



**PHARMACOGNOSTICAL AND PHYTOCHEMICAL SCREENING
OF *Cordia rothii* ROEM & SCHULT.**

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ABSTRACT

Cordia rothii is a small tree growing mostly in tropical and temperate regions of India, Pakistan, Srilanka, North Africa and Arabica. Morphological and microscopical examination of *Cordia rothii* leaf was carried out using the reported methods in standard texts. Physical constants of crude drugs like loss on drying, ash values and extractive values were determined. Leaf constants like stomatal number, stomatal index, pallisade ratio, vein islet numbers and vein termination numbers were also determined to establish the standards. The preliminary phytochemical screening with the various qualitative chemical tests revealed the presence of carbohydrates, glycosides, saponins, steroids, triterpenes, proteins, amino acids and mucilage in the leaf extracts of *Cordia rothii*. The thin layer chromatography was also carried out as per the standard texts.

KEYWORDS: *Cordia rothii*, Extractive values, Phytochemical screening, TLC profile.



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INTRODUCTION



Figure 1
Twig of Cordia rothii

The plant *Cordia rothii* Roem & Schult, Fig:1 belongs to the family Boraginaceae. It is a small tree growing mostly in tropical and temperate regions of India, Pakistan, Srilanka, North Africa and Arabica. In India, it is mostly found in Gujarat, particularly in the Kachchh and Junagarh region. The plant possesses good medicinal value and is used by the people for the treatment of various diseases. The leaf paste was applied on an open wound and also useful in inflammation of legs¹. The plant *Cordia rothii* possess antidote activity: the fruit pulp has astringent, antidiarrhoeal and antiseptic activities and reduces burning sensation of urinary tract and the root has abortifacient and anti-inflammatory activities, while the whole plant has antidiabetic and antileprotic activities²⁻⁵. It has been reported that leaf extracts of certain species of *Cordia* such as *Cordia myxa*, *Cordia francisci*, and *Cordia serratifolia* have significant analgesic, anti-inflammatory and antiarthritic activity in the rat⁶. The aerial parts of *Cordia verbenacea* plant are used in folk medicine as anti-rheumatic, anti-inflammatory, analgesic and healing properties in the form of alcoholic extracts, decoctions and infusions⁷. The pharmacognostical and phytochemical studies of leaf of this plant have not been

reported. Therefore, the present investigation was planned to study the pharmacognostical and phytochemical aspects of *Cordia rothii* Roem & Schult.

MATERIALS AND METHODS

Collection and authentication of the plant

Leaves of *Cordia rothii* Decne were freshly collected from the Kachchh district of Gujarat (India) in the month of July. The plant material was identified and authenticated by the Botany Department, University School of Sciences, Gujarat University, Ahmedabad, Gujarat (India). The voucher specimen CRZAH-01 was also preserved for future reference. The collected leaves were shade dried for 15 days and size reduced by mechanical grinder into coarse powder. It was then stored in a well closed container free from environmental climatic changes till usage.

Morphological and Microscopical Investigation⁸⁻¹³

The healthy plants and their normal parts were selected for the morphological investigation. Morphological examination of *Cordia rothii* leaf was carried out using the reported methods in standard texts.

Microscopical characters were studied by preparing the specimen leaves of *Cordia rothii* in FAA (Formalin-5ml + Acetic acid-5ml + 70% Ethyl alcohol-90ml). After 24 hours of fixing, the specimens were dehydrated with graded series of TBA (tertiary-Butyl alcohol) as per the schedule. Infiltration of the specimens was carried out by gradual addition of paraffin wax (melting point 58-60 °C) until TBA solution attained super saturation. The specimens were cast into paraffin blocks. Wherever necessary, sections were also stained with safranin, fast-green and N/10 iodine to identify the presence of lignified cells and starch grains.

Proximate analysis¹⁴⁻¹⁵

Physical constants of crude drugs like loss on drying, ash values and extractive values were determined by using official methods.

Determination of leaf constants¹⁴⁻¹⁵

Leaf constants like stomatal number, stomatal index, palisade ratio, vein islet numbers and vein termination numbers were also determined to establish the standards.

Method of extraction¹⁶⁻¹⁷

The powder of dried leaves of *Cordia rothii* was subjected to continuous extraction with soxhlet extractor using various organic solvents such as petroleum ether (60-80 °C), chloroform, ethyl acetate, ethanol and water

respectively. After concentration and drying of each extract, identification of phytoconstituents was carried out by performing different qualitative chemical tests. The colour, consistency and percentage yield of the extracts were also noted.

