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Phytochemical and analytical evaluation of Cordia dichotoma Linn. Leaves

Seema Bahuguna, Anubi Badhani, Archana Gautam
Assistant professor, Assistant professor, Pharmacy, JBIT, Dehradun, India

Abstract-Cordia dichotoma Linn, a plant that is significant from an ethnomedicinal perspective, is used in a number of indigenous medical practises and is popular among a diverse range of ethnic groups in India for the treatment of a wide range of conditions, including as an astringent, an anthelmintic, a diuretic, a demulcent, an anti-diabetic, and an expectorant. The ever-increasing customer demand makes it imperative that quality control measures be strictly adhered to at all times. Aims and Objectives: The primary purpose of this investigation was to establish a standard pharmacognostical, physicochemical, phytochemical, fluorescence, and HPTLC chromatographic profile of the leaves of Cordia dichotoma Linn (CD). Both the Materials and the Methods: Analyses of pharmacognostical, physicochemical, fluorescence, and high performance thin-layer chromatography (HPTLC) were carried out on CD in accordance with the standard methodology. The CD had previously been authenticated. The results and the conclusion are as follows: the last observations were written down. It was determined that the loss on drying at 105 degrees Celsius was 8.5% by weight, that the total ash value was 13% by weight, that acid-insoluble ash was 5.07% by weight, that water-soluble ash was 5.49% by weight, that water-soluble extractive was 9.2% by weight, that alcohol-soluble extractive was 5.81% by weight, and that the pH of 1 percent aqueous extract was 6.88. The phytochemical analysis of the methanolic extract revealed the presence of steroid, carbohydrate, alkaloid, saponin, cardiac glycosides, flavonoid, and phenolic components. Under UV light, the fluorescence of the CD could be seen, and depending on the solvent, it appeared to be a different colour. Analysis using HPTLC showed that there are five peaks at a wavelength of 366 nm, with maximum Rf values ranging from 0.3 to 0.93. The pharmacognostical, physicochemical, fluorescence, and HPTLC observations made in this study can be used to evaluate the purity and quality of the leaves of Cordia dichotoma or medicinal compositions prepared from them.

Keywords- Physicochemical parameters, HPTLC chromatogram, *Cordia dichotoma*, Fluorescence analysis.**Introduction -**Cordia dichotoma Linn is a small to moderate-sized deciduous tree that belongs to the family Boraginaceae. It has a short bole and a spreading crown, and it is found throughout India and Srilanka. 1 Commonly known as Indian cherry in English, Lasura/Bhokar/Borla in Hindi, and Vadgundo/Gunda in Spanish and Portuguese respectively (Gujarati). Ayurveda and Unani are two examples of indigenous medical practises that make use of various plant parts, such as the stem, bark, and leaves of the plant. These treatments are also popular across the many different ethnic communities that make up India.as a remedy for a wide range of conditions thanks to its properties as an expectorant, astringent, anthelmintic, diuretic, demulcent, and anti-diabetic. In the Dandakaranya region of Andhra Pradesh, India, the leaves of this plant have a long history of use as a traditional medicine for the treatment of jaundice. It has been suggested that it possesses pharmacological properties such as antioxidant, juvenomimetic, antifertility, and anti-inflammatory properties, amongst other properties. 2-4 Carotenoids, which are mostly found in their leaves and have powerful antioxidant action, are present in this plant. 5 The current research focuses on a comprehensive pharmacognostical investigation of the plant's leaves, as well as physicochemical testing, fluorescence analysis, and HPTLC chromatographic fingerprint profiling. Despite the availability of more sophisticated modern research tools for evaluating plants and plant-derived crude drugs, the microscopic method is still one of the easiest and most cost-effective ways to begin establishing the identity of the source materials. This is despite the fact that it is one of the oldest methods. This study will offer future scientists with standardised characteristics for the leaves of Cordia dichotoma Linn, which will help them correctly identify the plant and detect any adulteration that may have occurred

MATERIALS AND METHODS

Plant specimens are being collected and authenticated at this time.
For the purpose of the study, leaves of the plant Cordia dichotoma Linn were taken from a neighbouring location of Kukrail forest in Lucknow, Uttar Pradesh, and authenticated by the National Botanical Research Institute of Lucknow

Components and chemical substances

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

Pharmacognostical assessment

The evaluation of CD for pharmacognostic purposes includes organoleptic characteristics (such as colour, odour, taste, and texture) as well as microscopic analyses. Observation with the naked eyes was used to capture the organoleptic characteristics. Microscopic examinations, specifically a transverse section (T.S.) of leaf that was prepared by treating it with saffaranin and mounting it with glycerin water over a glass slide, were analysed using a Carl Zeiss microscope. Microphotographs were taken using a binocular microscope that was also equipped with a camera. 6

physiochemical and physicochemical analysis

Physicochemical parameters, such as loss on drying, total ash value, acid-insoluble ash, water-soluble ash, pH value, and extractive values, were utilised in order to conduct an analysis of CD.

