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Pharmacognostical standardization and HPTLC fingerprint of *Cardiospermum halicacabum* L. Stem

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ABSTRACT

The use of plant as medicine is as old as human civilization. Exploration of this traditional knowledge for cures to common diseases is an attractive prospect. *Cardiospermum halicacabum* L. commonly known as 'Balloon vine' has many associated utilities, including a remedy for joint pain. The tender, young shoots are used as a vegetable, fodder, diuretic, stomachic and rubefacient. It is used in rheumatism, lumbago, nervous diseases and as a demulcent in orchitis and in dropsy. In many regions this plant is harvested in backyards for both medicinal and food value. To get information about correct identification of the stem of this plant pharmacognostical and physico-chemical studies, as well as phytochemical analysis and HPTLC fingerprint were carried out. Anatomical studies revealed presence of covering trichomes, anomocytic stomata, pitted as well as spiral thickening of vessels and fibres. Various physico-chemical parameters such as total ash, water soluble ash, acid insoluble ash and extractive values were carried out. Preliminary phytochemical analysis and TLC showed presence of saponin, tannins, flavanoids, glycosides and cardiac glycosides. This study will provide referential pharmacognostical, physico-chemical and phytochemical information for correct identification of *Cardiospermum halicacabum* L. stem.
Key words: *Cardiospermum halicacabum* L., Stem, Pharmacognosy, Physicochemical analysis, HPTLC fingerprint.

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INTRODUCTION

In the last few decades there is an exponential growth in the field of herbal medicine. It is getting popularize in developing countries owing to its natural origin and lesser side effects. Nowadays, herbal medicines are being manufactured on a large scale in mechanical units, where manufacturers are facing many problems such as availability of good quality of raw material, authentication of raw material, availability of standards, proper standardization methodology of drugs and formulations, quality control parameters etc. [1].

Cardiospermum halicacabum L. (Family: Sapindaceae) 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 rheumatism, lumbago, nervous diseases and as a demulcent in arthritis and in dropsy. It is used for the treatment of skeletal fractures in Sri Lanka. The juice of the herb is used to cure ear-ache and to reduce hardened tumours. It exhibits significant analgesic, anti-inflammatory and vaso-depressant activity, which is transient in nature. *In vitro* studies have revealed its antispasmodic and curative actions confirming the use of the herb in Ayurvedic medicine [2].

Various products like gel, cream, shampoo, spray etc. of *Cardiospermum halicacabum* L. are available in the market. These products are useful for dry itchy skin and scalp. In the global market, balloon vine has been utilized as several products, 'Love in a Puff ', 'Balloon Vine' and 'Heartseed'. It is also one of the ingredients in "Allergy Relief Liquid ™" and "Bioforce Pollinosan® Tabs" marketed by Bioforce USA as a natural relief for hay fever, allergies, sneezing, watery eyes, and allergic reactions. Another US based company, Boericke and Tafel produces "Florasone Cardiospermum Cream" for skin ailments such as swelling, scaling, blisters/vesicles, burning and pain. These products are supported by the various claims concerning the many medicinal properties of balloon vine [3].

Though the plant species could be easily distinguished on the basis of the flowers, it becomes very difficult when the crude drug is in the form of dried and cut pieces [4]. Therefore, the main aim of the present study is to develop standard pharmacognostical parameters for stem of *Cardiospermum halicacabum* L.

Pharmacognostical evaluation included examination of morphological and microscopical characters, determination of ash values, extractive values, powder characters and fluorescence analysis. The preliminary phytochemical analysis was carried out by chemical and TLC methods. HPTLC fingerprint of methanolic extract of stem powder was also developed.

MATERIALS AND METHODS

Herbarium of *Cardiospermum halicacabum* L. was prepared and authenticated from Blatter Herbarium, St. Xavier's College, Mumbai. Whole plant of *Cardiospermum halicacabum* L. was collected from Haji Malang (Kalyan, M.S., India) (Plate No. 1: A). Stem was separated and washed under running tap water and blotted dry for further studies (Plate No. 1: B). The stems

were dried in preset oven at 40 ± 2˚C for about two weeks, ground into powder and used for further analysis.