Preliminary phytochemical screening of various extracts¹⁶⁻¹⁹

The leaf extracts of *Cordia rothii* obtained during the extraction process were subjected to preliminary phytochemical screening to determine the presence of various phytoconstituents using reported methods.

Thin layer chromatography of various extracts¹⁷⁻¹⁸

After concentration and drying of each extract in vacuum desiccator, identification of phytoconstituents was carried out by thin layer chromatography using different detecting reagents. The test extract was dissolved by using appropriate solvent in a concentration of 1 mg/ml and subjected for spotting. Silica gel G (mesh size 60) was used as a stationary phase and appropriate solvent systems to determine the presence of various phytoconstituents. The R_f value of compounds were noted for all the extracts. The R_f values were calculated by using the following formula:

$$R_f = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$

RESULTS AND DISCUSSION

Morphological and Microscopical Investigation

Morphological as well as microscopical studies of plants are the primary steps to establish its botanical standards before going to other studies. As per WHO norms, botanical standards are to be proposed as a protocol for the diagnosis of the herbal drug. The pharmacognostic parameters are helpful for the future identification and

authentication of the plant in the herbal industry. The leaf constants can be included as microscopical standards in Indian Herbal Pharmacopoeia. Phytochemical study was also useful to isolate the pharmacologically active principles present in the drug. Morphological examination of leaf reveals the following interpretations: The plant *Cordia rothii* is a shrub or small tree. Leaves opposite, subopposite or alternate, oblong to obovate, greyish-green with pale hairs on both surfaces; margins entire or with widely

spaced teeth on the apical half. Flowers in terminal clusters, white, and the calyx lobes are covered in soft rusty hairs. Fruits are ovoid with a long tip, embedded in the persistent calyx, orange-red when mature. Microscopy of leaf reveals presence of rectangular and polygonal epidermal cells with thick straight walls, unbranched multicellular trichomes, unbranched glandular trichomes, xylem elements arranged in dense parallel files, thin walled parenchymatous cells, uneven lamina, etc.

Physical evaluation

The physical standards, such as loss on drying, ash values, extractive values will be useful to identify the authenticity of the drug

even from the crushed or powdered plant materials. It will serve as a standard data for the quality control of the preparations containing this plant in the future. The information obtained from the ash values and extractive values are useful during the time of collection and also during extraction process. Using these standards, the plant can be differentiated from other related species. Physical evaluation revealed that loss on drying 8.56 %; total ash 7.92 %; acid insoluble ash 3.26 %; water soluble ash 4.66 %; alcohol soluble extractives 8.55 % and water soluble extractives 13.72 % w/w values were observed in leaves of *Cordia rothii*. The results were shown in Table 1.

Table 1
Physical parameters of leaves of *Cordia rothii*

Sr. No.	Parameters	Determined value* (% w/w)
1	Loss on drying	8.56 ± 0.12
Ash values		
2	Total ash	7.92 ± 0.09
	Acid insoluble ash	3.26 ± 0.06
	Water soluble ash	4.66 ± 0.04
Extractive values		
3	Alcohol soluble extractives	8.55 ± 0.27
	Water soluble extractives	13.72 ± 0.20

* Mean value of three counts

Determination of leaf constants

Leaf constants are fixed for all plant species, but they may vary from species to species. Determination of leaf constants is also one of the methods of standardization. It is helpful in identification of correct plant variety and also useful in predicting adulteration. The results of leaf constants determined were shown in Table 2.

Table 2
Determination of leaf constants

S. No.	Parameters	Range	Mean
1.	Palisade ratio	4.00 – 6.00	5.32 ± 0.16
2.	Stomatal number-upper surface	8.00 – 11.00	9.32 ± 0.27
3.	Stomatal number-lower surface	5.00 – 8.00	6.48 ± 0.09
4.	Stomatal index-upper surface	13.55 – 16.72	14.79 ± 0.12
5.	Stomatal index-lower surface	7.31 – 12.48	10.03 ± 0.16
6.	Vein-islet number	4.00 – 6.00	5.02 ± 0.15
7.	Vein-termination number	4.00 – 6.00	4.89 ± 0.18

Average extractive values (% w/w)

During successive solvent extraction, the percentage yields were determined as petroleum ether 12.68 %, chloroform 6.13 %, ethyl acetate 2.39 %, ethanol 6.52 % and water 8.70 % w/w. The colour and consistency of the each extract were also noted during the extraction process as shown in Table 3.