Examination of phytochemicals

The phytochemical analysis included looking for things like carbohydrates, proteins, alkaloids, steroids, and glycosides, among other things.

Examination with fluorescence

Through the use of fluorescence analysis, finely powdered CD that had been subjected to a variety of chemical treatments was investigated. In addition to that, it was carried out with each of the various extracts, but no chemical treatment was applied. 10,11

chromatographic examination with HPTLC

The banding technique was used to highlight a sample solution of methanolic extract of Cordia dichotoma leaves (MECD). a CAMAG Linomat 5 sample applicator being used in conjunction with a Hamilton syringe that has been placed on a precoated silica gel GF 60254 aluminium plate. The mobile phase consisted of a mixture of toluene, ethyl acetate, and formic acid at a ratio of 5:4:1. Following the completion of the development process, the plate was maintained in the CAMAG Reprostar 3 and a densitometric scan was carried out using a Camag TLC scanner3 in reflectance absorbance mode at UV detection as 254 nm and 366 nm under the direction of win-CATS software. 12,13

OBSERVATIONS AND RESULTS

Pharmacognostical assessment

The organoleptic characteristics of CD are depicted in figure 1. The leaf has a shape that is almost spherical and has a dentate border. Both the upper and lower surfaces have a rough texture and a colour and look of light green. The flavour has a mucilaginous quality, and the aroma is really delightful. Microscopy of CD revealed characteristics such as scattered vascular bundles with patches of perimedullary phloem, as well as unicellular and multicellular covering trichomes (see Figure 2 for further explanation). Powder microscopy revealed the presence of xylem vessels as well as calcium oxalate crystals.

physiochemical and physicochemical analysis

Physicochemical parameters were utilised in order to evaluate CD. It was discovered that the pH of an aqueous solution containing one percent (w/v) of powdered leaves was 6.88, which is about neutral pH. The following are some further observations, which are reported in Tables 1 and 2.

Physicochemical parameters		*% (with reference to air dried drug)*
Loss on drying		8.3
Ash value	Total ash	12
Acid insoluble ash		5.05
Water soluble ash		5.42

Examination of phytochemicals

The methanolic extract demonstrated the presence of components including steroids, carbohydrates, alkaloids, saponins, cardiac glycosides, flavonoids, and phenols. Table 3 outlines the degree of presence that was observed.

Examination with fluorescence

Tables 4 and 5 demonstrate the fluorescence characteristics of powdered leaves of the plant Cordia dichotoma and its numerous consecutive solvent extracts under daytime and ultraviolet light, respectively.

