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Pharmacological Evaluation of Selected Medicinal Plants and Isolation of Active Compounds

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Soxhlet extraction, TLC, FTIR, Antioxidant activity, DPPH assay, Flavonoids, Polyphenols

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ABSTRACT
The present study was undertaken to isolate, characterize, and evaluate bioactive phytoconstituents from the bark of *Cordia dichotoma*, a medicinal plant widely used in traditional systems of medicine. Medicinal plants serve as an important source of therapeutic agents due to the presence of diverse secondary metabolites such as alkaloids, flavonoids, tannins, and phenolic compounds, which exhibit significant pharmacological activities. The bark of *Cordia dichotoma* was collected, authenticated, and subjected to pharmacognostic and physicochemical evaluation to establish its identity, purity, and quality. Successive solvent extraction was carried out using petroleum ether, chloroform, ethyl acetate, and ethanol. Among the extracts obtained, the ethanolic extract showed the highest yield and was rich in phytoconstituents. Preliminary phytochemical screening confirmed the presence of various bioactive compounds including alkaloids, flavonoids, glycosides, tannins, and phenolic compounds. Further analysis through thin-layer chromatography (TLC) and spectroscopic techniques such as FTIR enabled the identification and characterization of isolated compounds, including naringenin chalcone and bopindolol. Quantitative estimation revealed that the ethanolic extract possessed the highest total polyphenolic and flavonoid content, indicating strong antioxidant potential. The antioxidant activity was evaluated using DPPH assay, where the extract exhibited significant free radical scavenging activity comparable to standard ascorbic acid. Additionally, in vitro studies demonstrated promising anti-asthmatic activity of the extract by inhibiting histamine-induced contractions. The overall findings validate the traditional use of *Cordia dichotoma* and highlight its potential as a natural source of bioactive compounds for therapeutic applications.

INTRODUCTION

Cordia dichotoma: (Nazim Hussain et. al., 2013)
Botanical Name: Cordia dichotoma
Kingdom: Plantae
Division: Magnoliophyta Class: Dicotyledons
Subclass: Astaridae
Order: Lamiales
Family: Boraginaceae
Genus: Cordia
Species: Cordia dichotoma Forst.
Common Names:
India: Bhokar, Gondani, Leshora, Gonda, Lasora
English: Soap berry, sebestan plum, fragrant manjack Sumatran: Nunang
Thailand: Paw man Assamese: Goborsuta Gujrati: Gunda, Vadgundo
Sanskrit: Shelu, Bahuvarka, Shleshmatak
Hindi: Lasura, Bhokar, Borla

Habitat:
It is found in diverse of forests ranging from the dry deciduous forests of Rajasthan to the moist deciduous forests of Western Ghats in India and forests in Myanmar. In Maharashtra, it grows in moist monsoon forest. It does not grow gregariously, but is found growing singly in moist shady valleys.

Description:
Cordia dichotoma is morphologically small to medium-size deciduous tree with a short-crooked trunk, short bole and spreading canopy. Bark look-alike simple, entire and slightly dentate, elliptical-lanceolate to broad ovate with a round and cordate base. The stem bark reveals greyish brown smooth or longitudinally wrinkled. Flowers appear short stalked, bisexual and white to pinkish in colour and in loose corymbose cymes. Fruits are generally edible with sticky flesh mass. It 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 (Erdtman, 1986).
Parts used: Bark

Traditional uses:
The whole plant of C. dichotoma is edible and is used as food. Immature fruits are pickled and are also used as vegetable. Mixture of flower and curd applied two times in a day used to protect body against heavy sun heat waves. The rural people of coastal areas of Orissa eat the ripe fruits raw. The seed kernels

of C. dichotoma contain high quantity of fatty oils and proteins which has potential as cattle feed. Amongst, all organs of Cordia dichotoma have been traditionally utilized in folk medicine, including wound healing, demulcent, anthelmintic, diuretic, astringent, emollient, expectorant, hepatoprotective, analgesic, immune modulator, hypoglycemic, anti-inflammatory, laxative, antioxidative stress, hypolipidemic.

