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Pharmacognostical and analytical studies of leaves of *Cardiospermum canescens* Wall

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Abstract

The present investigation deals with the Microscopical, physicochemical and preliminary phytochemical studies on the leaves of *Cardiospermum canescens* Wall. with the scope of Ethnic importance. Leaves of this plant are used by the tribes of Nellore district as a single drug remedy to treat for Dysentery and Rheumatoid arthritis. In the present work the leaf of the plant was subjected to various microscopical and physico- preliminary phyto chemical evaluations. In the microscopical studies, the different cell structures and arrangements were studied. Physicochemical parameters like loss on drying, total ash value, acid insoluble ash, water insoluble ash, various extractive values etc., were carried out. Further, qualitative tests for various functional groups like Triterpenoids, alkaloids, glycosides etc..., were carried out and HPTLC profile was also established with Methanolic extract.

Keywords: *Cardiospermum canescens* Wall. Macro and Microscopical characters of leaf, macerate, physical constants studies.

Introduction

One of the important and difficult problem encountered in all Traditional systems is the use of different botanical species under the same drug name, because their similarity in the morphological characters. Identification of those plants is very difficult in dried form. In such cases pharmacognastical studies is the only source to identify the genuine plant used, which prevent adulteration of drugs by using Standardization and microscopical methods. With this view the present work was taken on *Cardiospermum canescens* Wall. (Fig.No.1) The plant has been recorded throughout the tropical regions. In India it is recorded in the tropical and subtropical regions growing upto 1300 m in the Himalayan region. This species is globally distributed in the Pantropics. Within India, it is found in the tropical and subtropical regions throughout the plains, ascending upto an altitude of 1300 m. in the Himalayas. The plant has good medicinal value. Leaves of this plant are used as a single drug remedy to treat for Dysentery and Rheumatoid arthritis by the tribes of Nellore district in Andhra Pradesh of India. Past literatures also expose the same medicinal value [1-3].The plant has resemblance with Cardiospermum halicacabum L.(Fig.No.2), Family Sapindaceae. Hence identification of this plant in dried form with macroscopical characters is too hard and also for the pharmacognostical standardization it is new one. For the authentic identification of this plant pharmacognostical standardization is necessary, in this connection present work was taken.

Taxonomy of the plant: Climbing tendril- bearing herb, up to 5m, with wiry stem and branches, stems deeply furrowed. Leaves biternate; leaflets ovate-lanceolate, 1.5-5X 1-3 Cm. pubscent, base acute or attenuate, apex acuminate, petiolule to 1mm. Flowers white, in long peduncled umbellate cymes on tendrils. Male flowers: sepals suborbicular, 1.5 mm. petals 4, white, 6X4 mm; Capsules bloated not winged, 3.5 X 3 Cm; seeds globose, to 5mm (Fig.No.1) [4,5]

Materials and Methods

Fresh leaves of *Cardiospermum canescens* Wall. were collected from the Tribes of Nellore district. Identification and confirmation were done by Department of Botany Sri venkateswara University. The voucher herbarium specimen was processed followed by standard procedure present in Jain and Rao [6]. Microscopical studies of leaf using fresh plant material were carried out with standard procedures [7-10]. During these studies T.S of the leaf, Powder microscopical studies and Maceration were done to observe peculiar characters of the leaf. Physicochemical studies like, total ash, acid insoluble ash, water soluble ash; water soluble and alcohol soluble extractive values were computed according to the methods described in Indian Pharmacopoeia [11. Preliminary phytochemical investigation in leaf powder was performed as

described in Khandelwal et al. [12] and Kokate [10]. Fluorescence analysis was carried out according to methods of Kokoski et [8]. HPTLC fingerprinting Methanolic extract was also carried out. Pre-coated TLC plate of silica gel 60 F (Merck) was used as stationary phase. For sample application, DESAGA 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

Macro and Microscopical Characters of the Leaf

Macroscopical Characters of the Leaf: Leaves biternate; leaflets ovate-lanceolate, 1.5-5X 1-3 Cm. pubscent, base acute or attenuate, apex acuminate, petiolule to 1mm. (Fig.No.1)

