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Phytochemical and Pharmacognostic Evaluation of the Leaves of Cordia dichotoma

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Abstract:

Compared to other medications, plants constitute a major source of drugs for therapeutic purposes in the pharmaceutical sector. *Cordia dichotoma*, a small to moderately sized member of the Boraginaceae family, has been employed in studies on pharmacognostic evaluation to comprehend the color, taste, odor, surface, size, and shape of leaves. Leaves, which have anti-inflammatory properties, were the plant portion employed in the study. They also exhibit anti-ulcer and anti-diabetic properties. Phytochemical analysis of leaves reveals the presence of proteins, amino acids, carbohydrates, glycosides, triterpenoids, and some tannin with phenolic compounds. To verify the purity, a thin-layer chromatograph study must be conducted.

Keywords: Cordia dichotoma, TLC, Microscopical, Morphological, Phytochemical Analysis.

Introduction:

The pathophysiological reaction of living tissue to damage is inflammation, which causes plasmatic fluids and blood cells to build up locally. The intricate processes and mediators involved in the inflammatory response can cause, sustain, or worsen a number of illnesses even though it is a defense mechanism. However, research on inflammatory diseases is still ongoing, and the side effects of the anti-inflammatory medications currently on the market present a significant challenge when using them in clinical settings. Understanding the physiopathology of inflammation and the role of free radicals in its pathogenesis has advanced significantly. An imbalance between the body's antioxidant system and oxidizing molecules results from an abnormal increase in reactive oxygen species (ROS), which are created when free radicals react with molecular oxygen. The cellular components are harmed by the inflammatory cascades brought on by this oxidative stress. In many nations, pain and inflammation are traditionally treated using a variety of methods. Because of their affordability, accessibility, and environmental benefits, herbal remedies are frequently used to treat pain and inflammation in developing nations. *Cordia Dichotoma Forst*. Indian traditional medicine has made use of this. It also grows in the wet monsoon forests of Maharashtra. It grows alone in moist, shaded valleys and ravines rather than in groups. It grows up to a height of roughly 1500 meters in the sub-Himalayan tract and outer ranges. It can be found in a wide range of forests, including tidal forests in Myanmar, moist deciduous forests in the Western Ghats, and dry deciduous forests

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in Rajasthan. It has been found in Afghanistan, India, Pakistan, Sri Lanka, Thailand, and Malaysia. It grows best in humid environments or beside watercourses in wastelands and mixed open forests. It grows best near streams or depressions where moisture is present in regions with less than 500 mm of annual precipitation. Within the Boraginaceae family of borage trees and shrubs, the genus Cordia is occasionally subscandent. Worldwide, about 300 species—mostly found in warmer climates—have been identified. The majority of Cordia species, including *Cordia dichotoma*, C. myxa, C. oblique, C. verbenacea, C. martinicensis, C. salicifolia, C. spinescens, C. latifolia, and C. ulmifolia, have long been used in traditional medicine for cicatrizant, astringent, anti-inflammatory, anthelminthic, antimalarial, diuretic, febrifuge, appetite suppressant, cough suppressant, urinary infections, lung conditions, and leprosy. One of the traditional medicinally significant deciduous plants found throughout India is *Cordia dichotoma*. According to reports, the fruit has a high polysaccharide content. C. dichotoma produces a sticky, jelly-like mass when its fruit is ripe. Plants have antibacterial, antiviral, and antitussive properties in the Unani medical system. C. dichotoma is the main ingredient in joshandah, polyherbal formulations that are widely used in India to treat fever, respiratory distress, cough, catarrh, and common colds. Dyspepsia, fever, diarrhea, leprosy, gonorrhea, and burning sensations have all been treated with leaves and stem bark since ancient times.

Material and Method:

Collection and Authentication of Plant:

The leaves of *C. dichotoma* G. Forst., family Boraginaceae, were procured from the local area. The plant is authenticated and identified by experts from the Department of Botany and Research Centre. The voucher specimen has been preserved in the laboratory for future reference. The voucher specimen has been preserved in the laboratory for future reference.