Properties and uses:

The bark is bitter, astringent, and acrid after digestion, constipating, anthelmintic, cooling and depurative, and useful in dyspepsia, fever, and diarrhea, burning sensation, vitiated conditions of kapha and pitta, helminthiasis, leprosy and skin disease. The bark is aphrodisiac and useful in gonorrhea and ophthalmodynia. The fruits are sweet, cooling, emollient, anthelmintic, purgative, vulnerary, diuretic, expectorant, aphrodisiac, depurative and febrifuge, and are useful in vitiated conditions of vata and pitta, ulcers, leprosy, skin disease, hyperdipsia, burning sensation, bronchitis, dry cough, pectoral disease, strangury, urethralgia, urethritis, chronic fever, arthralgia, pharyngopathy, splenopathy and ring worm. Every part of the plant is recommended for the treatment of snake- bite and scorpion sting. The polysaccharide gum (97%) obtained from the plant used for various pharmaceutical purposes. Chromium present in the fruit has therapeutic value in diabetes. A fruit also contains some anti-nutritional factors such as phytic acid, phytate phosphorus and oxalic acid. New natural cellulose fabrics were identified from the branches of the C. dichotoma.

Fig.1: *Cordia dichotoma* plant with various parts

MATERIALS AND METHODS

Extraction Method:

The solid extraction of drug represents a solid from solid separation. The liquid- liquid extraction is one, in which any of the two immiscible liquids are used for the extraction (Solvent extraction). Extraction process comes to halt when the distribution of the extractive substance between miscella and drug residue reaches the value 'K', i.e., when the concentration gradient between miscella and drug residue has become zero. (Mukherjee,2002)

K = Concentration of extracted substances in the miscella / Concentration of extractive substance in the drug residue

Hot continuous extraction- Soxhlation:

Soxhlet extractor is the simplest way for preparation of extracts of crude drugs. Pure solvent is used in this technique. The crude drug used for extraction is kept in a 'thimble' made of cloth or cellulose in middle portion of the soxhlet apparatus. Siphon tube and a side arm both are connected to a lower portion. The solvent used for extraction is kept in the lower portion and a condenser is connected above the middle compartment.

The solvent is added in round bottom flask and heated to boil to form vapours. The vapours travel through the side arm into the reflux condenser. The vapour cools there and falls onto the thimble containing the crude drug kept for extraction. The hot solvent passes through the crude drug and extraction takes place. The extract gets deposited in the lower portion of middle compartment. As the height of extract reached to the top of the siphon tube, the extract gets deposited in the middle portion passes through it and goes into the lower container i.e., round bottom flask. The same process was repeated till complete extraction of crude drug takes place.

In this technique of extraction, the extract gets collected in the lower RBF, gradually becomes concentrated. The soxhlet extraction process is very helpful for the total extraction of crude drug with a specific solvent. Different solvents with increasing polarity can be used for the continuous total extraction, e.g., benzene, hexane, pet. ether, chloroform, methanol, ethanol, water. The crude drug was dried when it was subjected for extraction using another solvent. The previous solvent should be removed completely and powder should be dried totally. It prevents the mixing of the previous solvent into another solvent. (Mukherjee, 2002; Harbone1998)