Microscopical Characters: T.S of the leaf dorsiventral in structure, plano convex towards lower region and towards upper region slightly bulged, epidermal cells are rectangular covered by thick cuticle(Fig.No.19,24) and shows simple elongated trichomes. Upper epidermis followed by 3-4 durlayered Collenchymatous cells(Fig.No.20) and 1-2 layered Parenchymatous cells. Both the epidermal cells shows simple elongated trichomes (Fig.No.22,23). In the centre vascular bundle is represented by xylem and phloem. Lower epidermal region is represented by rectangular irregular cells covered by thick cuticle (Fig.No.24), followed by 1-2 layered Collenchymatous cells and closely arranged, rounded 3-4 layered Parenchymatous cells, and these cells are filled by prominent clustered Calcium Oxalate crystals and small starch grains(Fig.No.21). T.S of the leaf through laminar region shows both upper and lower epidermis covered by thick cuticle and some of the epidermal cells elongate to form elongated pointed trichomes. Palisade 1-2 layered and spongy tissue single to double layered, loosely arranged and shows clustered calcium oxalate crystals. Stomata present on lower side and of Ranunculaceous (Anomocytic) type of stomata (Fig 18).

Diagnostic Characters: Presence of pointed elongated trichomes, mucilage, prominent clustered calcium oxalate crystals in midrib, and laminar region and Rnunculaceous (Anomocytic) type of stomata in the lower region.

Powder microscopy of Leaf: Leaf powder is Green in colour, coarse to touch, with abundant fibers, smell agreeable and tastes slightly sweetish. By treating with chloral hydrate solution and water, fragments of different tissues were observed under the microscope they are Fragments of elongated uniseriate trichomes with pointed tip. (Fig.No.13), elongated Tracheids with simple pits. (Fig.No.7,17), Abundant uniseriate trichomes (Fig.No.6), thinwalled epidermal cells with clustered crystals. (Fig.No.12), Epidermal cells and clustered crystals (Fig.No.12), rounded thinwalled Parenchymatous cells. (Fig.No.10), abundant elongated fibers in groups. (Fig.No.5) and abundant trichomes with abundant mucilaginous cells.(Fig.No.19)

Diagnostic characters: Presence of uniseriate pointed trichomes in abundance, clustered calcium oxalate crystals abundantly, elongated tracheids with simple pits, Presence of mucilaginous cells abundantly,abundant fibers in groups or in singles and foaming nature of the drug powder when it is treated with water.

Fluorescence study :Fluorescence analysis were studied and recorded in Table 1

Physico-chemical details: Ash values of a drug give an idea of the earthy matter or the inorganic composition and other impurities present along with the drug [7,11,13-15]. The results are given in Table-2.

Preliminary phytochemical tests: These tests revealed the presence of Triterpenoids, Flavanoids, Alkaloids, Saponins, Carbohydrates, Proteins and Tannins listed in Table No-3

HPTLC analysis:

A densitometric HPTLC analysis was established for the development of characteristic fingerprint profile, which may be worked as marker for quality evaluation and standardization of the drug. Rf values and the relative percentage of the separated compounds are given in Table No.4.HPTLC fingerprint and densitogram are given in Fig.No 26 and Fig.No 27 respectively.

Discussion

During this study the microscopical, anatomical, physico-chemical and Preliminary phytochemical analysis were performed in the leaf. HPTLC profile was also established with Ethyl acetate and Methanolic extract. Transverse section of the leaf shows the Presence of pointed elongated trichomes, mucilage, prominent clustered calcium oxalate crystals in midrib, and laminar region and Rnunculaceous (Anomocytic) type of stomata in the lower region. Powder microscopy of Leaf revealed the presence of uniseriate pointed trichomes, clustered calcium oxalate crystals, elongated tracheids with simple pits, mucilaginous cells and abundant fibers in groups or in singles. Maceration studies of leaf powder brought out the presence veinlets and Xylem elements with Spiral thickenings. This anatomical study brought to light diagnostic features that revealed a characteristic pattern of arrangement of the cellular components of leaf of Cardiospermum canescens Wall. 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 255 nm and 365 nm wavelength. To determine extent of adulteration as well as to establish the quality and purity of drug, ash values were calculated. Total ash was

Table No.1—Behavior of the drug (Leaf) powder with different Chemical reagents. [7,13]

