 g of powder of dried leaves of *Cardiospermum halicacabum* Linn. and powder of dried seed hulls of *Hydnocarpus pentandra* (Buch.-Ham.) Oken. were accurately weighed and transferred to different pre weighed silica crucibles. The crucibles were ignited with a flame of Bunsen burner, for about 1 hour. The silica crucibles were then kept in a muffle furnace at $550^{\circ}\text{C} \pm 20^{\circ}\text{C}$, till a white carbon free ash was formed. The silica crucibles were then cooled in desiccators and weighed. The total ash obtained for powder of dried leaves of *Cardiospermum halicacabum* Linn. and powder of dried seed hulls of *Hydnocarpus pentandra* (Buch.-Ham.) Oken were taken in two different beakers and 25 mL of dilute hydrochloric acid (2N HCl) was added in each beaker. Both the beakers were then heated for 10 minutes and allowed to cool. The contents of the beakers were filtered through Whatman filter paper no. 41. The residues were then washed with water, till washings were free from chloride. The filter papers along with the residues of plant powders were placed in different silica crucibles and ignited in a muffle furnace, at $550^{\circ}\text{C} \pm 20^{\circ}\text{C}$, for 1 hour. The crucibles were cooled and weighed to a constant weight. The percentage of acid insoluble ash was then calculated for powder of dried leaves of *Cardiospermum halicacabum* Linn. and powder of dried seed hulls of *Hydnocarpus pentandra* (Buch.-Ham.) Oken and the result obtained is given in Table 1.

Water soluble ash

About 2.0 g of powder of dried leaves of *Cardiospermum halicacabum* Linn. and powder of dried seed hulls of *Hydnocarpus pentandra* (Buch.-Ham.) Oken. were accurately weighed and transferred to different pre weighed silica crucibles. The crucibles were ignited with a flame of Bunsen burner, for about 1 hour. The silica crucibles were then kept in a muffle furnace at $550^{\circ}\text{C} \pm 20^{\circ}\text{C}$, till a white carbon free ash was formed. The silica crucibles were then cooled in desiccators and weighed. The total ash obtained for powder of dried leaves of *Cardiospermum halicacabum* Linn. and powder of dried seed hulls of *Hydnocarpus pentandra* (Buch.-Ham.) Oken

were taken in two different beakers and 25 mL of distilled water was added in each beaker. Both the beakers were then heated for 10 minutes and allowed to cool. The contents of the beakers were filtered through Whatman filter paper no. 41. The filter papers along with the residues of plant powders were placed in different silica crucibles and ignited in a muffle furnace, at $550^{\circ}\text{C} \pm 20^{\circ}\text{C}$, for 1 hour. The crucibles were cooled and weighed to obtain water soluble ash. The percentage of water soluble ash was then calculated by subtracting water soluble ash from total ash for powder of dried leaves of *Cardiospermum halicacabum* Linn. and powder of dried seed hulls of *Hydnocarpus pentandra* (Buch.-Ham.) Oken. The results obtained are given in Table 1.

Moisture content

About 100 mg of accurately weighed powder of dried leaves of *Cardiospermum halicacabum* Linn. and powder of dried seed hulls of *Hydnocarpus pentandra* (Buch.-Ham.) Oken were transferred to the reaction vessel. Titration with Karl Fischer reagent was carried out as described in Indian Pharmacopeia and the results obtained are given in Table 1.

Loss on drying

About 2.0 g of powder of dried leaves of *Cardiospermum halicacabum* Linn. and powder of dried seed hulls of *Hydnocarpus pentandra* (Buch.-Ham.) Oken were accurately weighed and transferred to different pre weighed silica crucibles. The crucibles were kept in the hot air oven, for about 2 hours, at $100^{\circ}\text{C} \pm 2^{\circ}\text{C}$. The silica crucibles were then cooled in desiccator and weighed. The percent loss on drying was then calculated. The results obtained are given in Table 1.

Alcohol soluble extractives

About 1.0 g powder of each, dried leaves of *Cardiospermum halicacabum* Linn. and dried seed hulls of *Hydnocarpus pentandra* (Buch.-Ham.) Oken, were accurately weighed in two separate stopper conical flasks. To each flask, 10.0 mL of ethanol was added and was allowed to stand for 18 hours with occasional shaking. The contents of each flask were then filtered through Whatman No.1 filter paper in separate pre-weighed dry beakers and each filtrate was evaporated to dryness on a water bath. Each dried residue was then weighed and the percentage extractive values were calculated.

Water soluble extractives

About 1.0 g powder of each, dried leaves of *Cardiospermum halicacabum* Linn. and dried seed hulls of *Hydnocarpus pentandra* (Buch.-Ham.) Oken, were accurately weighed in two separate stopper conical flasks. To each flask, 10.0 mL of water was added and was allowed to stand for 18 hours with occasional shaking. The contents of each flask were then filtered through Whatman No.1 filter paper in separate pre-weighed dry beakers and each filtrate was evaporated to dryness on a water bath. Each dried residue was then weighed and the percentage extractive values were calculated.