Plant Information: Scientific classification, vernacular names and local names of the C. dichotoma:

a. Scientific classification:

Kingdom: Plantae, Division: agnoliophyta,Class: Dicotyledons, Subclass: Asteridae, Order: Lamiales, Family: Boraginaceae, Genus: Cordia L. – cordia, Species: *Cordia dichotoma* G. Forst., Fragrant: Manjack, Synonym: *Cordia myxa* forsk, *Cordia obliqua* Willd, *Cordia myxa* Roxb., Common name: Bhokar, Shleshmantaka.

b. Vernacular names:

Malaysia: Sekendal, sekendai, petekat, English: Sebestan plum, soap berry, fragrant Manjack, India: Gonda, lasora, leshora, Javanese: Kendal Sumatran: Nunang Thailand: Paw man.

c. Local names:

Hindi: Lasura, Bhokar, Borla., English: Sebesten, Clammy Cherry, Indian cherry., Bangali: Buhal, Bahubara., Gujarati: Vadgundo, Gunda. Javanese: Kendal, Lao: Man, Sino-Tibetan, Man khok., Malay: Petekat, Sekendai. Tamil: Kalvirusu, Vidi, Naruli. Nepali: Bohori, Kalobohori., Thai: Mandong, Manma, Phakmong., Sanskrit: Shelu, Bahuvarka, Shleshmatak.

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Botanical Description:

The plant of *Cordia dichotoma,* belonging to family Boraginaceae., small to moderate-sized deciduous tree with ashort bole, short crooked trunk and spreading crown. The stem bark is grayish brown smooth or longitudinally wrinkled. Leaves simple, entire and slightly dentate, elliptical-lanceolate to broad ovate with a round and cordate base. These flowers are followed by 1 in (25mm) long dull pinkish edible fruits with sticky flesh flowers are short stalked, bisexual and white in colour, appear in loose corymbose cymes. The fruit is a yellow or pinkish-yellow shining globose or ovoid drupe seated in a saucer-like enlarged calyx. It turns black on ripening and the pulp gets viscid.

Pharmacognostic Evaluation:

1. Morphological Evaluation:

C. dichotoma belongs to family Boraginaceae is a small to moderate size deciduous tree with a short bole, short crooked trunk and spreading crown. 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.

2. Microscopy Evaluation:

A thin transverse section (TS) of the leaf was cut by free hand sectioning and stained with different stains (Phloroglucinol, Sudan red III). The transverse section shows the cuticle,xylem, and phloem of the leaf after utilizing the stains.

3. Surface Preparation:

Take fresh or dried leaflet and boil it with water or chloral hydrate solution for 30 to 60 min (because the surface of the leaves is rough at both sides). Then take a sharp razor, glass slides, and freshwater. The leaf surface matter was cleared by freehand and the matters were separated by using the water and observe under the microscope.

4. Evaluation of Leaf Constant:

The leaf constant like Stomatal number, Stomatal Index, Vein-Islet number, Vein termination number, Palisade cells number were determined.

5. Powder Microscopy:

To a little quantity of finely ground plant powder taken on a microscopic slide, 1-2 drops of phloroglucinol solution was added along with a drop of concentrated HCl, alsoSudan red III was added for staining. It was mounted in glycerol and observed under microscope. The characteristic features of the powder were noted. Analysis of powder microscopy of leaf was done by standard procedure as. The dried leaf powder was brown to green in color. Microscopy of the powder detected the presence of fragments of unicellular trichomes, stomata, fibers, crystal differ in shape.

6. Scanning Electron Microscopy (SEM):

Leaf material was examined by Scanning Electron Microscopy (SEM). Small pieces (c. 7 mm2) of dried mature leaf material were fixed to SEM stubs using double-sidedadhesive tape,

examined under the 500X in the Department of Chemistry at Savitribai Phule Pune University, Pune.

Physicochemical Evaluation:

The physical constants like moisture content, foreign organic matter, ash value (total ash, acid insoluble ash value) and extractive values (water soluble extractive and alcohol soluble extractive value) were determined.