Morphological characters of the stem were studied using fresh plant material. In anatomical studies, section of young and old stem (T.S. and L.S.) as well as powder characteristics were observed. Maceration was done to observe peculiar characters present in the stem as per the method describe in Khandelwal [5]. Histochemical tests were also carried out using methods described by Madhavan *et al*. [6].

Physicochemical constants *viz.* total ash, acid insoluble ash, water soluble ash; water soluble and alcohol soluble extractive values were calculated according to the methods described in Indian Pharmacopoeia [7]. Preliminary phytochemical analysis of stem was performed as described in Khandelwal [5] and Kokate [8]. Fluorescence analysis was carried out according to methods of Kokoski [9]; Chase and Pratt [10]. Phytochemical analysis was carried out using Thin Layer Chromatography as per methods described by Wagner and Bladt [11].

HPTLC fingerprinting of methanolic extract was also carried out. Pre-coated HPTLC plate of silica gel 60 F_{254} (Merck) as stationary phase was used. For sample application, CAMAG Linomat V sample applicator was employed. The plates were then developed in glass twin trough chamber. The developed plates were scanned using TLC Scanner 3 (CAMAG).

RESULTS

Macroscopic Characters

The macroscopical characters of the stem can serve as diagnostic parameters for the plant.

Color: Light green
Texture: Smooth
Extra features: Slender, striated and pubescent

Microscopy

Surface preparation shows presence of covering trichomes and anomocytic type of stomata (Plate No. 1: F, G). Transverse section of young stem is polygonal in outline. Epidermis is single layered and is composed of horizontally flattened cells. The cells are compactly arranged. Covering trichomes (simple and glandular) are present on epidermal cells. The peripheral layers in the ground tissue are composed of collenchymatous and chlorenchymatous cells. These cells occur as bands and they alternate with each other thus forming a continuous layer next to epidermis. A continuous ring of pericyclic fibers is present in the ground tissue. Vascular bundles are arranged in a ring in the ground tissue and are collateral. The rest of ground tissue is parenchymatous (Plate No. 1: C).

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The transverse section of old stem is wavy in outline with distinct ridges and furrows. Epidermis is composed of single layer of cells. Hypodermis consists of 3-4 layers of collenchyma and 1-2 layers of chlorenchyma. The collenchyma is present below ridges, while chlorenchyma is present below furrows. These cells occur as bands and they alternate with each other thus forming a continuous band next to epidermis. Below the hypodermis there are few layers of sclerenchymatous cells. Stele consists of vascular bundles and pith. Xylem and phloem are found in the form of continuous cylinder and traversed by medullary rays. Pith is parenchymatous and is hollow in the centre (Plate No. 1: D). Longitudinal section of stem shows presence of covering trichomes, spiral and pitted thickening of vessel (Plate No. 1: E).
Maceration of stem shows presence of pitted and spiral thickening of vessels and fibers (Plate No. 1: L, N, K).

Table 1: Microscopical features of the powdered stem of *Cardiospermum halicacabum* L.

Sr. No.	Observations	Plate
1.	Trichome	H
2.	Anomocytic stomata	M
3.	Spiral thickening of vessel	I
4.	Pitted thickening of vessel	J
5.	Fiber	K

Table 2: Histochemical analysis of stem of *Cardiospermum halicacabum* L.

Sr. No.	Reagent	Test for	Color	Histological zone
1	Weak Iodine Solution	Starch Grain	Blue	Pith
2	Aqueous $FeCl_3$	Tannin	Black	Cortex
3	Sudan III	Oil Globules	-	-
4	Conc. HCl	Crystals	-	-
5	Phloroglucinol + Conc. HCl	Lignin	Pink	Sclerenchyma, Xylem vessel

Powder characteristics

Preliminary examination of powder

Stem powder is light green in color with slightly bitter taste.