S.N0.	Treatment	Colour observations Under		
		Ordinary light	U.V Light	
			255nm	365nm
1.	Powder +Distilled water	Light Green , mucilaginous with foamy nature.	Dark Green	Green
2.	Powder+5%Aqueous FeCl	No change	No change	No change
3.	Powder+Glacial acetic acid	No change	No change	No change
4.	Powder+5% HNO_3	No change	No change	No change
5.	Powder+N/10 Iodine Solution	Blue colour	Dark Blue	Pale Blue
6.	Powder+ConHCL	Light Green	Dark Green	Green
7.	Powder+ConH_2SO_4	Black	Black	Brownish Black
8.	Powder+Ammonia solution	Light green	Dark Green	Green
9.	Powder+5%Aqueous NaOH	No change	No change	No change
10.	Powder+5%Aqueous KOH Solution.	Green	Black	Blackish green

Table No.2.Physicochemical Parameters (% W/W)

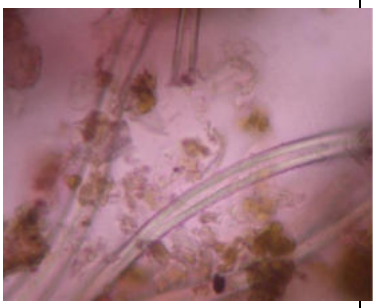
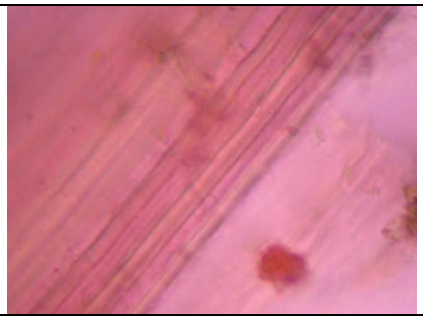
S.No	Reaction Name:	Values
1.	Total ash	9.5%
2.	Acid insoluble ash	1.12%
3.	Water soluble ash	2.25%
4.	Water insoluble ash	5.9%
5.	Moisture content (LOD) at 110 C	21.64%
6.	Water soluble extractive values	21.52%
7.	Alcohol soluble extractive values	16.43%

Table No.3 Preliminary phytochemical tests revealed the presence of Triterpenoids, Flavanoids, Alkaloids, Saponins, Carbohydrates, Proteins and Tannins.

S.No	Chemical compound name	Chemical test	Result
1.	**Triterpenoids**	a)Leibermann buchard test	+
		b)salkowsky test	+
2.	**Flavanoids**	a)Lead acetate test	+
3.	**Glycosides**	a)Baljet test	_
		b)Legal test	_
4.	**Steroids**	a) Leibermann buchard test	_
		b)Salkowsky test	_
5	**Alkaloids**	a)Dragendroffs test	+
		b)Hagers test	+
		c)wagers test	-
		d)Mayers test	+
8.	**Proteins**	Biuret test	+
9.	**Tannins**	Ferric chloride test	+

Table No.4 Peak list of *Cardiospermum canescens* Wall. at 366nm

Peak no.	Y-Pos	Area	Area (%)	Height	Rf value
1	10.7	1493.80	67.9	738.09	0.02
2	20.7	5.58	0.3	4.76	0.16
3	25.8	123.37	5.6	53.57	0.23
4	30.3	13.67	0.6	6.92	0.30
5	40.2	62.75	2.9	21.91	0.43
6	47.5	3.74	0.2	2.54	0.53
7	53.8	80.92	3.7	22.41	0.62
8	67.8	13.12	0.6	6.15	0.82
9	78.2	402.55	18.3	81.62	0.96

		
Fig.No.1. ***Cardiospermum canescens*** Wall.	Fig.No.2.Cardiospermum halicacabum L.	Fig.No.3. Abundant Mucilaginous cells
		
Fig.No.4.Macroscopy Leaf Powder	Fig.No.5 Fragments of elongated fibers.	Fig.No.6. Uniseriate trichomes

