



Pharmacognostical and physico-chemical evaluations of *Cardiospermum halicacabum* L. seeds

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Abstract

Background and objectives: *Cardiospermum halicacabum* L. is a climber plant of Sapindaceae family with certain medicinal properties that have made it to be used traditionally in different systems of medicine such as Ayurveda, Homeopathy and Unani medicine. Literature survey has revealed the reports about some investigations but references were not available about the seeds of *C. halicacabum*; hence, the seeds were selected for the present investigation. **Methods:** Detailed macroscopic and microscopic studies of *C. halicacabum* seeds and powder were carried out, physico-chemical parameters, preliminary and quantitative phytochemical analyses of different extracts of the seeds performed. Besides, the amount of total phenols and flavonoids in different extracts were determined. **Results:** The phytochemical screening gave a positive test for presence of various phytoconstituents like alkaloids, flavanoids, and tannins. The amount of total phenols was found to be between 29.697 ± 0.232 to 187.372 ± 0.615 mg pyrogallol equivalent/g and the content of flavonoids between 27.833 ± 0.412 to 139.261 ± 0.412 mg quercetin equivalent/g in various extracts. **Conclusion:** The present work has reported the detailed macroscopic, microscopic and physio-chemical evaluations of the seeds of *C. halicacabum* and provided information which would be helpful for better identification and standardization of *C. halicacabum* seeds.

Keywords: balloon vine, *Cardiospermum halicacabum*, microscopic evaluation, phytochemical evaluation, seeds

Introduction

Cardiospermum halicacabum L. of Sapindaceae family is an annual or sometimes perennial climber plant. It extensively grows in tropical and subtropical areas of the world and is commonly found as a weed throughout India. It is known as *love in a puff* or *balloon vine* in English and *karnasphoṭa* in Hindi. The whole plant of *C. halicacabum* is used in folk remedies for its

nutritional values and is known as a medicinal plant which possesses medicinal properties for treatment of various ailments; it has been used in various traditional systems of medicine such as Ayurveda, the Indian system of medicine, [1] Unani [2] and Chinese medicine [3]. In Indian system of medicine *C. halicacabum* L. has been used for treatments of chronic bronchitis,

stiffness of limbs and snakebites. In Chinese medicine, it has been used for treatment of rheumatism, lumbago, nervous diseases, and as a demulcent in orchitis and in dropsy [4-5]; in Unani medicine the seeds are mentioned as tonic and for treatment of cancer [6]. It is also helpful in case of dry itchy skin and scalp and is available in market as herbal products like cream, gel, shampoo, spray, etc [7].

In spite of all the reported therapeutic properties of different parts of the plant, none of them has been evaluated so far; therefore, the seeds that were easily available in a good condition were selected for the present study.

Experimental

Plant material

Cardiospermum halicacabum plant with its leafy flowering branches and mature seeds were collected from Junagadh district of Gujarat, for preparation of the herbarium sheet (figure 1). The correct identity and authenticity of the species was done by studying the morphological characters of various parts of the plant and comparing them with the characters mentioned in various floras [8-11]. For further confirmation the herbarium sheet were also sent to Raw Material, herbarium and museum division CSIR-NISCAIR, Delhi. Voucher specimen was lodged at the Herbarium unit of Department of Pharmaceutical Sciences, Saurashtra University for further documentations.

Pharmacognostical evaluation

The seeds were washed properly under running water and stored in a jar containing FAA (formalin, glacial acetic acid and ethyl alcohol (5:5:90) preservative solution of formalin, glacial acetic acid and ethyl alcohol (5:5:90) and used for taking free hand sections. Seed sections were cleared with chloral hydrate, stained with phloroglucinol and hydrochloric acid and mounted in glycerin to observe the lignification of the elements. Powder of the seeds was prepared finally by grinding the seeds in a mechanical pulverizer and passing through a 40-

mesh sieve. The powder was stored in an airtight jar, protected from the light and was subjected for studying the organoleptic, microscopic and physico-chemical characters according to the WHO guidelines [12]. Microphotographs were taken for showing the distribution of various tissues in transverse section, and free hand drawings of various macerated and powder tissue were done with help of camera lucida [13,14]