1. Moisture Content (Loss on drying):

Place the 10 gm of drug in evaporating dish and dry at 105^0 C for 3 hours. Dry the substance to constant mass. The drying is carried out over diphosphorous pentoxide oranhydrous silica gel at room temperature.

2. Foreign Organic Matter:

Weigh 100 to 500 gm or the quantity specified in the individual monograph, of the original sample and spread it out in a thin layer. Inspect the sample with unaided eye or with the use of 6X lens and separate the foreign organic matter manually as completely aspossible. Weigh and determine the percentage of foreign organic matter from the weight of the drug taken.

Ash Value Determination:

1. Total Ash Value Determination:

Weigh accurately 2 to 3 gm of the air-dried crude drug in tared silica crucible and incinerate, gently at first and gradually increase the temperature upto 675 ± 25^0C, until free from carbon. Collect the residue and cool in dessicator, weigh the ash, and calculate the percentage of ash with reference to air-dried crude drug.

2. Acid-insoluble Ash Value:

Boil the obtained total ash with 25 ml of 2 M hydrochloric acid for 5 minutes, collect the insoluble matter on ashless filter papers, wash with hot water, ignite, cool in a dessicator and weigh. Calculate the percentage of acid-insoluble ash on the dried drug basis.

3. Water-soluble Ash Value:

Boil the obtained total ash for 5 minutes with 25 ml of water, collect the insoluble matter on ashless filter paper, wash with hot water, and ignite for 15 minutes at a temperature not exceeding 450^0 C. Subtract the weight of the insoluble matter from the weight of the ash; the difference in weights represent the water-soluble ash. Calculate the percentage of water-soluble ash on dried basis.

Extractive Value Determination:

1. Alcohol-Soluble Extractive Value:

Macerate 5 gm of the air-dried drug, coarsely powdered, with 100 ml of alcohol of specified strength in a closed flask for 24 hours, shaking frequently during first 6 hours and allowing standing for 18 hours. Thereafter, filter rapidly taking precautions against loss of ethanol; evaporate 25 ml of the filtrate to dryness in a tared flat-bottomed shallow dish, dry at105^0 C

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and weigh. Calculate the percentage of ethanol-soluble extractive with reference to air-dried drug.

2. Water-Soluble Extractive Value:

Macerate 5 gm of the air-dried drug, coarsely powdered, with 100 ml of 5% Chloroform -water of specified strength in a closed flask for 24 hours, shaking frequently during first 6 hours and allowing standing for 18 hours. Thereafter, filter rapidly taking precautions against loss of ethanol; evaporate 25 ml of the filtrate to dryness in a tared flat- bottomed shallow dish, dry at 105^0 C and weigh. Calculate the percentage of water-soluble extractive with reference to air-dried drug.

Extraction of *C. dichotoma* Leaves:

The collected plant Material was washed with water to remove other undesirable material and dried under shade. Air dried leaf powder of *Cordia dichotoma* (100g) was subjectedto successive Soxhlet extraction by solvents in increasing order of polarity *viz.* petroleum ether (60- 80°C), chloroform, and methanol. Before each extraction the powdered material (marc) was airdried in shade. Then the extract was evaporating to dryness on the water-bath. All the extracts wereweighed and percentage was calculated in terms of the air-dried weight of the plant material andpreserved in vacuum desiccators.

Preliminary Phytochemical Screening on Extract:

Phytochemical Tests: The different extracts of leaves of *Cordia dichotoma* were tested for various components as follows:

Test for Alkaloids:

Small portion of solvent free extract was stirred with few drops of dil. HCl and filtered. The filtrate was then tested for following color test:

Mayer's test:

To 2mg of methanolic extracts 3-4 drops of the Mayer's reagent was added. Alkaloidspresence was confirmed after the formation of white or pale yellow precipitate.

Wagner's Test:

1.27 gm of iodine and 2 gm of potassium iodide was dissolved in 5 ml of water and make up the volume to 100 ml with distilled water. Appearance of reddish brown precipitate with Wagner's reagent showed the presence of alkaloids.

Hager's test:

Take 20 ml of saturated solution of picric acid and add few drops of it to 2-3 ml of extract. A yellow color was observed.