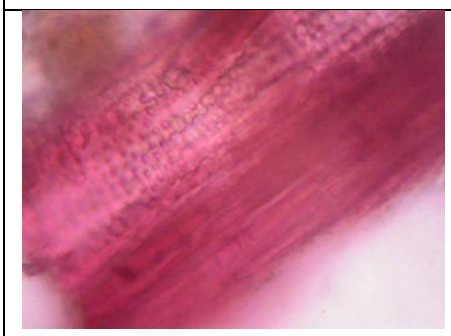
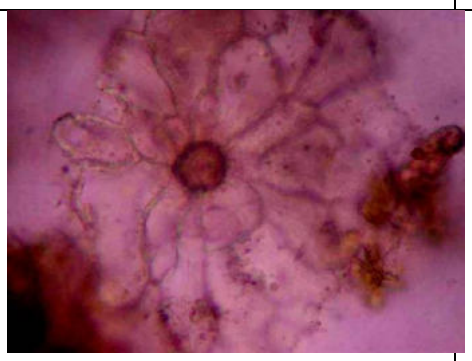
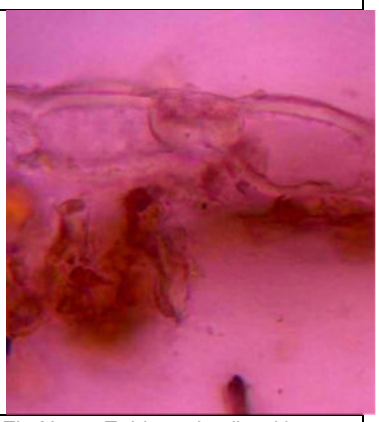

		

Fig.No.7 Tracheids with simple pits and fibers.	Fig.No.8 Epidermal cells and clustered crystals.	Fig.No..9. Epidermal cells with mucilage.

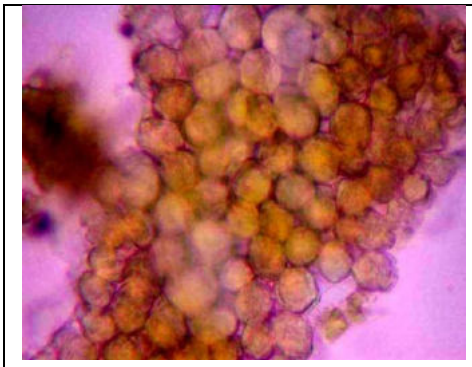
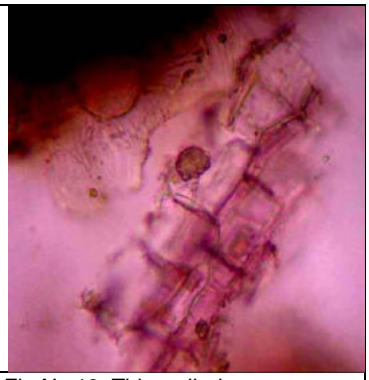
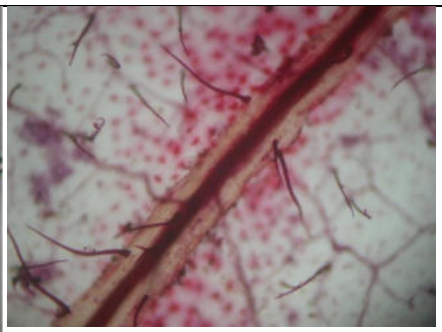
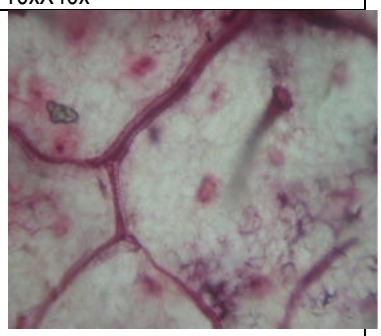
		

Fig.No.10. Parenchymatous cells 10xX40x	Fig.No.11 Clustered crystal 10xX40x	Fig.No.12. Thin walled Parenchymatous cells with crystals 10xX40x
		
Fig.No.13. Single trichome with pointed tip.	Fig.No.14. Trichomes and veinlets.	Fig.No.15. Veinlets with Spiral thickenings.