Preliminary phytochemical analysis of dried leaf powder of *Cardiospermum halicacabum* Linn. and dried seed hull powder of *Hydnocarpus pentandra* (Buch.-Ham.) Oken.

Following tests were carried out for preliminary photochemical analysis and the results are listed in Table 2^{4,10,11,14}.

Tannins

About 0.2 g of each plant material was weighed and 10.0 mL distilled water was added. The solution was filtered through Whatman filter paper no. 41 and aqueous filtrate was collected. 2.0 mL of alcoholic FeCl_3 was added to the 2.0 mL of the above aqueous filtrate.

Formation of blue precipitate indicated the presence of tannins.

Alkaloid

About 0.2 g of each plant material was weighed and 10.0 mL methanol was added. The solution was filtered through Whatman filter paper no. 41 and methanolic filtrate was collected. 1.0 mL of 1 % HCl and 6 drops of Dragendorff's reagent was added to the above aqueous filtrate. Formation of brown or red precipitate indicated presence of alkaloids.

Saponins

To 0.5 mL of above methanolic filtrate, 5.0 mL of distilled water was added. Persistent frothing on shaking indicated presence of saponins.

Terpenoids

To 2.0 mL of methanolic filtrate, 2.0 mL of acetic anhydride was added and 1.0 mL of conc. H_2SO_4 was slowly poured along the walls of the test tube. Formation of blue ring indicates presence of terpenoids.

Cardiac glycosides

To 2.0 mL of methanolic filtrate, 1.0 mL of glacial acetic acid and 1 drop FeCl_3 was added. 1.0 mL of conc. H_2SO_4 was slowly poured along the walls of the test tube. Formation of green ring indicated presence of cardiac glycosides.

Steroids

About 0.2 g each plant material was weighed and 10.0 mL chloroform was added to it. The solution was filtered through Whatman filter paper no. 41 and the filtrate was collected. In 2.0 mL of filtrate, 2.0 mL of acetic anhydride and 1.0 mL of conc. H_2SO_4 was added. Formation of blue ring indicated presence of steroids.

Flavonoids

About 0.2 g each plant material was weighed and 10 mL of ethanol was added to it. The solution was filtered through Whatman filter paper no. 41 and the filtrate was collected. In 2.0 mL of filtrate 1.0 mL conc. HCl and a small piece of magnesium ribbon was added. Formation of pink color indicated presence of flavonoids.

RESULTS AND DISCUSSION

Microscopic evaluation of dried leaf powder of *Cardiospermum halicacabum* Linn.

The powder of dried leaves of *Cardiospermum halicacabum* Linn. showed the presence of acicular crystals (Figure 1.1) and rosette crystals (Figure 1.3) of calcium oxalate. Acicular crystals are needle like, slender, long pointed at ends and are also present in clusters (Figure 1.2). Fragmented oil cells were also observed (Figure 1.4). The powder also showed the presence of trichomes in abundance. Glandular trichomes (Figure 1.5)

were observed along with warty, long, unicellular trichomes with pointed ends and bulbous base (Figure 1.6). Glandular trichome observed was simple, unicellular and with one-celled stalk (Figure 1.5). Parenchyma cells containing mucilage were also observed (Figure 1.7 and Figure 1.8). Sclerenchyma tissue was also observed whose cells appeared polygonal in shape with thickened and stratified walls and without intercellular spaces (Figure 1.9). The plant powder also showed presence of other characters such as xylem vessel having spiral thickening (Figure 1.10 and Figure 1.11).



Figure 1.1: Acicular crystal of calcium oxalate

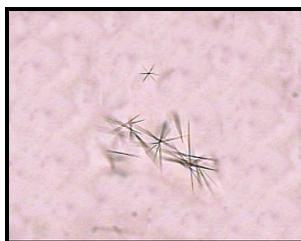


Figure 1.2: Clusters of acicular crystals of calcium oxalate



Figure 1.3: Rosette crystal of calcium oxalate



Figure 1.4: Fragmented oil cells



Figure 1.5: Glandular trichome



Figure 1.6: Warty, unicellular trichome



Figure 1.7: Parenchyma cells containing mucilage



Figure 1.8: Parenchyma cell containing mucilage



Figure 1.9: Sclerenchyma tissue



Figure 1.10: Xylem vessel with spiral thickening

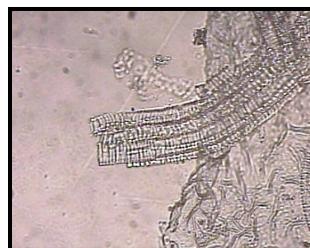


Figure 1.11: Xylem vessels with spiral thickening

Figure 1: Microscopic evaluation of *Cardiospermum halicacabum* Linn.

