Phytochemical and analytical evaluation of *Cordia dichotoma* Linn. leaves

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ABSTRACT

Background: An ethnomedicinally important plant, *Cordia dichotoma* Linn is practiced in various indigenous systems of medicine and popular among the various ethnic groups in India for the cure of variety of ailments as an astringent, anthelmentic, diuretic, demulcent, anti-diabetic and expectorant. Because of the increasing demand, maintaining quality standards is the need of the day. **Aims and Objectives:** The present study was designed to set standard pharmacognostical, physicochemical, phytochemical, fluorescence and HPTLC chromatographic profile of the leaves of *Cordia dichotoma* Linn (CD). **Materials and Methods:** CD, which was previously authenticated, was subjected to pharmacognostical, physicochemical, fluorescence and high performance thin-layer chromatography (HPTLC) analysis as per standard protocol. **Results and Conclusion:** The final observations were recorded. The loss on drying at 105°C was found to be 8.5% w/w, total ash value 13% w/w, acid-insoluble ash 5.07% w/w, water-soluble ash 5.49% w/w, water-soluble extractive 9.2% w/w, alcohol-soluble extractive 5.81% w/w and pH (1% aqueous extract) 6.88. Phytochemical screening showed the presence of steroid, carbohydrate, alkaloid, saponin, cardiac glycosides, flavonoid and phenolic compounds in methanolic extract. The CD fluorescence was seen in UV light and it was of different colour in different solvents. HPTLC analysis revealed 5 peaks at wavelength 366 nm with max R_f values in the range of 0.3 to 0.93. The purity and quality of the leaves of *Cordia dichotoma* or pharmaceutical preparations prepared from it can be tested by pharmacognostical, physicochemical, fluorescence and HPTLC observations of the present study.

Keywords: *Cordia dichotoma,* Fluorescence analysis, Physicochemical parameters, HPTLC chromatogram.

INTRODUCTION

Cordia dichotoma Linn belonging to the family Boraginaceae is a small to moderate-sized deciduous tree with a short bole and spreading crown widely distributed in India and Srilanka.[1] It is commonly named as Indian cherry (English), Lasura/Bhokar/Borla (Hindi), Vadgundo/Gunda (Gujarati). The various parts of the plant viz., stem bark and leaves are practiced in various indigenous systems of medicine viz., Ayurveda and Unani and popular among the various ethnic groups in India for the cure of variety of ailments as an astringent, anthelmentic, diuretic, demulcent, anti-diabetic and expectorant. Their leaves are traditionally used for the treatment of jaundice at Dandakaranya area, Andhra Pradesh in India. It is reported to have antioxidant, juvenomimetic, antifertility, anti-inflammatory and various other pharmacological activities.[2-4] Carotenoids are mainly present in their leaves which have potent antioxidant activity.[5] Present study deals with the detailed pharmacognostical study of its leaves, physicochemical evaluation, fluorescence analysis and HPTLC chromatographic fingerprint profiling. Microscopic method is one of the simplest and cheapest methods to start with for establishing the identity of the source materials despite of the availability of sophisticated modern research tools for evaluation of the plant and plant derived crude drugs. This study will provide standardized parameters for the leaves of *Cordia dichotoma* Linn to future scientists for its correct identification and adulteration, if any.

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MATERIALS AND METHODS

Collection and authentication of plant specimen

Leaves of the plant *Cordia dichotoma* Linn for the study were collected from nearby region of Kukrail forest, Lucknow, Uttar Pradesh and authenticated by National Botanical Research Institute, Lucknow (authentication reference number NBRI/CIF/306/2012 dated 18/06/2012).

Materials and reagents

Soxhlet apparatus, rotavapor, CAMAG HPTLC system (Muttenz, Switzerland) equipped with Linomat5 applicator, Reprostar3, TLC scanner3, twin trough plate development chamber, Hamilton syringe (100µl, Reno, Nevada, USA), win-CATS software, analytical grade chemicals (Fischer Scientific and E. Merck, India), HPLC grade methanol (E. Merck, India).