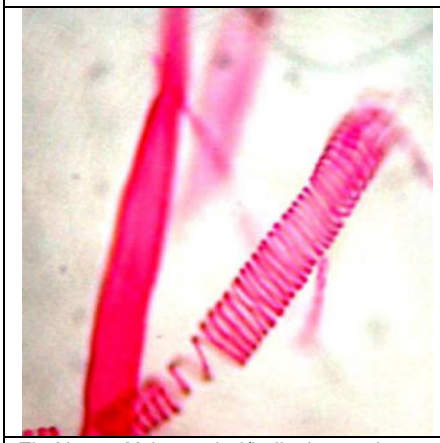
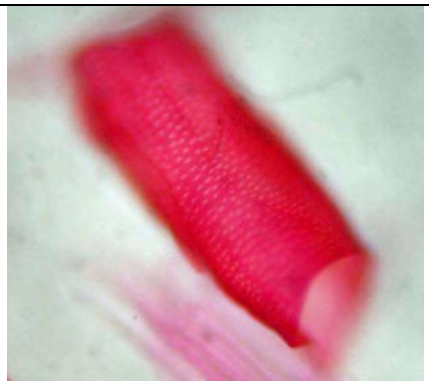
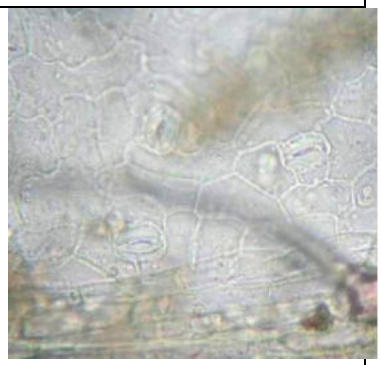

		

Fig.No.16. Xylem spiral/helical vessel .	Fig.No.17. Xylem vessel with simple pits.	Fig.No.18. Ranunculaclous type of stomata in the lower region

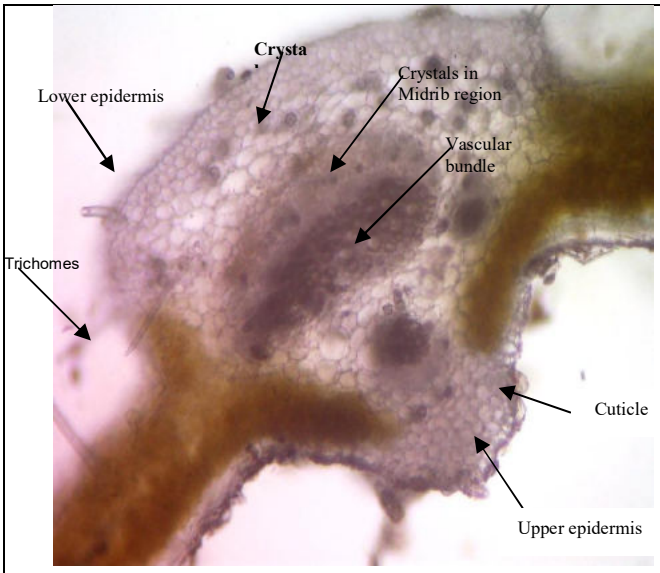


Fig.No.19. T.S of the leaf (Ground plan)

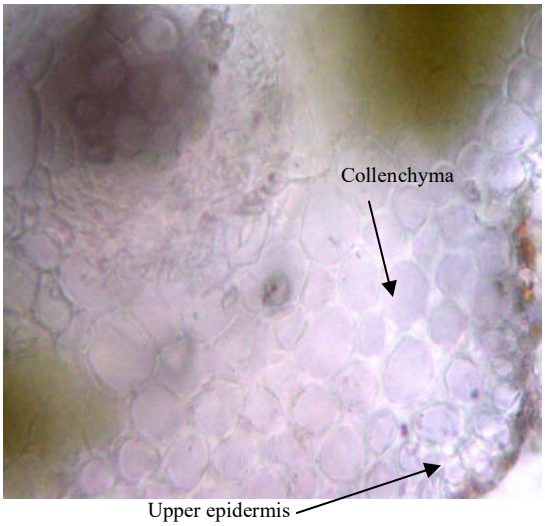


Fig.No.20. Upper Epidermis enlarged

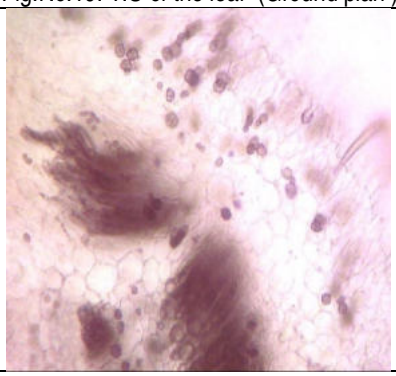


Fig.No.21. Parenchymatous region showing abundant crystals and starch grains towards the lower epidermis.