Microscopic examination of powder

The various diagnostic characteristics of stem powder are shown in Table 1 (Plate No. 1: H, M, I, J, K). The histochemical colour reactions on the stem were performed for the identification of various cell components like starch grains, oil globules, tannin, crystals and lignin using appropriate reagents. The colour change in the histochemical zone was observed under microscope and results are shown in Table 2.

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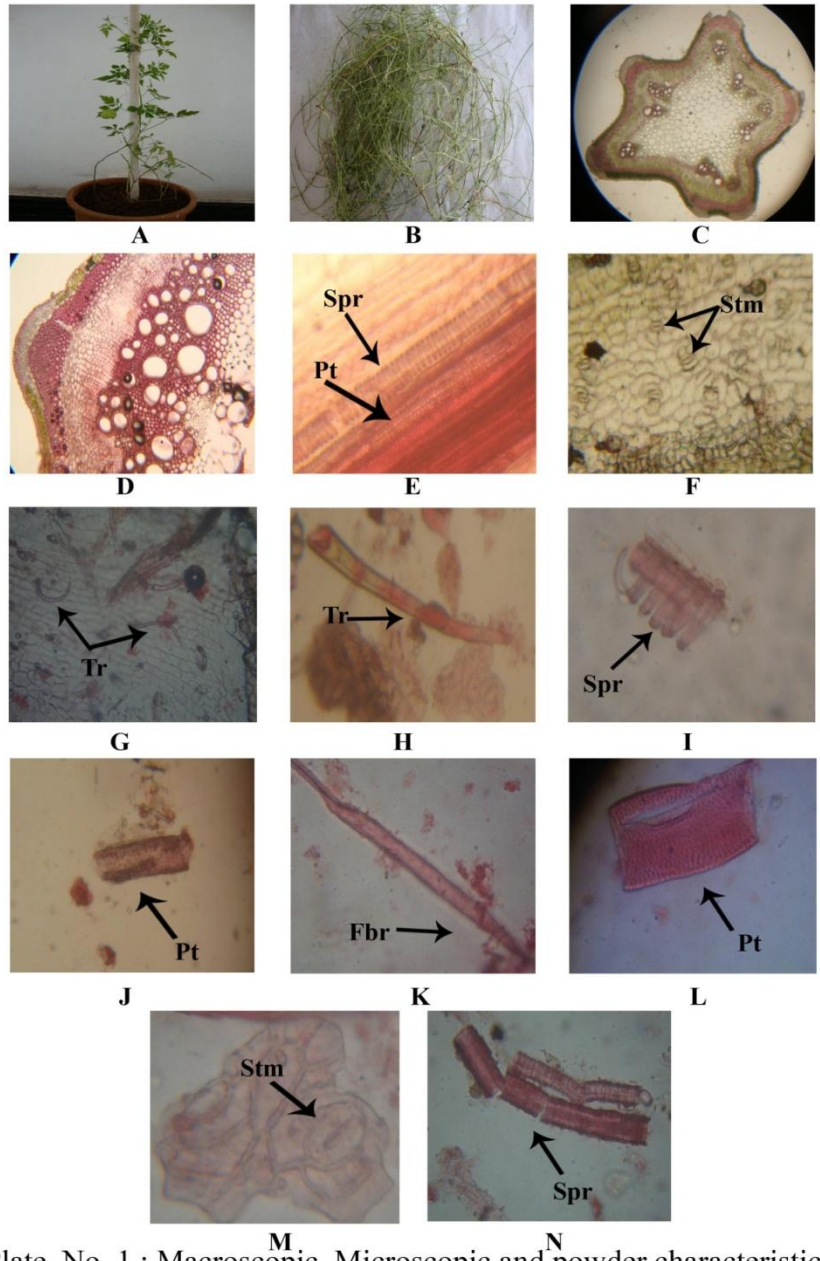

Plate No. 1 : Macroscopic, Microscopic and powder characteristic of *Cardiospermum halicacabum* L. stem.

