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Pharmacognostic, physicochemical and phytochemical screening of the leaves of *Abrus precatorius* (Linn.) and *Cordia wallichi* (Don.) for Quality control assessment

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Abstract
Objective: To evaluate the pharmacognostic properties, including the macroscopic, microscopic, physicochemical characteristics and phytochemical screening of the leaves of *Abrus precatorius* (Linn.) *and Cordia wallichi* (Don).
Methods: Microscopic and macroscopic characteristics of dried leaf samples were analyzed. Organoleptic evaluations and physicochemical studies were performed using WHO-recommended parameters, and fluorescence behavior of the leaf samples were also analyzed. Extraction was done with cold maceration process using various of polar solvents like Aqueous, 70% hydroalcohol and methanol. The phytochemical analysis was done by using the standard procedure.
Results: Microscopic studies revealed the presence of three-lobate leaves, with the arrangements of spongy and palisade tissues. Physicochemical parameters such as
foreign matter, moisture content, extractive values, ash content, pH, and fluorescence behavior of leaf powder were also determined. The results revealed that the leaves extracts contain Flavonoids, Terpenoids, Tannins, Phlobatannins, Saponins, Cardiac glycosides, Carbohydrate, Protein and Anthraquinones in major proportion.
Conclusions: The present study revealed pharmacognostic and phytochemical parameters of of the leaves of *Abrus precatorius*(L.) and *Cordia wallichi*(D.), which would be beneficial for its standardization and future scope of the work.

Keywords: *Abrus precatorius* (Linn.), *Cordia wallichi* (Don.) Pharmacognostic analysis, physicochemical standards, phytochemical analysis

1. Introduction

According to the World Health Organization (WHO), 80% of the rural population in the developing countries depend on traditional medicines to meet their primary health-care needs [1]. Since long, plants have been used for medicinal purposes across a globe. Indian system of medicine is based on effective use of plants for the therapeutic purposes. Large numbers of plants are well documented in ancient medical literature of India. Among those plant Abrus precatorius (L.) and *Cordia wallichi* (D.) also documented in various literature for their medicinal properties.

A.precatorius is found in South Africa, China, Islands, West Indies, India, Brazil, etc. Plant found all throughout the plains of India, from Himalaya down to Southern India [2, 3]. Secondary metabolites present in A. precatorius include alkaloids [4], flavonoids/flavones such as luteolin, abrectorin, orientin, isoorientin, and desmethoxycentaviridin-7-O-rutinoside [5, 6] triterpene glycosides, saponins, steroids and other terpenoids, fixed oil carbohydrate, protein, tannins, anthocyanins and amino acids. The plant roots and leaves contain sweet-tasting glycyrrhizin as a major phytoconstituent [7] The leaves are used reported to posses neuromuscular, antiepileptic, antidiabetic, and many more activities [8, 12]. Traditionally leaves are used as nerve tonic, applied on cuts and swellings and mouth ulcer.

The Boraginaceae family consists of about 2,700 species, which are distributed in tropical, sub-tropical and warmer regions around the world. It is composed of about 130 genera and six sub families, in which *Cordioideae* is one. It contains the genus *Cordia*, which is comprised of evergreen trees and shrubs. About 300 species of genus *Cordia* have been identified worldwide. There are 13 species of this genus found in India.One of them is *Cordia wallichi.* Willd About 300 species of genus *Cordia* have been identified worldwide [13]. There is number of resercher work on species Cordia obliqua, cordia myxa, cordia dichotoma, but less are reported on wallichi species [14, 19].

In spite of presence of vital chemicals, Cordia leaves are quite unexplored. Till today, there are no reports of pharmacognostic and phytochemical analysis of *Cordia wallichi (L.)* leaves. Considering the therapeutic potential and prospective utility of the leaves in near future, its pharmacognostic and phytochemical studies were planned. Moreover, this is the first report of the above-mentioned studies with respect to *Cordia wallichi (L.)* leaves.