Figure 1: Leaves of *Cordia dichotoma* Linn

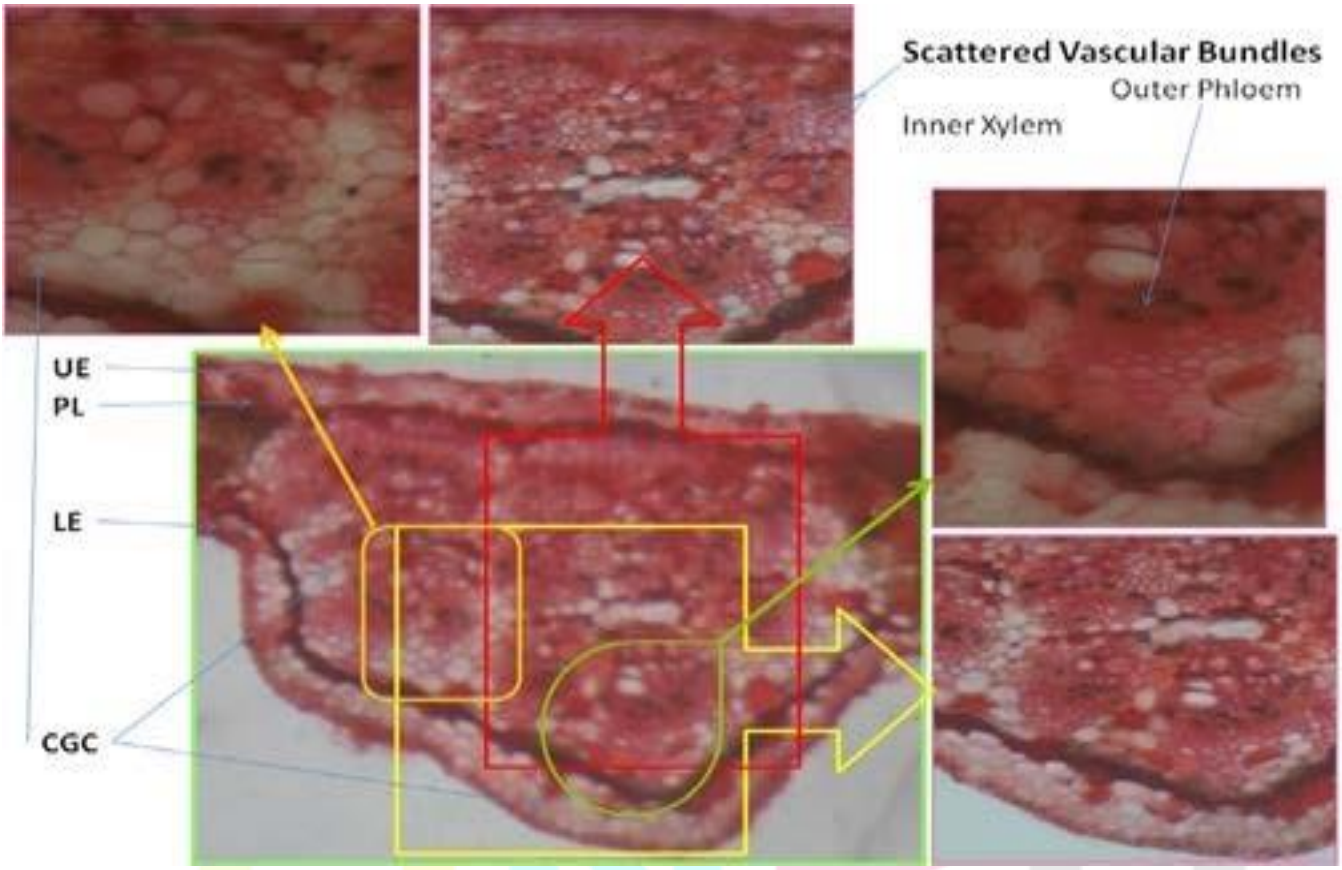

Figure 2: T.S. of leaf of *Cordia dichotoma* Linn in midrib region under 4X, 10X and 28X views of microscope showing scattered vascular bundles [UE: Upper Epidermis, LE: Lower Epidermis, PL: Palisade Layer, CGC: Collenchymatous Ground Cells].

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	0.62
Chloroform extract	dark yellowish green	non-sticky	Soluble	0.85
Methanol extract	dark green	non-sticky	highly soluble	5.82
Aqueous extract	brown	dry powder	highly soluble	9.28

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

chromatographic examination with HPTLC

Following examination with a densitometer at UV 254 nm and 366 nm, the HPTLC chromatogram showed the presence of many peaks (Figure 3, Figure 4, Table 6 and Table 7). At a wavelength of 254 nanometers, it showed three peaks. While doing so, it showed five peaks at UV 366 nm with maximum Rf values in the range of

a value in the range of 0.3 to 0.93, which indicates the presence of at least 5 distinct components in an amount of MECD measuring 5 l. It was determined that four of the components with maximum Rf values of 0.3, 0.53, 0.60, and 0.65 were prominent because the percentage area was greater for each of these four components: 30.97 percent, 13.18 percent, 26.23 percent, and 20.91 percent correspondingly. It was discovered that one of the components with a maximum Rf value of 0.93 had a percentage area that was less than 10 percent

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

Chemical treatment	Day light	254 nm	Fluorescence UV light 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