Test for Carbohydrates and Glycosides:

Molisch's test:

10 gm of alpha naphthol was dissolved in 100 ml of 95 % alcohol. Extract was treatedwith this solution and 0.2 ml of conc. sulphuric acid was slowly added through the sides ofthe test

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tube, purple or violet color appeared at the junction.

Benedicts test:

The test solution was treated with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and upon boiling on water bath, reddish brown precipitate formed if reducing sugars were present.

Fehling's Test:

6.932 gm of copper sulphate was dissolved in distilled water and make volume up to 100 ml (solution A). 34.6 gm of potassium sodium tartarate and 10 gm of sodium hydroxide was dissolved in distilled water and make volume up to 100 ml (solution B). Two solutions was mixed in equal volume prior to use and few drop of sample was added and boiled, abrick red precipitate of cuprous oxide was formed, if reducing sugars were present.

Barfoed's Test:

2-3 drops of Barfoed's reagent is added to 1 ml of extract sample in a test tube and boiled for 30 seconds and then allowed to cool. If a red precipitate occurs, a monosaccharide is present.

Test for Sterols and Triterpenoids:

Salkowski test:

Extract was treated with few drops of conc. Sulfuric acid, shake well and allowed to stand for some time, red color appear at the lower layer indicated the presence of steroids andformation of yellow colored lower layer indicated the presence of triterpenoids.

Test for Terpenoids:

Sulphur powder test:

Small amount of sulphur powder was added to the test solution, it sinks at the bottom.

Test for Proteins and Amino Acids:

Ninhydrin test:

1 gm of ninhydrin (indane1, 2, 3 trione hydrate) was dissolved in n-butanol and makethe volume to 100 ml. Extract treated with this solution gave violet color on boiling.

Biuret test:

To 3 ml test solution 4 % w/v NaOH and few drops of 1 % w/v copper sulphatesolution were added. A blue color was observed.

Test for Saponins:

Foam test:

1 ml of extract was diluted with distilled water to 20 ml and shake in a graduated cylinderfor 15 minutes. A one centimeter layer of foam indicated the presence of sponins.

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Test for Tannins and Phenolic Compounds:

Ferric chloride test:

Extract was treated with ferric chloride solution, blue color was appeared if hydrolysable tannin was present and green color was appeared if condensed tannins were present.

Vanillin hydrochloride test:

1 gm Vanillin was dissolved in 10 ml alcohol and 10 ml concentrated hydrochloride solution. Extract was treated with this solution gave pink or red color due to the presence of tannins and phenolic compounds.

Lead Acetate Test:

10 mg of leave extract was taken and 0.5 ml of 1% lead acetate solution was added and the formation of precipitate indicates the presence of tannins and phenolic compounds.

Result and Discussion:

Pharmacognostic Evaluation:

1. Morphological Evaluation:

The morphological evaluation was done by observing the leaflet of *Cordia dichotoma* G. Forst., and the observation are as follow:

Table 1: Morphological and Organoleptic Evaluation

Sr. No.	Evaluation Parameter	Description
1.	**Colour**	Light Green
2.	**Odour**	Pleasant
3.	**Taste**	Mucilaginous
4.	**Surface**	Upper and Lower surface are rough
5.	**Shape**	Ovoid with dentate margin
6.	**Size**	Length upto 14-16 cm; Width upto 6-7 cm.

2. Microscopy Evaluation:

The microscopic evaluation was done by cutting of transverse section; staining withdifferent reagents and the observation is as follows:

Table 2: Staining/ Micro-Chemical Tests

Sr. No.	Reagents	Observations	Characteristics
1.	Phloroglucinol + conc. HCl (1:1)	Red/Pink	Lignified tissues: xylem (vascular bundle) Sclerenchyma (pericycle)
2.	Sudan red III	Red/Pink	Cutin/Cuticle