Table 3
Colour, consistency and percentage yield of leaf extracts of *Cordia rothii*

Sr. No.	Solvent used	Colour and consistency	Average percentage yield (% w/w on dry weight basis)
1	Petroleum ether	Greenish sticky mass	12.68
2	Chloroform	Brown	6.13
3	Ethyl acetate	Brown	2.39
4	Ethanol	Dark brown	6.52
5	Water	Dark brown	8.70

TLC profile of extracts

The preliminary phytochemical studies with the help of thin layer chromatography revealed the presence of saponins in water extract (R_f value 0.49, 0.62); phytosterols in chloroform and alcohol extract (R_f value 0.41); triterpenes in chloroform and ethyl

acetate extract (R_f value 0.66); carbohydrates in alcohol and water extract (R_f value 0.32, 0.35, 0.60, 0.76); fats and oils in petroleum ether, chloroform, ethyl acetate and alcohol extract (R_f value 0.65, 0.70). The results were shown in Table 4.

Table 4
Qualitative TLC analysis of phytoconstituents in *Cordia rothii*

Phytoconstituents	Solvent system	Visualizing reagent	No. of Spots	R_f Value	Colour
Saponins	Ethyl acetate: Methanol (50:50)	Phenol sulphuric acid	2	0.49, 0.62	Yellow-brown
Phytosterols	Pet. ether: Acetone (90:10)	Antimony trichloride	1	0.41	Blue
Triterpenes	Chloroform : Acetone (80:20)	Liebermann-Burchard reagent	1	0.66	Brown pink
Carbohydrates	Butanol: Glacial acetic acid: Ether: Water (45:30:15.5)	Phenol Sulphuric acid	3	0.35, 0.60, 0.76	Brown black
Amino acids	Butanol: Glacial acetic acid: Water (40:10:50)	Ninhydrin reagent	3	0.45, 0.67, 0.74	Pink
Fats and oils	Toluene: Ethyl acetate (93:7)	Vanillin sulphuric acid	2	0.65, 0.70	Yellow-brown

Preliminary phytochemical screening of *Cordia rothii* leaves

The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, saponins, tannins, triterpenes, sterols etc. Thus the preliminary screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development. Further, these tests

facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds. The preliminary phytochemical screening with the various qualitative chemical tests revealed the presence of carbohydrates, glycosides, saponins, steroids, triterpenes, proteins, amino acids and mucilage were present in the leaf extracts of *Cordia rothii*. The results were shown in Table 5.

Table 5
Preliminary phytochemical screening of leaf extracts of *Cordia rothii*

Plant constituents Tests/reagents used	Extracts				
	P	C	E	A	W
1 Alkaloids					
Dragendorff's reagent	-	-	-	-	-
Mayer's reagent	-	-	-	-	-
Hager's reagent	-	-	-	-	-
Wagner's reagent	-	-	-	-	-
2 Carbohydrates and glycosides					
Molisch's reagent	-	-	-	+	+
Fehling solution	-	-	-	+	+
Benedict's reagent	-	-	-	+	+
Barfoed's test	-	-	-	-	-
Baljet test	-	-	-	-	-
Legal's test	-	-	-	-	-
Bortrager's test	-	-	-	+	+
3 Phytosterols					
Salkowski test	-	+	+	+	-
Liebermann-Burchard's test	-	+	+	+	-
4 Fixed oils and fats					
Spot test	+	+	+	+	-
Saponification test	+	+	+	+	-
5 Saponins					
Foam test	-	-	-	-	+
6 Phenolic compounds					
Ferric chloride solution	-	-	-	-	-
Bromine water	-	-	-	-	-
Lead acetate solution	-	-	-	-	-
7 Tannins					
Gelatin test	-	-	-	-	-
Ferric chloride test	-	-	-	-	-
Vanillin hydrochloride test	-	-	-	-	-
Alkaline reagent test	-	-	-	-	-
8 Triterpenes					
	-	+	-	-	-
9 Flavonoids					
Shinoda test	-	-	-	-	-
Zinc-HCl reduction test	-	-	-	-	-
Alkaline reagent test	-	-	-	-	-

1	Proteins and aminoacids					
0	Millon's reagent	-	-	-	-	+
	Biuret test	-	-	-	-	+
	Ninhydrin reagent	-	-	-	-	+
1	Gums and mucilages					
1		-	-	-	-	+
1	Volatile oils					
2		-	-	-	-	-