2. Material and methods

2.1 Collection and authentication of plant material

The authentic plant materials were collected from Kamrup district, Assam and identified and authenticated by Dr. T.G. Gohil, taxonomist and HOD of Botany, Botanist in B.K.M Science College, Valsad (Gujurat). Voucher specimen of the collected plants were prepared and maintained in the Botany department of BKM Science College. Valsad, Gujarat. for further reference. Botanical identity was confirmed by correlating their morphological and microscopical characters.

2.2 Chemicals and reagents

All the chemicals and reagents used in the present study were of analytical grade and were obtained from Himedia, Sigma, Merk and SD Fine. Absolute alcohol,

Phloroglucinol, acetic acid, chloral hydrate, H_2SO4, NaOH, HNO_3, $FeCl_3$, distilled water, Conc. HCl and chloroform.

2.3 Microscopical studies

2.3.1 Transverse section of leaves

Section cutting done by with help of blade for both the fresh leaves of *Abrus precatorius* (Linn.), and *Cordia wallichi (Don.)* placed in between potato to obtain a thin section. Fluloroglucinol and hydrochloric acid in the ratio 1:1 was used as a stain and mounted on a glass slide and focused under a microscope. A thin transverse section was taken and studied

2.3.2 Powder Microscopy

Shade dried leaves powdered with the help of an electric grinder till a fine powder was obtained. This fine powder of the leafs, subjected to powder microscopy, as per standard procedures mentioned. Powder of leavess, was taken in watch glass and stained with quantities of phloroglucinol and hydrochloric acid was taken in the watch glass. Slides were prepared with help of a brush. Focused under microscope and taken photographs.

2.4 Physicochemical screening as per WHO guidelines

The respective plant leaves were shade dried and finely powdered using laboratory blender and fine powder was used for further studies. The physico-chemical parameters such as Total ash, acid insoluble ash, alcohol soluble extractive, water soluble extractive, LOD, Foreign organic matter, Foaming index,Crude fibre content and pH (1%,10%aqueous), were determined [20, 21, 22].

2.4.1 Determination of total ash

The powdered material (2 g) was accurately weighed and placed in a crucible. The material was spread in an even layer and it was ignited to a constant weight by gradually increasing the heat to 500-600 °C until it was white indicating the absence of carbon. The residual ash was allowed to cool in a desiccator. The content of total ash (g) of air-dried material was calculated as follows:

Determination of total ash value formula: Total ash value of the sample %=(Z-X) X100

X= weight of empty dish Y

Y= weight of the drug taken

Z= weight of the dish + ash (after complete incineration)

2.4.2 Determination of acid insoluble ash

HCl (2 N; 25 mL) was added to the crucible containing the total ash, covered with a watch glass, and boiled gently for 5 min. The watch glass was rinsed with 5 mL of hot water and the rinsed contents were added to the crucible. The acid insoluble matter was collected on an ashless filter paper and washed with hot water until the filtrate was neutral. The filter paper containing acid insoluble matter was transferred to the original crucible, dried on a hot plate, and ignited to a constant weight. The residue was allowed to cool in a desiccator and weighed. The content of the acid insoluble ash (in mg/g) of air-dried material was calculated as follows:

Acid insoluble ash %(W/W) = $\frac{\text{Weight of ash x100}}{\text{Weight of the sample}}$

2.4.3 Determination of water-soluble ash

Water (25 mL) was added to the crucible containing the total ash, covered with a watch glass and boiled gently for 5 min. The watch glass was rinsed with 5 ml of hot water and added to the crucible. The water insoluble matter was collected on an ash less filter paper and washed with hot water. The filter paper containing the water insoluble matter was transferred to the original crucible, dried on a hot plate, and ignited to a constant weight. The water-soluble ash content was calculated using the following equation.