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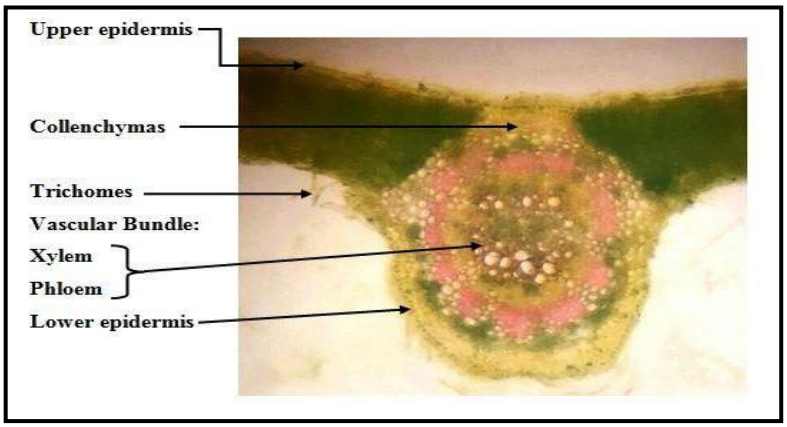


Figure 1: T. S. of *Cordia dichotoma* G. Forst. Plant Leaves

Transverse section of leaf shows upper and lower epidermis with straight wall cells, few of which contain mucilage. Paracytic stomata and nonlignified unicellular and multicellular covering trichomes are found on both the surfaces. Midrib shows the vascular bundles having patches of perimedullary xylem and phloem, almost surrounded by lignified pericyclic fibres. It is shows collenchymatous cells at upper and lower side of Transverse section.

3. Surface Preparation:

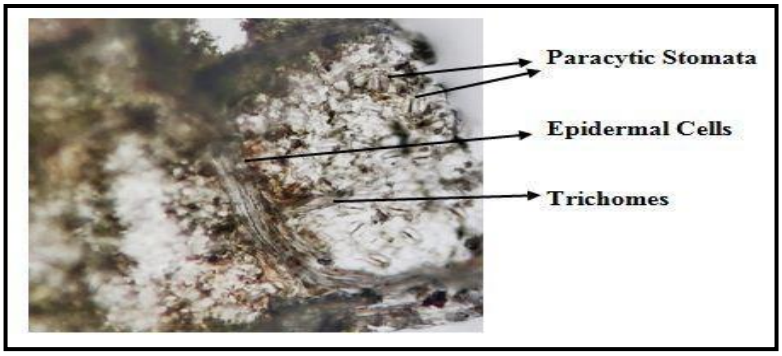


Figure 2: Surface Preparation of *Cordia dichotoma* G. Forst. Plant Leaves.

Paracytic Stomata: Rubiaceous or parallel called stomata with two subsidiary cells around the guard cells.

Epidermal Cell: Polygonal, thin and straight walled, parenchymatous.

Trichomes: Covering unicellular, conical, bulbous base, thick walled, lie appressed to the epidermis.

4. Evaluation of Leaf Constant:

Table 3: Evaluation of Leaf Constants of *Cordia Dichotoma* G. Forst. Plant Leaves.

Sr. No.	Ratio Values	Constant
1.	Stomatal Number	6
2.	Stomatal Index	20.8
3.	Vein-Islet Number	15
4.	Vein Termination Number	7
5.	Palisade Ratio	-

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5. Powder Microscopy:

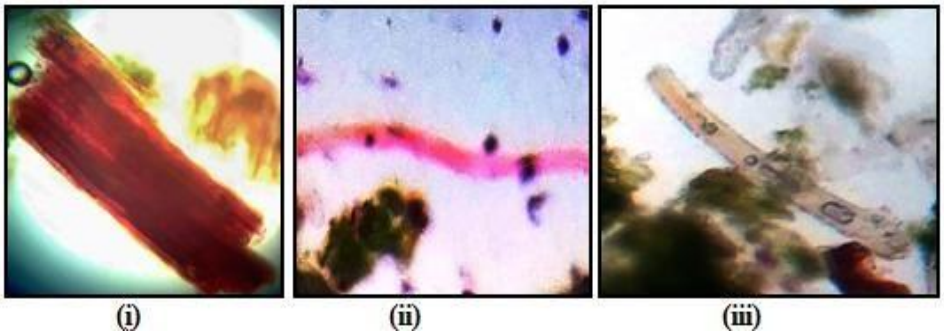


Figure 3: Powder Microscopy of *Cordia dichotoma* G. Forst Leaves.