Pharmacognostical evaluation

Pharmacognostical evaluation of CD comprises of organoleptic characters [i.e., color, odor, taste, and texture] and microscopic studies. Organoleptic characters were recorded by observing with naked eyes. Microscopic studies, i.e., transverse section (T.S.) of leaf by treating with saffaranin and mounting with glycerin water over glass slide was observed under Carl Zeiss Binocular microscope attached with camera and microphotographs were taken.[6]

Physicochemical evaluation

CD was analyzed through physicochemical parameters i.e., loss on drying, total ash value, acid-insoluble ash, water-soluble ash, pH value and extractive values.[7,8]

Phytochemical screening

Phytochemical screening for carbohydrate, protein, alkaloid, steroid, glycoside, etc., had been carried out.[9]

Fluorescence analysis

Finely powdered CD after treatment with different chemicals was analyzed through fluorescence analysis. It was performed for the different extracts too but without any chemical treatment.[10,11]

HPTLC chromatographic analysis

Sample solution of methanolic extract of *Cordia dichotoma* leaves (MECD) was spotted in the form of band using a Hamilton syringe on precoated silica gel GF 60_{254} aluminium plate by means of CAMAG Linomat 5 sample applicator. Toluene: ethyl acetate: formic acid (5:4:1) was used as the mobile phase. After development, plate was kept in CAMAG Reprostar 3 and densitometric scan was performed with a Camag TLC scanner3 in reflectance absorbance mode at UV detection as 254 nm and 366 nm under the control of win-CATS software.[12,13]

OBSERVATIONS AND RESULTS

Pharmacognostical evaluation

Organoleptic features of CD are shown in **Figure 1**. Shape of leaf is almost ovoid with dentate margin. Upper and lower surfaces are rough with light green in color and appearance. Odor is pleasant and taste is mucilaginous. Microscopy of CD showed features like scattered vascular bundles having patches of perimedullary phloem, unicellular and multicellular covering trichomes as shown in **Figure 2**. Xylem vessels and calcium oxalate crystals were seen too in powder microscopy.

Physicochemical evaluation

CD was analyzed through physicochemical parameters. pH of 1% (w/v) aqueous solution of powdered leaves was found to be 6.88, approximately of neutral pH. Other observations are presented in **Table 1 and Table 2**.

Table 1: Loss on drying and ash values for the powdered leaves of the plant *Cordia dichotoma*

Physicochemical parameters		*% (with reference to air dried drug)*
Loss on drying		8.5
Ash value	Total ash	13
	Acid insoluble ash	5.07
	Water soluble ash	5.49

Phytochemical screening

Methanolic extract showed the presence of steroid, carbohydrate, alkaloid, saponin, cardiac glycosides, flavonoid and phenolic compounds. The intensity of presence is shown in **Table 3**.

Fluorescence analysis

Fluorescence characteristics of powdered leaves of the plant *Cordia dichotoma* and its various successive solvent extracts under day and ultraviolet light are presented in **Table 4 and Table 5**.