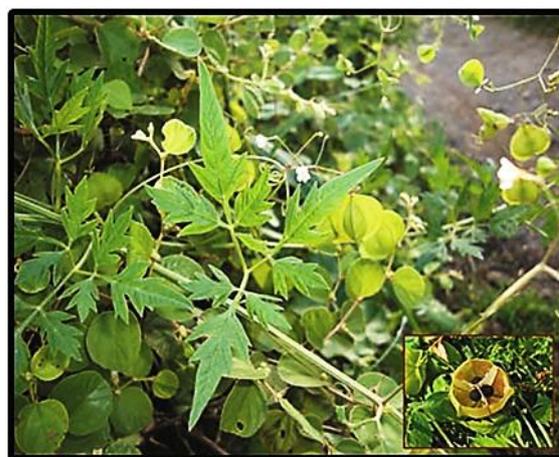


Figure 1 . *Cardiospermum halicacabum* plant

Phytochemical investigations

Two-hundred g of powder was used for preparation of the various extracts by solvents with different polarities like *n*-hexane, chloroform, ethyl acetate, methanol and aqueous methanol (methanol 60 %). The extracts were tested for the presence of various constituents, some of which were further subjected for quantitative estimation [15-17].

Results and Discussion

The present work described the detailed macroscopic and microscopic evaluations of the seeds of *C. halicacabum* L. (Sapindaceae); it also dealt with the preliminary physico-chemical analysis of the seeds.

The macroscopic evaluations showed that the seeds were hard and globose in shape, black in color with grayish white colored heart shape spots. Their size was 4 to 6 mm in diameter, with

smooth surface – finely pitted with occasionally few depressions. The seeds were pachychalazal. Seed coats developed from the chalazal tissue. In fact the white spots were the expansion of the chalaza seated on the head of the expanded funicular vestigial aril, the spot lying near the grooved being the micropyle and next to it lied the spot of the hilum. The taste was astringent and later slightly bitter, with no odor (figure 2).

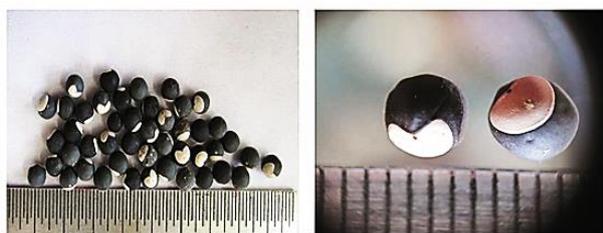


Figure 2. *Cardiospermum halicacabum* seeds

Microscopic studies of Longitudinal sections (LS) and Transverse sections (TS), showed that the seeds were circular in outline showing an outer palisade layer of pachychalaza resembling to testa, encircling the narrow parenchymatous band of the chalazal tissue and the centrally located wide embryo consisting of sigmoid cotyledons and a small radical at one end which occupied the major area of the section.

Detailed TS showed an outer pachychalazal seed coat consisting of a layer of radially elongated, thick walled cells of palisade with dark brown colored lumen getting narrower at the top and with linea lucida crossing across; a layer of hypodermis lying underneath this consisted of brown colored rectangular, radially elongated cells embedded at the base with a row of irregular globular masses of volatile oil globules, followed by rows of elongated thick walled parenchymatous cells, embedded with fixed oil globules and aleurone grains. Section passing through the hilum showed an outer layer of epidermis covered with thin cuticle, enclosing the inner, curved, hilar plate consisting of 4 to 6

rows, of thin walled, small sized, parenchymatous cells embedded with cluster and rosette crystals of calcium oxalate, underneath this lied a discontinuous layer of broad lumened palisade cells which were short in height and each was embedded with a huge prismatic crystal of calcium oxalate. Figures 3 and 4 show the details of seed sections.

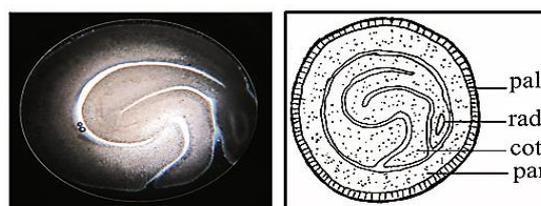


Figure 3. LS *Cardiospermum halicacabum* seeds
pal:palisade, rad: radical, cot:cotyledon, par:parenchyma

Powder was observed as whitish brown colored powder scattered with black fragments of testa, with astringent taste which later on turned into bitter with slightly aromatic odor. The diagnostic characters of the powder were: fragments of palisade cells of epidermis, brown colored parenchymatous cells, aleurone grains with cluster, rosette and few prismatic crystals of calcium oxalate and fixed oil globules (figure 5). Various physical parameters of seeds and powder such as moisture content, total ash, acid insoluble ash, water-soluble ash, pH values and extractive value, were determined. The results of this study were shown in tables 1 and 2. Results of preliminary phytochemical screening are compiled in table 3.