Xylem vessel: Angular thickening, lignified (Pink Colour).

i.Cutin/Cuticle: Straight, lignified fibres (Pink Colour).

ii. Covering Trichomes: Unicellular, thick warty walls, acute apex, bulbous base, narrow lumen, conical shape.

Physicochemical Evaluation:

Table 4: Physicochemical Evaluation of *Cordia Dichotoma* G. Forst. Leaves

Sr. No.	Physicochemical Properties	Result (%w/w)
1.	Moisture Content (loss on drying)	11
2.	Foreign Organic Matter	02
3.	Total Ash Value	14
4.	Acid-Insoluble Ash Value	7.98
5.	Water-Soluble Ash Value	5.23
6.	Alcohol-Soluble Extractive Value	27
7.	Water-Soluble Extractive Value	13

Extraction of *C. Dichotoma* G. Forst, leaves:

Table 5: Extractives values of different extracts leaves of Cordia dichotoma powdered drug

Sr. No.	Type of Extracts	Colour	Odour	Consistency	Extractive Value (w/w)
1.	**Pet. Ether**	Light green	Characteristics	Greasy	4.13
2.	**Chloroform**	Green	Characteristics	Sticky	4.31
3.	**Methanol**	Green	Characteristics	Sticky	3.89

Preliminary phytochemical screening on extract:

Table 6: Qualitative Phytochemical Chemical Analysis of Extract of Cordia dichotoma G. Forst, Plant Leaves

Sr. No.	Test Performed	Petroleum Ether Extract	Chloroform Extract	Methanol Extract
Test for Alkaloids				
1.	Mayer's test	-	-	-
2.	Wagner's Test	-	-	+
3.	Hager's test	-	-	+
Test for Carbohydrates and Glycosides				

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4.	Molisch's test	-	-	+
5.	Benedicts test	-	+	-
6.	Fehling's Test	-	+	+
7.	Barfoed's Test	-	-	+
Test for Sterols and Triterpenoids				
8.	Salkowski test	-	-	+
Test for Terpenoids				
9.	Sulphur powder test	-	-	-
Test for Proteins and Amino Acids				
10.	Ninhydrin test	-	-	+
11.	Biuret test	-	-	+
Test for Saponins				
12.	Foam test	-	-	-
Test for Tannins and Phenolic Compounds				
13.	Ferric chloride Test	-	-	+
14.	Vanillin HCL acid Test	-	+	+
15.	Lead Acetate Test	-	-	+

Thin Layer Chromatography Study on Extract:

Sr. No.	Plant Extract	Test Solution	Stationary Phase	Mobile Phase	Visualization	Rf Value
1.	Cordia dichotoma G. Forst. Leaves	Petroleum Ether Extract	Silica Gel G Plate	Chloroform: Dichloromethane: Benzene **(2:2:6)**	UV Chamber (254nm)	**0.92**
2.	Cordia dichotoma G. Forst. Leaves	Methanol Extract	Silica Gel G Plate	Chloroform: Methanol **(9:1)**	UV Chamber (254nm)	**0.57**
3.	Cordia dichotoma G. Forst. Leaves	Chloroform Extract	Silica Gel G Plate	Benzene: Acetone **(9:1)**	UV Chamber (254nm)	**0.61**

Conclusion:

The aforementioned study aids in comprehending *Cordia dichotoma's* pharmacognostic assessment. Microscopic examination of the transverse section of leaves reveals the presence of trichomes, vascular bundles, epidermis, and collenchymas, whereas macroscopic analysis of leaves is useful in understanding their size and shape. Identification of the moisture content, foreign organic materials, etc. is aided by the physicochemical analysis. Thin layer chromatography and phytochemical analysis are useful for confirming identity, quality, and purity. The information above is helpful for assessing plant extract's medicinal potential.

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