Key words:- Spr-Spiral thickening, Pt- Pitted thickening, Stm-Stomata, Tr-Trichome, Fbr-Fiber

Fluorescence study

The reaction of certain drugs either in powdered form or on their smooth sectioned surfaces with filtered ultra violet light is of importance in several cases. They help in determining adulterants [12]. Colour reaction of powdered drug with different reagents and their fluorescence analysis were studied and recorded in Table 3.

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Table 3: Fluorescence analysis of powdered stem of *Cardiospermum halicacabum* L

Sr. No.	Treatment	Observation under		
		Ordinary light	UV light	
			254nm	365nm
1.	Powder as such	Light green	Dark green	Green
2.	Powder + Nitrocellulose	Light green	Dark green	Green
3.	Powder + 1N NaOH in methanol	Green	Dark green	Green
4.	Powder + 1N NaOH in methanol + Nitrocellulose in amyl acetate	Green	Dark brown	Greenish brown
5.	Powder + 1N HCl	Light brown	Brown	Greenish brown
6.	Powder + 1N HCl + Nitrocellulose in amyl acetate	Brown	Dark brown	Brown
7.	Powder + 1N NaOH in water	Green	Brown	Greenish brown
8.	Powder + 1N NaOH in water, dried and mounted in Nitrocellulose in amyl acetate	Brownish green	Black	Dark Green
9.	Powder + HNO_3 (1:1)	Brown	Dark brown	Black
10.	Powder + H_2SO_4 (1:1)	Dark green	Black	Dark brown

Physico-chemical Parameters

Table 4: Physico-chemical studies of powdered stem of *Cardiospermum halicacabum* L.

Sr. No.	Parameter	Observation
1.	**Ash values**	
a.	Total ash content	11.1 %
b.	Acid insoluble ash	0.5 %
c.	Water soluble ash	4.0 %
2.	**Extractive values**	
a.	Water soluble extractive values	20.08 %
b.	Alcohol soluble extractive values	15.04 %
3.	**Loss on drying**	48.96 %

Ash values of a drug give an idea of the earthy matter or the inorganic composition and other impurities present along with the drug [13]. Extractive values are primarily useful for the determination of exhausted or adulterated drugs. The extractive value of the crude drug determines the quality as well as purity of the drug [14]. Thus, alcohol and water soluble extractive values were determined. Loss on drying, percentage of total ash, acid insoluble ash, water soluble ash and different extractive values are tabulated in Table 4.

Phytochemical Evaluation

Preliminary phytochemical screening is tabulated in Table 5. Results for TLC are tabulated in Table 6.

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Table 5: Preliminary phytochemical screening of *Cardiospermum halicacabum* L. stem

Sr. No.	Tests for Phytoconstituents	WE	AE	CE
1.	Carbohydrate	+	+	+
2.	Proteins	+	-	-
3.	Amino acid	+	-	-
4.	Saponins	+	-	-
5.	Tannins	+	+	-
6.	Hydrolysable Tannins	-	-	+
7.	Alkaloid	-	-	-
8.	Flavonoids	+	-	-
9.	Steroid	-	-	+
10.	Glycosides	+	+	-
11.	Cardiac glycosides	+	+	+
12.	Anthraquinone	+	-	-
13.	Volatile oil	-	-	-

WE - Water Extract
AE - Alcohol Extract
CE - Chloroform Extract
+: Present
- : Absent