Figure 1: Leaves of *Cordia dichotoma* Linn

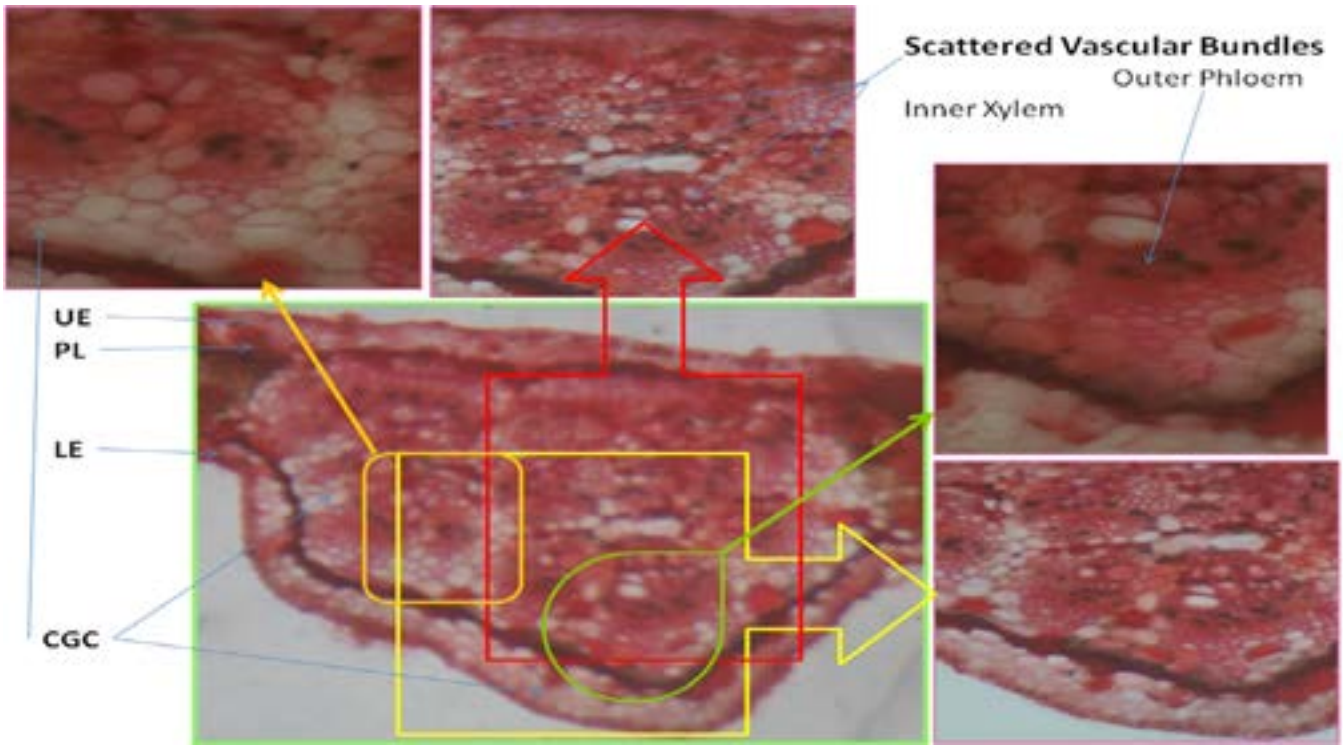

Figure 2: T.S. of leaf of *Cordia dichotoma* Linn in midrib region under 4X, 10X and 28X views of microscope showing scattered vascular bundles [UE: Upper Epidermis, LE: Lower Epidermis, PL: Palisade Layer, CGC: Collenchymatous Ground Cells].

Table 2: Extractive values with color, consistency and solubility in water of different extracts for the powdered leaves of the plant *Cordia dichotoma*

Solvent extracts	*Color*	*Consistency*	*Solubility in water*	*Extractive values (% yield)*
Petroleum ether extract	yellowish green	sticky	insoluble	0.60
Chloroform extract	dark yellowish green	non-sticky	soluble	0.80
Methanol extract	dark green	non-sticky	highly soluble	5.81
Aqueous extract	brown	dry powder	highly soluble	9.20

Table 3: Preliminary phytochemical screening of the methanolic extract of *Cordia dichotoma* leaves

Chemical Tests	Results	Chemical Tests	Results
1. Tests for phenolics and flavonoids		C. Cardiac Glycosides	
a) Lead acetate test	++	a) Legal test	–
b) Ferric chloride test	++	b) Keller-Killiani test	+
c) Sodium hydroxide test	++	**D. Test for steroids** Salkowski test	++
d) Shinoda test	++	**4. Test for Carbohydrates:**	
2. Test for Alkaloids		**A. Reducing sugar**	
a) Mayer's test	–	a) Molisch's test	–
b) Dragendorff's test	–	b) Fehlings test	+
c) Wagner's test	++	**B. Starch** (iodine test)	–
d) Hager's test	++	**5. Test for Proteins and free amino acids**	
3. Test for Glycosides:		a) Biuret test	+
A. Saponin Glycosides		b) Millions test	++
a) Foam Test	++	c) Xanthoprotein test	++
b) Sodium bicarbonate test	+	d) Ninhydrin test	+
B. Anthraquinone Glycosides Borntrager's test	–	**(++) indicates medium presence, (+) weak and (–) absence.**	

Table 4: Fluorescence characteristics of powdered leaves of the plant *Cordia dichotoma*

Chemical treatment	Fluorescence		
	Day light	UV light	
		254 nm	366 nm
Powder as such	greenish brown	purple	black
Powder + water	brown	light green	brown
Powder + 1 N HCl	brown	light green	brown
Powder + 5% NaOH	light green	dark green	purplish brown
Powder + 1 N NaOH (Alc.)	green	green	black
Powder + 50% HNO_3	reddish green	green	purplish brown
Powder +1M H_2SO_4	brown	green	brown
Powder +25% liquid ammonia	green	green	purplish black
Powder +acetic acid	light green	dark green	purple
Powder+iodine solution	yellow	dark green	black
Powder + 5% $FeCl_3$ in ethanol	light green	dark green	black