[P - Petroleum ether extract; C - Chloroform extract; E - Ethyl acetate extract; A - Alcohol (Ethanol) extract; W - Water extract]

CONCLUSION

This study on micro-morphological features of *Cordia rothii*, proposed a set of anatomical parameters may enable those who handle this plant to maintain its quality control. Morphological as well as microscopical studies of plants are the primary steps to establish its botanical standards before going to other studies. As per WHO norms, botanical standards are to be proposed as a protocol for the diagnosis of the herbal drug. The pharmacognostic parameters are helpful for the future identification and authentication of the plant in the herbal industry. The physical standards, such as loss on drying, ash values, extractive values will be useful to identify the authenticity of the drug even from the crushed or powdered plant materials. It will serve as a standard data for the quality control of the preparations containing this plant in the future. The information obtained from the ash values and

extractive values are useful during the time of collection and also during extraction process. Using these standards, the plant can be differentiated from other related species. The leaf constants can be included as microscopical standards in Indian Herbal Pharmacopoeia. The curative properties of medicinal plants are perhaps due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, saponins, sterols, etc.²⁰ Thus the preliminary screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development. Further, these tests facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds. Phytochemical study was also useful to isolate the pharmacologically active principles present in the drug.

REFERENCES

1. Thakar JI. Plants of Kachchh and their utility, Pravin Publications, Rajkot, 80 (1926).
2. Vaidya BG. Nighantuadarsha (Uttarardh), Swami Atmanand Saraswati Ayurvedic Pharmacy Ltd, Surat, 2nd Edn, 724 (1965).
3. Chunekar K and Pandey G. Bhavaprakash Nighantu, Chaukhamba Oriental, Varanasi. 8th Edn, 584 (1988).
4. Watt JM and Breyer-Branwjick MG. The Medicinal and Poisonous Plants of Southern and Eastern Africa. E & S Livingstone Ltd, Edinburg and London, 2nd Edn, 145 (1962).
5. Thaker JJ. Vanaspati Varnan (Flora of Baroda Mountain). Sastu Sahitya Vardhak Karyalaya, Ahemadabad, 2nd Edn, 478 (1952).
6. Ficarra K, Ficarra P and Tommsini S. Leaf extracts of some *Cordia* species: analgesic and anti-inflammatory activities as well as their chromatographic analysis. Pharmacology, 4: 245 (1995).
7. Akisue MK, Oliveira F, Moraes MS, Akisue G and Mancini B. Pharmacognostical characterization of

- Cordia verbenacea*. Rev Cienc Farm, 5: 69-82, (1983).
8. Wallis TE. Text Book of Pharmacognosy, CBS Publishers and Distributors, Shahdara, Delhi 111 (1985).
 9. Sass JE. Elements of Botanical Microtechnique. Mc Graw Hill Book Co; New York, 222 (1940).
 10. Johansen DA. Plant Microtechnique. Mc Graw Hill Book Co; New York, 523 (1940).
 11. O'Brien TP, Feder N and Mc Cull ME. Polychromatic Staining of Plant Cell Wall by Toluidine Blue, 59: 364-373 (1964).
 12. Easu K. Plant Anatomy, John Wiley and sons, New York, 767 (1964).
 13. Easu K. Anatomy of Seeds, John Wiley and sons, New York, 550 (1964).
 14. Indian Pharmacopoeia, Ministry of Health and Family Welfare, Government of India, Controller of publication, New Delhi, 2: 55 (1996).
 15. The Ayurvedic Pharmacopoeia of India, Government of India, 188 (2001).
 16. Kokate CK, Practical Pharmacognosy, Vallabh Prakashan, New Delhi, 115 (1994).
 17. Harborne JB, Phytochemical methods, A guide to modern technique of plant analysis, Chapman and Hill, London, 90 (1998).
 18. Kapoor LD, Singh A, Kapoort SL and Strivastava SN, Survey of Indian Medicinal Plants for Saponins, 297 (1969).
 19. Evans WC, Trease and Evan's Pharmacognosy. 14th Edn. WB Saunders Company Limited, London, 545 (1996).
 20. Alwashli A, Al-sobarry M, Cherrah Y, Alaoui K. Anti-Inflammatory and analgesic effects of ethanol extract of *Dracaena Cinnabari* Balf, as endemic plant in Yemen International Journal of Pharma and Bio Sciences, 3(2): 96-100, (2012).