Table 6: Phytochemical analysis of *Cardiospermum halicacabum* L. stem by TLC

Sr. No.	Compound	Type of extract	No. of spots	R*f* value
1.	Arbutin	Methanolic	4	0.05, 0.44, 0.78, 0.98
		Aqueous	2	0.05,0.44
2.	Cardiac glycoside	Methanolic	3	0.11, 0.26, 0.76
		Aqueous	1	0.11
3.	Alkaloid	Methanolic	-	-
		Aqueous	-	-
4.	Essential oil	Methanolic	5	0.14,0.26, 0.29, 0.42, 0.56
		Aqueous	-	-
5.	Bitter principle	Methanolic	3	0.16, 0.35, 0.53
		Aqueous	3	0.16,0.32, 0.46
6.	Pungent principle	Methanolic	4	0.50, 0.64, 0.78, 0.87
		Aqueous	-	-
7.	Anthracene	Methanolic	2	0.25, 0.58
		Aqueous	2	0.25,0.52
8.	Saponin	Methanolic	3	0.29, 0.48, 0.85
		Aqueous	2	0.29, 0.48

HPTLC Studies

A densitometric HPTLC analysis was performed for the development of characteristic fingerprint profile, which may be used as marker for quality evaluation and standardization of

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the drug. R*f* values and the relative percentage of the separated compounds are recorded in Table 7. HPTLC fingerprint and densitogram are given in Plate 2 and 3 respectively.