Table 1. Physicochemical parameters of *C. halicacabum* seeds

Parameter	w/w %
Loss on drying	7
Total ash	4
Acid insoluble ash	2
Water soluble ash	1.5
pH (1%)	7.25
pH (10%)	5.85

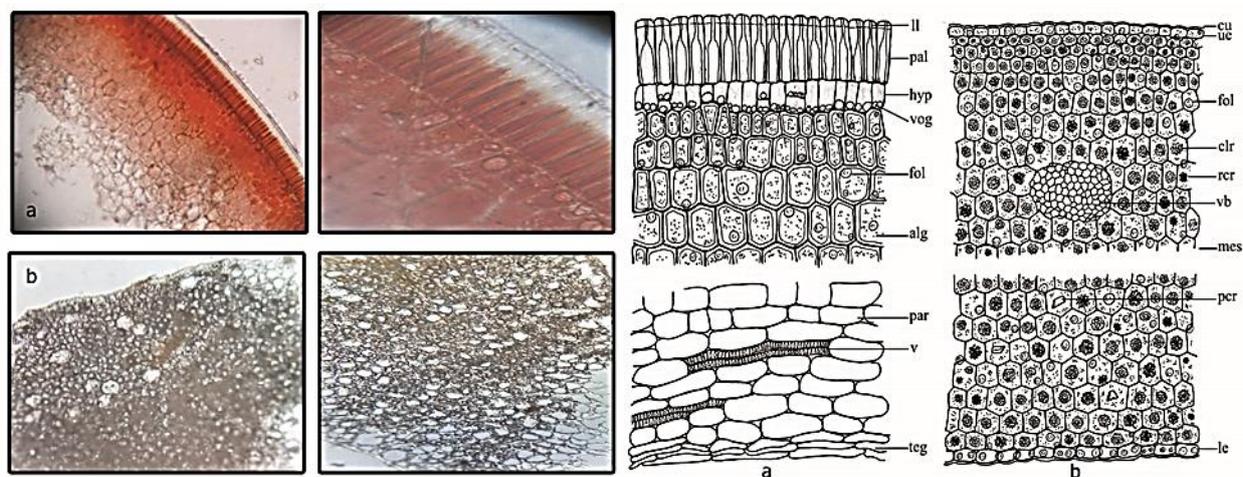


Figure 4. Sections of *Cardiospermum halicacabum* seeds. **a)** Detailed TS of testa; **b)** detailed TS of cotyledon *alg: aleurone grains, clr: cluster crystal of calcium oxalate, cu: cuticle, fol: fixed oil globule, hyp: hypodermis, lc: lower epidermis of cotyledon, ll: linea lucida, mes: mesophyll, pal: palisade, par: parenchyma, pcr: prismatic crystal of calcium oxalate, rcr: rosette crystal of calcium oxalate, teg: tegment, ue: upper epidermis of cotyledon, v: vessels, vb: vascular bundle, vog: volatile oil globules

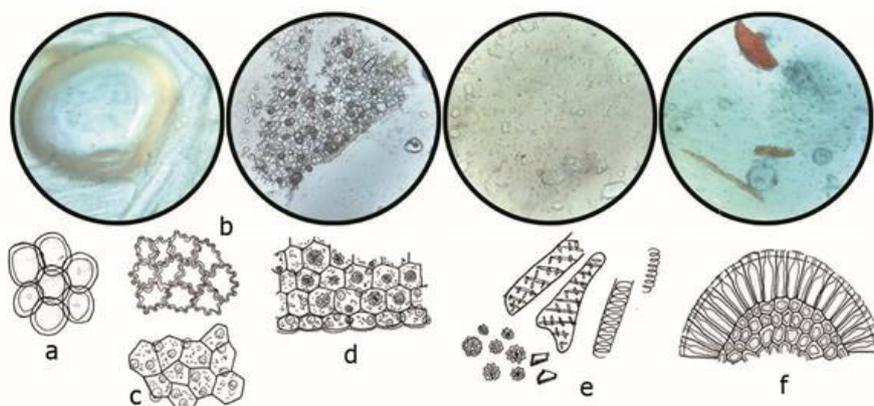


Figure 5. Powder of *cardiospermum halicacabum* seeds. **a)** Cells underneath hypodermis embedded with fixed oil and aleurone grains. **b)** Palisade cells of testa in surface view from above. **c)** Epidermis of cotyledon in surface view embedded with fixed oil and aleurone grains. **d)** Transversely cut fragment of cotyledon showing lower epidermis and underlined cells of mesophyll. **e)** Cluster, rosette and prismatic crystals of calcium oxalate and fragments of tracheids and vessels from hilar and testa. **f)** A row of palisade cells in sectional view with underlined cells of hypodermis.