HPTLC chromatographic analysis

On analyzing under densitometer at UV 254 nm and 366 nm, the HPTLC chromatogram revealed several peaks **(Figure 3, Figure 4, Table 6 and Table 7)**. It revealed 3 peaks at UV 254 nm. While, It revealed 5 peaks at UV 366 nm with max R_f values in the range of 0.3 to 0.93 indicating the occurrence of atleast 5 different components in 5 µl of MECD. Four of the components with max R_f values 0.3, 0.53, 0.60 and 0.65 were found to be predominant as the percentage area was more with 30.97%, 13.18%, 26.23 and 20.91% respectively. One of the components with max R_f value of 0.93 was found to have the percent area less than 10%.

Table 5: Fluorescence characteristics of various successive solvent extracts of powdered leaves of the plant Cordia dichotoma

Chemical treatment	Day light	Fluorescence	
		UV light	
		254 nm	366 nm
Petroleum ether extract	yellowish green	green	Black
Chloroform extract	light green	light green	greenish black
Methanolic extract	green	dark green	black
Aqueous extract	reddish brown	chocolate colored	black

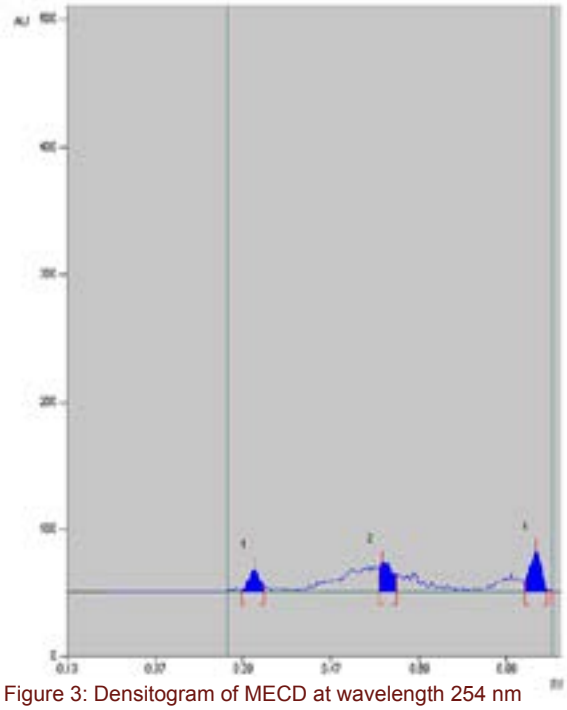

Figure 3: Densitogram of MECD at wavelength 254 nm

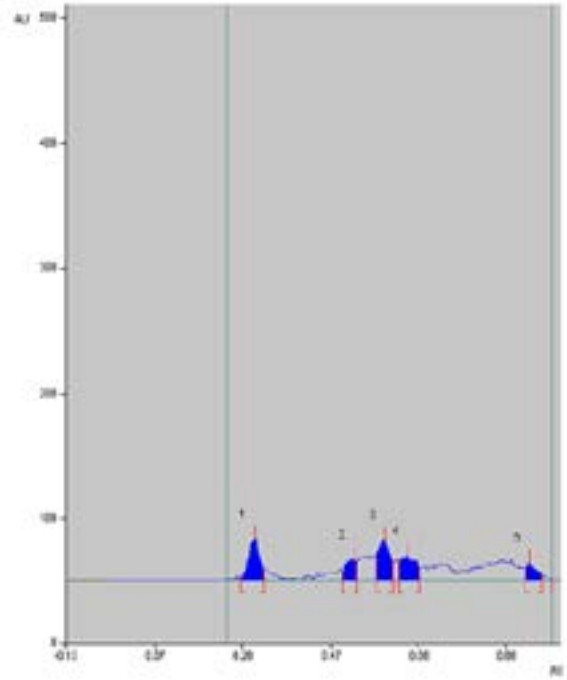

Figure 4: Densitogram of MECD at wavelength 366 nm

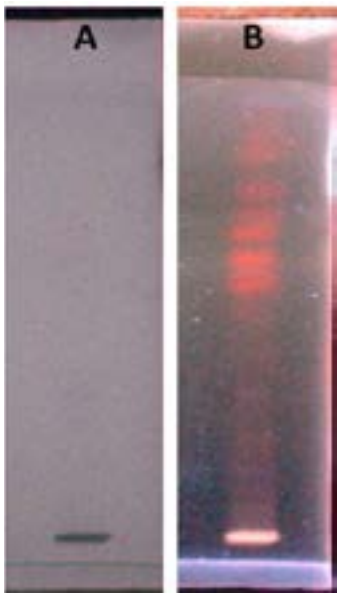

Figure 5: TLC photographs of MECD [under UV 254 nm (A), and UV 366 nm (B)].

DISCUSSION

Standardization of plant material is a complex task due to their heterogeneous composition, which is in the form of whole plant, plant part or extracts obtained thereof. Thus, to solve the task, there has been a rapid increase in the standardization of selected medicinal plants of potential therapeutic significance in recent years. To ensure reproducible quality, proper identification and authentification of starting material is first and essential step. Thus, care was given during collection of plant material for proper plant part and proper time of collection. Then, it was authenticated by expert scientist of the field. Physicochemical parameters were generally employed for deciding the identity, purity and adulterants, if any, present in the plant materials. Despite the modern techniques, identification of plant materials by the pharmacognostic evaluation and HPTLC chromatography analysis is

Table 6: Densitogram table of MECD for measurement at wavelength 254 nm

Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Area	% Area
1	0.27	0.8	0.30	17.4	24.29	0.33	2.9	412.8	23.47
2	0.59	18.2	0.59	23.1	32.26	0.63	12.8	594.9	33.83