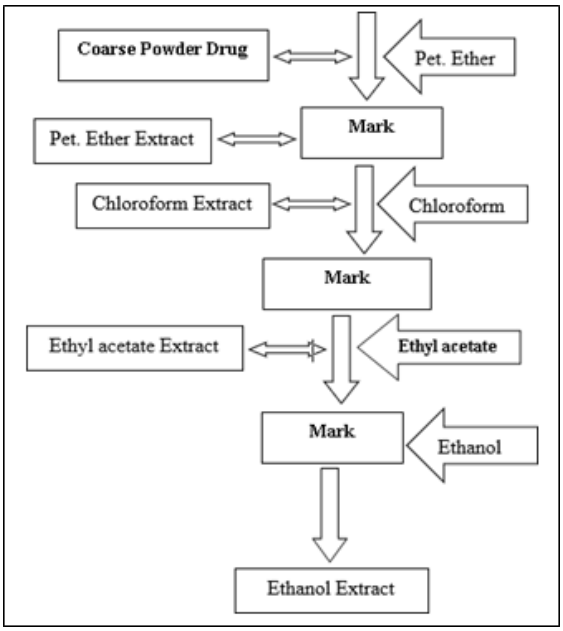

Figure 2: Scheme of successive extraction of Cordia dichotoma Bark

Extraction Procedure:

The dried powder of bark used for extraction procedure was sieved through 60- 120 mesh to separate fine and course powder. This course powder (1000g) was utilized for further extraction. *Cordia dichotoma* was exhaustively defatted using petroleum ether (60-80ºC) and extracted successively with chloroform, ethyl acetate, and ethanol using Soxhlet apparatus. The confirmation of complete extraction was done by taking a drop of extract from exit of side tube on TLC, drying and exposing to iodine vapours. If extraction is completed then it shows absence

of colored spot on TLC plate. After the complete extraction, solvents were evaporated on rotary evaporator and solvents were removed. The extracts thus obtained with different solvents were measured and the extracts were stored in desiccator. The percentage yield was calculated for all solvent extracts of both the plants by using following formula (Mukherjee, 2002):

$$\% \text{ yield} = \frac{\text{Weight of extract obtained after extraction}}{\text{Weight of powder drug used for extraction}}$$

Figure 3: Extraction of *Cordia dichotoma* bark by Soxhlet Apparatus

FTIR Method:

The FTIR spectroscopy was used to identify the functional groups based on the peak values. The toluene and acetone fractions of methanol fractions of Cordia dichotoma ethanolic extract were subjected to FTIR analysis and the functional groups of the components were separated based on the peaks. For the FTIR study Shimadzu FTIR Model No. 8400 instrument was used. The spectra were collected between 400-4000cm-1 on each fraction. The sample was mixed with KBr uniformly in the ratio 30:70 by trituration in a mortar. Autozero in the instrument was set running pure KBr and then sample and KBr mixture was run and peaks were recorded. (S.S.Bhokare et. al. 2022)