Table 2. Fluorescence analysis and yield of extracts in different solvent of *C. halicacabum* seeds

Solvents	Day light	UV light 366 nm	Extractive yield in % w/w	Consistency
<i>n</i> -hexane	Greenish yellow	Orange	24.18	Oily
Chloroform	Golden brown	Amber orange	0.96	Semi oily
Ethyl acetate	Nordic olive	Sulphuric yellow	0.71	Semi oily
Methanol	Red brown	Beige	2.72	Semi Solid
aqueous methanol	Dark brown	Brown	1.15	Solid

Table 3. Preliminary phytochemical screening of *C. halicacabum* seeds

Phytochemicals	<i>n</i> -hexane	Chloroform	Ethyl acetate	Methanol	aqueous methanol
Alkaloid	-	-	+	+	+
Saponin	-	-	-	+	+
Steroid	-	-	-	-	-
Flavonoid	+	+	+	+	+
Tanins and Phenolics	+	+	+	+	+

The results of the quantitative *phytochemical* estimations indicated the amount of phenols and flavonoids in different extracts; it indicated the levels of phenol expressed as milligrams of pyrogallol equivalents 29.697±0.232 in chloroform to 187.372±0.615 in the aqueous methanol extracts. The content of flavonoids was expressed as quercetin equivalents varied from 27.833±0.412 in ethyl acetate to 139.261±0.412 in the aqueous methanol extract. Table 4 summarizes the amounts of total phenolic and total flavonoid compounds in different extracts.

Table 4. Total phenolics and flavonoid content of *C. halicacabum* seeds extracts

Seeds extracts	Total phenolics (mg pyrogallol equivalent/g)	Total flavonoid (mg quercetin equivalent/g)
<i>n</i> -hexane	52.178±0.484	33.071±0.412
Chloroform	29.697±0.232	57.119±0.412
Ethyl acetate	96.906±0.232	27.833±0.412
Methanol	137.294±0.134	63.309±0.412
aqueous methanol	187.372±0.615	139.261±0.412

Each value in the table is represented as mean±SD

According to the World Health Organization (WHO), the determination of macroscopic and microscopic characters of the plants are the first steps towards establishing the identity and purity and has to be carried out before any other tests [12]. In addition, the results of macroscopic and microscopic study strengthen the identification of original drug and will help to identify it from its substitutes and adulterants in the market. In review of the literature, a few studies on different parts of the plant has been reported but the pharmacognostical study and phytochemical investigation on seeds of *C. halicacabum* have been carried out for the first time in the present study. Earlier pharmacognostical studies was reported by Zalke *et al.* on leaf and stem part of *C. halicacabum* which showed the characteristics of leaf and stem of the plant [18]. Previously

reported phytochemical study have revealed the presence of various phytoconstituents like flavones, glycosides, carbohydrates, fatty acids and volatile compounds in the extracts of the plant [19,20]. Other studies from aerial parts have reported different biological activities like antimicrobial and antibacterial properties [21,22]. Recent studies on seeds of *C. halicacabum* reported by Mohaddesi *et al.*, for its anticancer activity on various cancer cell lines have indicated the anticancer activity of *C. halicacabum* seed oil on breast cancer [23], which pointed out the potential medicinal value of *C. halicacabum* seeds and the need of the present work for standardization of seeds as it had not been reported so far. In this study, The preliminary phytochemical screenings showed positive tests for presences of different phytoconstituents in various solvents, which are known to have a range of therapeutic importance and therefore can be utilize for the biological activities. The results here indicated that the seeds have high extractive yield in *n*-hexane extract in the form of oil, which is an important product from seeds and is considered as a good source of nutrition and essential oils. The results indicated that the seeds have high extractive yield for the *n*-hexane extract in the form of oil, which is an important product from seeds and considered as a good source of nutrition and essential oils [24]. In addition, the amount of the total phenols and flavonoids present in the seeds showed a potential for medicinal values. The presented data from this study would be beneficial for quantitative and qualitative standardization of herbal preparations from *C. halicacabum* seeds.

Declaration of interest

The authors declare that there is no conflict of

interest. The authors alone are responsible for the content of the paper.

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