Fig.No.22. Laminar region showing abundant Trichomes.

Fig.No.23. Laminar region showing abundant Pointed Trichomes.

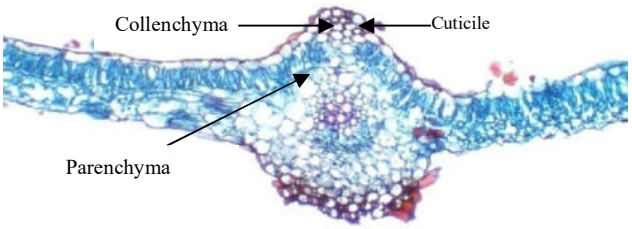


Fig.No.24. T.s of the leaf showing laminar region, vascular bundle, upper and lower epidermis

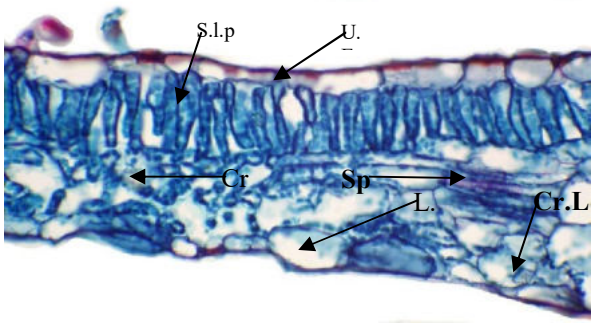


Fig.No.25. T.S. of leaf with short glandular trichome in the adaxial. S.l.p : Single layered palisade tissue; U.E: Upper epidermis; L.E: Lower epidermis; Cr: Clustered crystals; Cr.l: Crystals in Lower epidermis; Sp:Tracheids with spiral thickenings

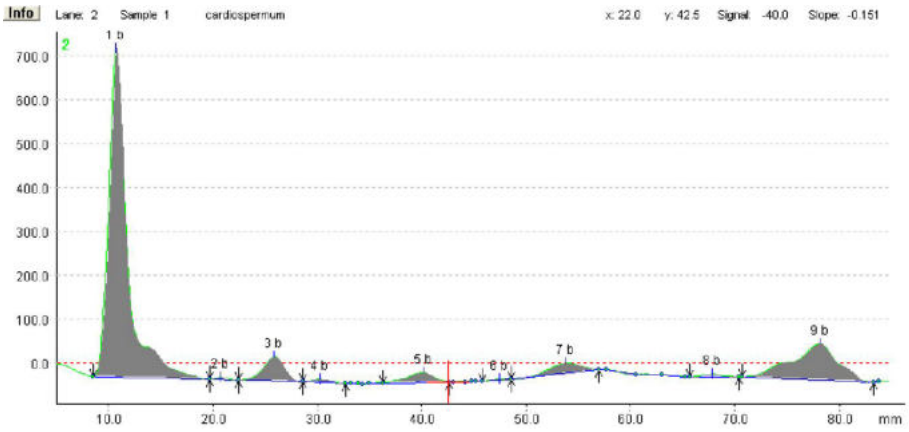
Fig.No 26. Finger Print TLC of methanolic extract of *Cardiospermum canescens* Wall. applied in triplicate as Track 1, 2 & 3 .

UV 366nm

Under Iodine vapors

Solvent system: Toluene: Ethyl Acetate: Methanol = 7: 2: 1 Spots: Nine under UV 366nm Solvent Run: 81mm

Fig.No 27 Densitogram of *Cardiospermum canescens* Wall. at 366nm



found to be 9.5%, of which, 1.12% was acid insoluble ash, and 2.25%was water soluble ash and 5.9%water insoluble . The extractive values were found to be 21.528% and 16.43%for water and alcohol respectively, which indicated higher extractive value for water compared to alcohol. The moisture content was found to be 21.64%. Preliminary phytochemical screening revealed the presence of Triterpenoids, Flavanoids, Alkaloids, Saponins, Carbohydrates, Proteins and Tannins. HPTLC fingerprint was established with Ethyl acetate and Methanolic extract. The pharmacognostical and phytochemical screening on the Leaf Cardiospermum canescens Wall. furnish useful information for identification and authentication of plant. It can also assist as an important source of information to insure the identity and to determine the quality and purity of the plant material in future studies.

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