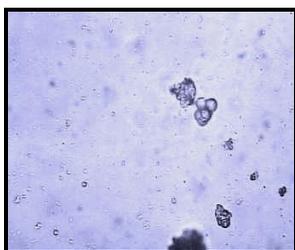


Figure 2.1: Compound starch grains



Figure 2.2: Fragmented oil cells

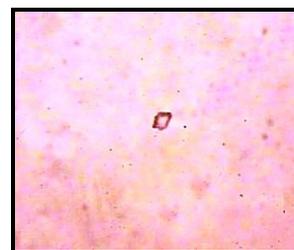


Figure 2.3: Prismatic crystal of calcium oxalate



Figure 2.4: Acicular crystals of calcium oxalate



Figure 2.5: Stones cells



Figure 2.6: Stone cell

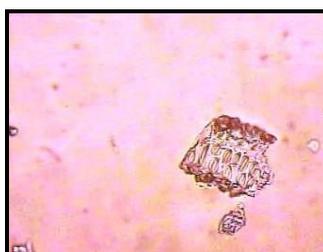


Figure 2.7: Pitted parenchyma



Figure 2.8: Xylem vessel with spiral thickening

Figure 2: Microscopic evaluation of dried seed hull powder of *Hydnocarpus pentandra* (Buch.-Ham.) Oken

Microscopic evaluation of dried seed hull powder of *Hydnocarpus pentandra* (Buch.-Ham.) Oken.

The powder of dried seed hulls of *Hydnocarpus pentandra* (Buch.-Ham.) Oken. showed the presence of compound starch grains (Figure 2.1) and fragmented oil cells (Figure 2.2). Prismatic crystal (Figure 2.3) and acicular crystals (Figure 2.4) of calcium oxalate were observed. Acicular crystals are needle like, slender, long pointed at ends and are singly present. Stone cells of various shapes were found to be present in abundance (Figure 2.5 and Figure 2.6). Pitted parenchyma (Figure 2.7) and xylem vessel having spiral thickening were also observed (Figure 2.8).

Physico-chemical Parameters

The physico-chemical parameters are important for identifying adulterants and improper handling of drugs. The moisture content of the drug is not too high, thus it could discourage bacteria, fungi or yeast growth. Determination of total ash is important in evaluation of purity of drugs because it indicates the presence or absence of foreign inorganic matter such as metallic salts and/or silica.¹⁵ Table 1 reveals the result of physico-chemical parameters of powdered drugs, carried out by using standard procedures.

Table1: Physico-chemical parameters for dried leaf powder of *Cardiospermum halicacabum* Linn. and dried seed hull powder of *Hydnocarpus pentandra* (Buch.-Ham.) Oken.

Physico-chemical parameters	<i>Cardiospermum halicacabum</i> Linn.	<i>Hydnocarpus pentandra</i> (Buch.-Ham.) Oken
Total ash (%)	7.26 ± 0.53	3.15 ± 0.22
Acid insoluble ash (%)	1.11 ± 0.17	0.45 ± 0.16
Water soluble ash (%)	4.08 ± 0.04	3.07 ± 0.28
Moisture content (%)	4.08 ± 0.34	4.84 ± 0.56
Loss on drying (%)	6.73 ± 0.21	3.72 ± 0.29
Water soluble extractive (%)	10.2 ± 0.23	11.5 ± 0.16
Alcohol soluble extractive (%)	13.4 ± 0.33	14.8 ± 0.20

Preliminary phytochemical analysis

Results of preliminary phytochemical analysis indicated the presence of alkaloids, tannins, flavonoids and saponins in both the plant materials. However, steroids were found to be present only in dried leaf powder of *Cardiospermum halicacabum* Linn. Terpenoids and

cardiac glycosides were absent in both the plant materials. It is due to the presence of these phytochemicals that make these plants useful for treating different ailments and having a potential of providing useful drugs of human use.¹⁵ The results of preliminary phytochemical analysis are listed in Table 2.

Table 2: Preliminary phytochemical analysis for dried leaf powder of *Cardiospermum halicacabum* Linn. and dried seed hull powder of *Hydnocarpus pentandra* (Buch.-Ham.) Oken

Phytochemicals	<i>Cardiospermum halicacabum</i> Linn.	<i>Hydnocarpus pentandra</i> (Buch.-Ham.) Oken.
Alkaloids	+	+
Terpenoids	-	-
Tannins	+	+
Flavonoids	+	+
Saponins	+	+
Cardiac glycosides	-	-
Steroids	+	-

(+): Present (-): Absent

CONCLUSION

Establishment of pharmacognostic profiles of leaves of *Cardiospermum halicacabum* Linn. and seed hulls of *Hydnocarpus pentandra* (Buch.-Ham.) Oken. will supplement the existing information with regard to their identification and standardization, even in the powdered form. The study will provide a guideline to quality control methods for determination of substitutes, adulterants, if any, in the powder of dried leaves of *Cardiospermum halicacabum* Linn. and the powder of dried seed hulls of *Hydnocarpus pentandra* (Buch.-Ham.) Oken.

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