RESULTS AND DISCUSSION

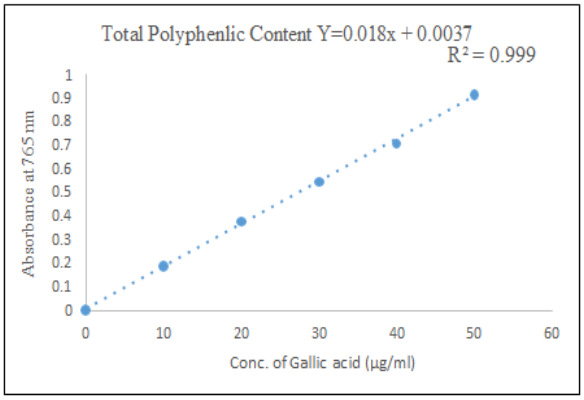

Figure.5: Calibration Curve of Gallic acid

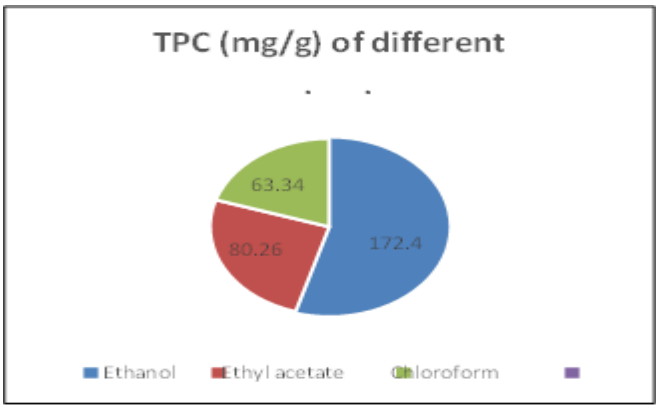

Figure 4: Total Polyphenolic content of *Cordia dichotoma* bark extracts

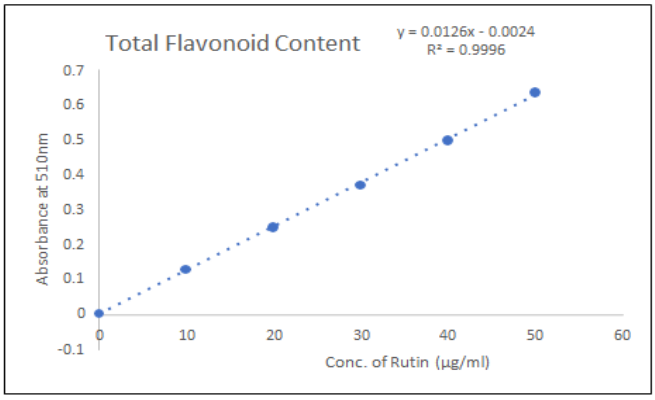

Figure.5: Calibration Curve of Rutin

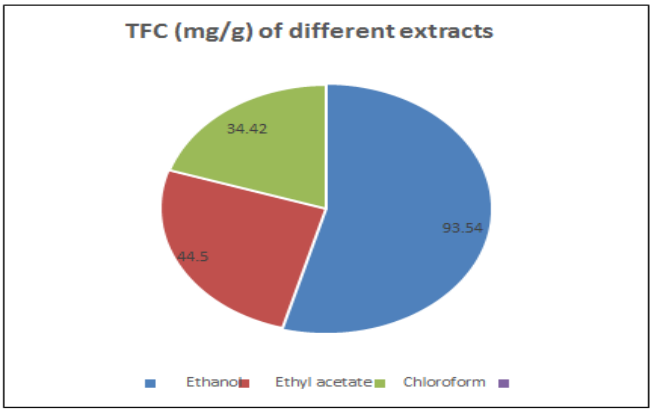

Figure 6: Total Flavonoid content of *Cordia dichotoma* bark extracts

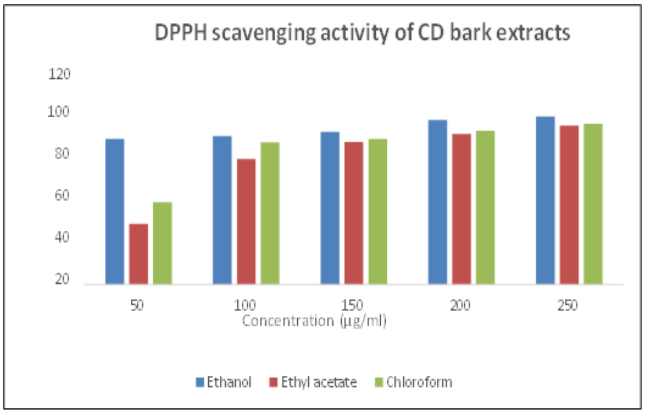

Figure 7: DPPH scavenging activity of Cordia dichotoma bark extracts

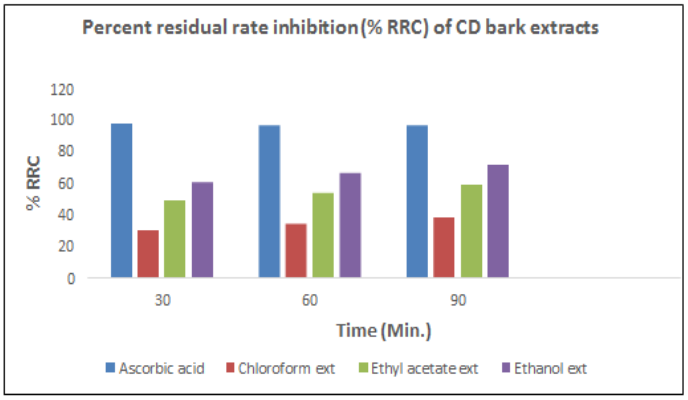

Figure 8: Percent residual rate inhibition (% RRI) of *Cordia dichotoma* bark extracts