Plate 2: HPTLC fingerprint of *Cardiospermum halicacabum* L. Stem

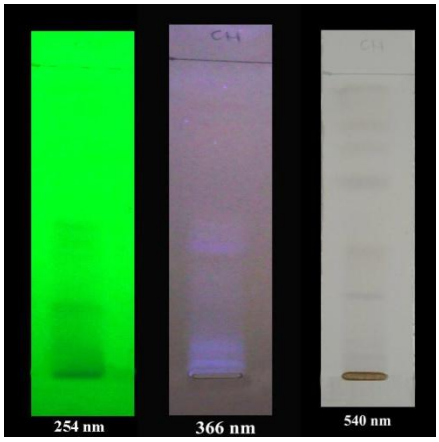

Plate 3: Densitogram of *Cardiospermum halicacabum* L. Stem

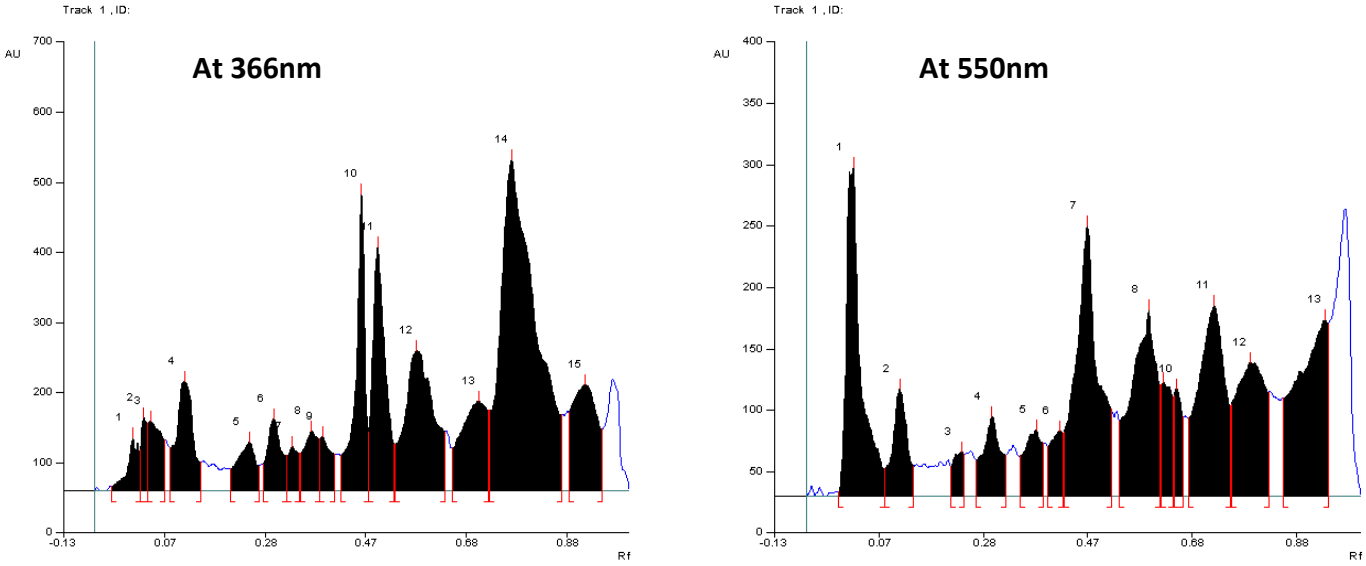

Table 7: R*f* values and relative percentage of the separated phytoconstituents by HPTLC fingerprint.

No. of peaks	366 nm		550 nm	
	Max. R*f*	Relative %	Max. R*f*	Relative %
1.	0.01	1.74	0.03	13.08
2.	0.04	1.26	0.12	4.22
3.	0.05	2.71	0.23	1.34
4.	0.12	5.40	0.29	3.81
5.	0.25	2.60	0.38	3.06

6.	0.29	2.99	0.42	2.19
7.	0.33	1.29	0.47	16.06
8.	0.37	2.53	0.59	12.30
9.	0.39	1.74	0.62	3.35
10.	0.47	8.03	0.65	2.25
11.	0.50	9.15	0.72	13.39
12.	0.58	11.94	0.79	10.52
13.	0.70	6.80	0.93	14.43
14.	0.77	34.08	-	-
15.	0.91	7.75	-	-

DISCUSSION

The standardization of crude drug is an integral part of establishing its correct identity. Despite the modern techniques, identification and evaluation of plant drugs by pharmacognostical studies is still more reliable, accurate and inexpensive means.

Transverse section of *C. halicacabum* stem shows presence of trichomes (simple and glandular), collenchyma, chlorenchyma and collateral vascular bundles whereas longitudinal section showed presence of trichomes, pitted and spiral thickening of vessels. Powder microscopy revealed presence of trichomes, spiral and pitted thickening of vessels and anomocytic stomata. Maceration and surface preparation also showed presence of pitted and spiral thickening, fibers, trichomes and anomocytic stomata respectively. This anatomical study showed diagnostic features that revealed a characteristic pattern of arrangement of the cellular component of stem of *Cardiospermum halicacabum* L.

Histochemical tests revealed the presence of starch grains, tannin and lignin whereas oil globules and crystals were absent. The fluorescence characters of powdered drug plays a vital role in the determination of quality and purity of the drug material. In the present study, powder treated with various reagents shows characteristic fluorescence at 254 nm and 366 nm wavelength.

To determine extent of adulteration as well as to establish the quality and purity of drug, ash and extractive values were calculated. Total ash was found to be 11.1 %, of which, 0.5 % was acid insoluble ash, and 4 % was water soluble ash. The extractive values were found to be 20.08% and 15.04% for water and alcohol respectively, which indicated higher extractive value for water compared to alcohol. The moisture content was found to be 48.96%.

For phytochemical screening by chemical tests, three different extracts *viz.* aqueous, chloroform and alcoholic extracts were used. Presence of carbohydrate, tannins, saponin, glycosides and cardiac glycosides were detected in all the three extracts used. Thin layer chromatography is carried out using two solvents *viz.* methanolic and aqueous. It shows presence of arbutin, cardiac glycoside, essential oil, bitter principle, pungent principle, anthracene and saponin. Results obtained from phytochemical analysis could make the plant useful for treating different ailments and having a potential of providing useful drugs for human

use. HPTLC fingerprint was carried out and their R*f* values as well as their relative percentage were recorded.

CONCLUSION

The pharmacognostical and phytochemical evaluation of *Cardiospermum halicacabum* L. stem can provide useful information for identification and authentication of plant. It can also serve as an important source of information to ascertain the identity and to determine the quality and purity of the plant material in future studies.

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