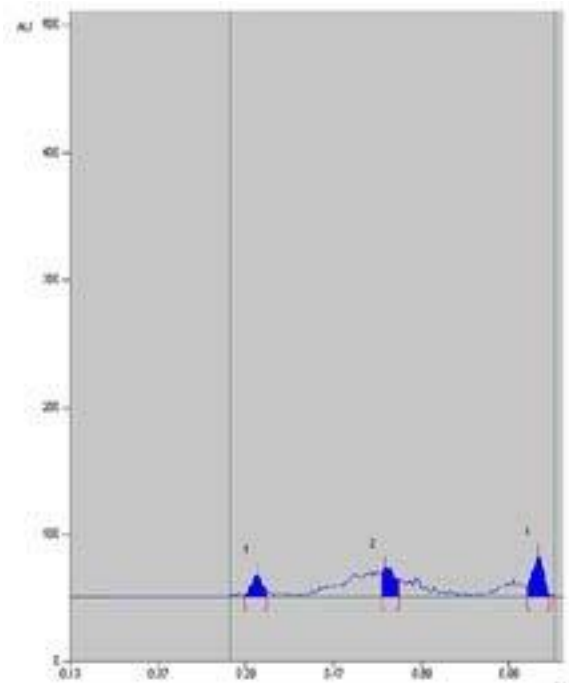

Figure 3: Densitogram of MECD at wavelength 254 nm

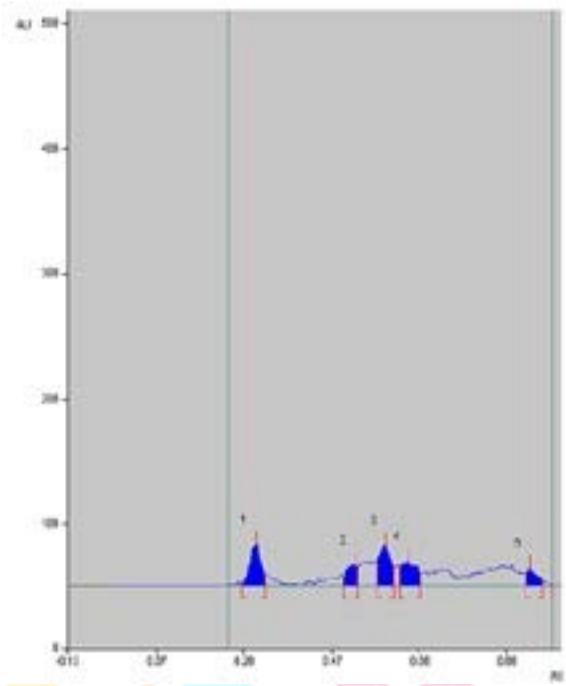

Figure 4: Densitogram of ECD at wavelength 366 nm

Discussion- The standardisation of plant material is a difficult process because of the diverse composition of plant material, which might be in the form of the complete plant, a part of the plant, or extracts obtained from the plant itself. As a result, in recent years there has been a rapid increase in the standardisation of many medicinal plants that have the potential to have therapeutic importance. This has been done in order to remedy the problem. The first and most important step in the process of achieving quality that can be replicated is to correctly identify and authenticate the initial material. Consequently, during the process of collecting plant material, attention was taken to gather the appropriate plant portion at the appropriate time. After that, it was validated by a specialist in the relevant scientific subject. In general, physicochemical parameters were used in order to determine the identity of the plant materials, as well as their purity and the presence or absence of any adulterants. In spite of the advances in technology, pharmacognostic evaluation and HPTLC chromatography analysis are still the most reliable methods for identifying plant materials.

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	0.26	0.8	0.30	17.4	24.29	0.33	2.9	412.8	23.47
2	0.58	18.2	0.59	23.1	32.26	0.63	12.8	594.9	33.83
3	0.93	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	0.51	6.2	0.53	16.5	14.33	0.54	14.6	373.1	13.18
3	0.56	19.6	0.60	31.7	27.50	0.62	14.6	742.8	26.23
4	0.65	15.4	0.65	20.0	17.32	0.68	9.1	592.1	20.91
5	0.93	8.9	0.93	13.5	11.75	0.96	3.3	246.5	8.71

more reliable. Evaluation of the organoleptic properties was carried out as part of the process of standardisation. An organoleptic evaluation is a method of qualitative analysis that is focused on the investigation of morphological and sensory components of substances, such as trichomes. Characteristics of the leaf's organoleptic makeup that can be used as diagnostic instruments The evaluations at the microscopic and physicochemical levels had been carried out. The percentage of extractives found in various solvents, such as petroleum ether, chloroform, alcohol, and water, provides information regarding the quantity and composition of the compounds found in the extracts. Extractive values are also helpful in estimating the amount of a certain ingredient that is soluble in a specific solvent. An HPTLC chromatographic analysis was performed, which is particularly beneficial since chromatographic fingerprinting is a sensible alternative to satisfy the requirement for more effective and powerful quality evaluation of traditional Chinese herbal medicine and traditional Indian medicine. The concluding observations have been written down. The loss on drying at 105 degrees Celsius was 8.5% by weight, the total ash value was 13% by weight, the acid-insoluble ash was 5.07% by weight, the water-soluble ash was 5.49% by weight, the water-soluble extractive was 9.2% by weight, and the alcohol-soluble extractive was 2% by weight. 5.81 percent by weight and a pH of 6.88 in an aqueous extract of 1 percent. Under UV light, the fluorescence of the CD could be seen, and depending on the solvent, it would have a distinct colour. Analysis using HPTLC showed that there were five

peaks at a wavelength of 366 nm, and their maximum Rf values ranged from 0.3 to 0.93. This result is helpful in supplementing the information that is already available with regard to the identification and standardisation of the powdered form of the plant medication in order to differentiate it from adulterants. and identification in order to determine the identity and purity of the leaves of CD or medicinal preparations prepared from it in order to carry out additional research studies.

CONCLUSION

Based on the findings of this study, it is possible to draw the conclusion that the pharmacognostical characters, along with their physicochemical parameters, fluorescence characteristics, and HPTLC chromatographic profiling of the MECD leaf, resulted in a set of standards that have the potential to serve as an important source of information with regard to the standardisation of the MECD leaf.as well as identification in order to confirm the identity and purity of the leaves of CD or the pharmacological formulations that were prepared from it in later research studies.

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