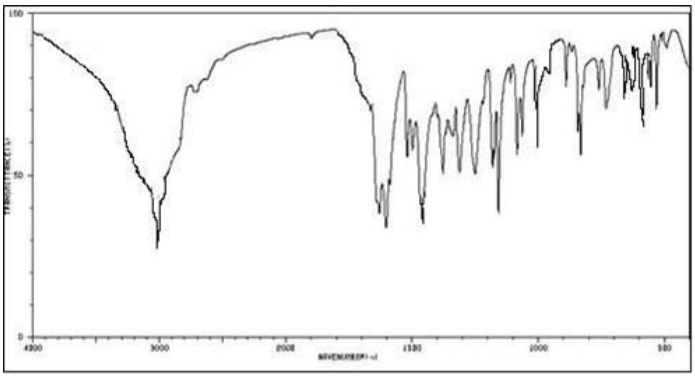

Figure 9: IR-Spectra of *Naringenin Chalcone* (Standard)

Table 1: Morphological characteristics of *Cordia dichotoma*

Sr. No.	Characteristic	Description
1.	Texture	Longitudinally wrinkled, rough, fissured surface
2.	Color	Externally brown, internally pale brown
3.	Odour	Characteristic
4.	Taste	Characteristic

Table 2: Physicochemical parameters of bark of *Cordia dichotoma*

Sr. No.	Physicochemical Parameter	Results
1.	% Foreign Organic Matter (w/w)	<2
2.	% Total Ash (w/w)	8.65
3.	% Acid Insoluble Ash (w/w)	0.26
4.	%Water Soluble Ash (w/w)	1.74
5.	Sulphated Ash Value (%)	0.985
6.	Moisture Content (w/w)	1.367
7.	% Extractive Values (w/w)	1.24
8.	Alcohol Soluble	24.86%
9.	Water Soluble	41.71%

Table 3: Calibration Curve of Gallic acid

Sr. No.	Concentration (µg/ml)	Abs. at 765nm
1.	0	0
2.	10	0.185±0.001
3.	20	0.375±0.009
4.	30	0.542±0.011
5.	40	0.706±0.012
6.	50	0.914±0.012

In-vitro Anti-Asthmatic activity:

Isolated goat trachea chain preparation:

In the present study, it was observed that Cordia dichotoma ethanolic extract, acetone fraction and methanolic fraction inhibits contraction produced by histamine in the tissue preparation. Histamine (30µg/ml) was taken in different dose level and DRC was plotted. Study showed that Cordia dichotoma ethanolic extract, acetone fraction and methanol fraction exhibit significant (***p<0.001) percentage decreased contraction at concentration 100µg/ml in goat tracheal chain preparation. *In vitro* isolated goat tracheal chain preparation study carried out and tracheal contraction was measured. Control group shown the maximum contraction of 99.91% at histamine dose of 3.2ml. Standard group (Salbutamol 4mg/kg) shown significant reduction in maximum contraction i.e., 43.56%. Acetone fraction and ethanolic extract shown significant reduction in maximum contraction, i.e., 73% and 75% respectively whereas methanol fraction shown highly significant reduction in maximum contraction (50%) which was a non-significant difference when compared to standard indicating the activity similar to the standard.