3	0.92	7.8	0.94	31.1	43.46	0.97	0.8	751.0	42.70

Table 7: Densitogram table of MECD for measurement at wavelength 366 nm

Peak	Start R_f	Start Height	Max R_f	Max Height	Max %	End R_f	End Height	Area	% Area
1	0.27	1.2	0.30	33.5	29.10	0.34	4.0	876.9	30.97
2	0.50	6.2	0.53	16.5	14.33	0.54	14.6	373.1	13.18
3	0.58	19.6	0.60	31.7	27.50	0.62	14.6	742.8	26.23
4	0.63	15.4	0.65	20.0	17.32	0.68	9.1	592.1	20.91
5	0.92	8.9	0.93	13.5	11.75	0.96	3.3	246.5	8.71

more reliable. As a part of standardization, organoleptic evaluation was carried out. Organoleptic evaluation is a technique of qualitative analysis based on the study of morphological and sensory parts like trichomes of drugs. The organoleptic characters of the leaf serve as diagnostic tools. The microscopic and physicochemical evaluation had been carried out. The percent extractives in different solvents like petroleum ether, chloroform, alcohol, water indicate the quantity and nature of constituents in the extracts. Extractive values are also helpful in estimation of specific constituent soluble in particular solvent. HPTLC chromatographic analysis was carried out which is particularly most valuable because, chromatographic fingerprint is a rational option to meet the need for more effective and powerful quality assessment to Indian traditional medicine and Chinese traditional herbal medicine. The final observations were recorded. The loss on drying at 105ºC was 8.5% w/w, total ash value 13% w/w, acid-insoluble ash 5.07% w/w, water-soluble ash 5.49% w/w, water-soluble extractive 9.2% w/w, alcohol-soluble extractive 5.81% w/w and pH (1% aqueous extract) 6.88. The CD fluorescence was seen in UV light and it was of different colour in different solvents. HPTLC analysis revealed 5 peaks at wavelength 366 nm with max R_f values in the range of 0.3 to 0.93. This finding is useful to supplement existing information with regard to identification and standardization in the powdered form of plant drug to distinguish it from adulterants.

CONCLUSION

From the present analysis, it can be concluded that the pharmacognostical characters along with their physicochemical parameters, fluorescence characteristics and HPTLC chromatographic profiling of the MECD leaf yielded a set of standards that may serve as an important source of information with regard to its standardization and identification to ascertain the identity and purity of the leaves of CD or pharmaceutical preparations prepared from it in further research studies.

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