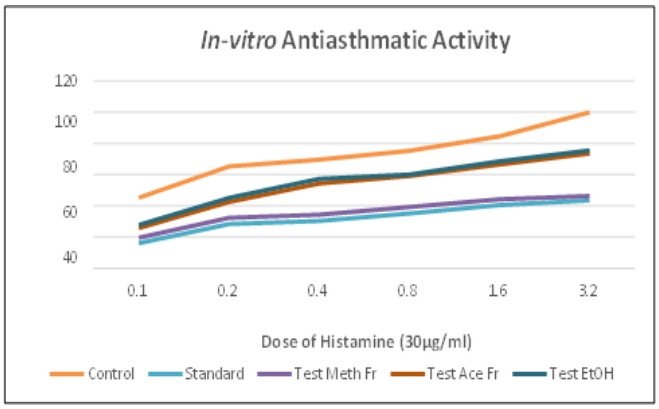

Figure 10: Anti-Asthmatic effect of extract and fractions on histamine induced contraction in isolated goat tracheal chain preparation

DISCUSSION

The present study was systematically designed to evaluate the pharmacognostic, phytochemical, and biological potential of Cordia dichotoma bark. The plant material was collected from the Nellore district and authenticated using morphological and microscopic characteristics. Proper processing of the crude drug, including drying, powdering, and sieving, ensured uniformity for further experimental analysis. Pharmacognostic evaluation confirmed characteristic features such as cork cells, fibers, and vessel elements, establishing the identity of the plant material. Standardization parameters including ash values, extractive values, and moisture content were found to be within acceptable limits, indicating the purity and quality of the crude drug. Successive solvent extraction using petroleum ether, chloroform, ethyl acetate, and ethanol yielded different extracts, among which the ethanolic extract showed the highest percentage yield (21.22%). Preliminary phytochemical screening revealed the presence of various bioactive constituents such as alkaloids, flavonoids, tannins, glycosides, and phenolic compounds, with the ethanolic extract exhibiting the richest phytochemical profile. TLC fingerprinting further confirmed the presence of multiple phytoconstituents, with the solvent system Toluene: Chloroform: Acetone providing better resolution. Quantitative phytochemical analysis demonstrated that the ethanolic extract contained the highest total polyphenolic (172.40 mg/g GAE) and flavonoid content (93.54 mg/g RE), suggesting strong antioxidant potential. Antioxidant studies

using DPPH radical scavenging and % RRI methods showed dose-dependent activity, with the ethanolic extract exhibiting maximum inhibition (95.63%), comparable to the standard ascorbic acid . Based on these results, the ethanolic extract was selected for further fractionation and isolation studies. Phytoconstituents such as naringenin chalcone and bopindolol were successfully isolated and characterized using FTIR spectroscopy, where the observed functional groups matched standard reference spectra. Furthermore, in vitro studies using isolated goat tracheal chain preparation demonstrated significant anti-asthmatic activity of the extract and its fractions by inhibiting histamine-induced contractions, with results comparable to the standard drug.

CONCLUSION

In conclusion, the study provides comprehensive scientific validation of *Cordia dichotoma* bark as a valuable source of bioactive phytoconstituents with significant pharmacological potential. The pharmacognostic, physicochemical evaluations confirmed the authenticity, purity, and quality of the plant material, supporting its use in herbal drug formulations. The extraction and phytochemical investigations revealed that the ethanolic extract is particularly rich in polyphenols and flavonoids, which are known contributors to antioxidant activity. The strong antioxidant activity observed in the ethanolic extract highlights its potential role in combating oxidative stress-related disorders. The successful isolation and characterization of key compounds such as naringenin chalcone further strengthen the phytochemical significance of the plant. Additionally, the demonstrated in vitro anti-asthmatic activity indicates that *Cordia dichotoma* possesses promising therapeutic potential in respiratory disorders. Overall, this study establishes a scientific basis for the traditional use of *Cordia dichotoma* and suggests its potential for further development as a natural antioxidant and anti-asthmatic agent. Future studies focusing on advanced spectroscopic characterization, in vivo pharmacological evaluation, and formulation development are recommended to explore its full therapeutic potential.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL: Not applicable

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AI TOOL DECLARATION

The authors declare that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

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