Water soluble ash %(W/W) = $\frac{\text{(Total ash- Water insoluble residue in total ash) x100}}{\text{Weight of the sample}}$

2.4.4 Extractive values of the leaves powder

Determination of extractive values Extracting values are useful for determining of crude drugs & it gives an idea about the nature of the chemical constituent's present. The solvent used for the extraction should be in position to dissolve appropriate quantities of desired substances**.**

2.4.5 Determination of alcohol soluble extractive

Accurately weighed powdered material (4 g) and was placed in a glass stoppered round bottle flask (RBF). Ethanol (100 ml) was added to the RBF and then, it was shaken well and allowed to stand for 1 h. A reflux condenser was attached and boiled gently for 1 h, and then it was cooled and filtered. The flask was shaken well and filtered rapidly through a dry filter paper. After that, 25 ml of the filtrate was transferred to a tarred flat-bottomed dish and evaporated to dryness on a water bath. Then the dish was dried at 105 °C for 6 h and cooled in a desiccator and weighed. The content of extractable matter (% w/w) air-dried material was calculated as follows:

Alcohol-Soluble Extractive = $\frac{\text{Wt. of extractive X4 X 100}}{\text{Wt. of sample}}$

2.4.6 Determination of hydro-alcoholic and water-soluble extractives

The same procedure as described for the ethanol soluble extractive matter was followed for the determination of hydro-alcoholic and water-soluble extractive matter using ethanol-water (1:1) and distilled water instead of ethanol.

About 5gm of air-dried powdered drug was taken & macerated with 100 ml of chloroform water in a closed flask for 24 hrs shaking frequently during the first 6 hrs. And then allowed to stand for 18 hrs. Thereafter, it was filtered rapidly taking precautions against loss of the solvent. 25 ml of the filtrate was evaporated to dryness in a tarred flat-bottomed shallow dish, dried at 105°C & weighed. The percentage of the water-soluble extractive value was calculated with reference to the air-dried drug

Water-Soluble Extractive = $\frac{\text{Wt. of extractive X 100}}{\text{Wt. of drug}}$

2.4.7 Determination of loss on drying

The loss on drying is the loss of weight in percentage w/w resulting from water and volatile matter of any kind that can be driven of under specified conditions. The test was carried out on well mixed sample of the substance.1 gm of leaves of the plants were transferred into a petridish plate and the contents were distributed evenly to a depth not exceeding 10

mm. The loaded plate was heated at 105^0C in hot air oven for 1 hr and then cooled in desiccators, loss in weight was recorded as moisture content. Respective moisture content percentage of the samples was calculated.

Moisture content (% w/w) = (Initial Wt. of the sample-Final Wt.of the sample) X 100
Wt. of the sample

2.4.8 Determination of pH
The pH of 1 and 10 % aqueous solution were determined by making appropriate concentration of powdered drug in aqueous solution, filtered and checked the pH of the filtrate with a standardized glass electrode (Anonymous, 1988).

2.4.9 Determination of Foreign organic matter
Medicinal plant materials should be entirely free from visible signs ofcontamination, *i.e.* moulds, insects, and other animal contamination, including animal excreta. It is seldom possible to obtain marketed plant materials that are entirely free from some form of innocuous foreign matter. However, no poisonous, dangerous or otherwise harmful foreign matter or residue should be allowed. Any soil, stones, sand, dust and other foreign organic matter must be removed before medicinal plant materials are cut or ground for testing. Macroscopic examination can conveniently be employed for the determination of foreign matter in whole or cut plant materials.

2.4.10 Determination of Foaming index
The foaming index measured the foaming ability of an aqueous decoction of plant material. 1g coarse powder of leaves of each plant was accurately weighed and transferred to a 500 ml conical flask containing 100 ml of boiling distilled water and moderate boiling was maintained for 30 minutes, cooled and filtered into 100 ml volumetric flask and the volume was made up to 100 ml with distilled water. The decoction was transferred into 10 labelled stopper test tubes in successive portions of 1 ml, 2 ml, 3 ml and up to 10 ml and volume was made up to 10 ml in each tube with distilled water. Tubes were shaken in length wise motion for 15 seconds with two shakes per second and were allowed to stand. for 15 minutes and the height of foam in each tube was measured.

2.4.11 Crude fiber content
About 2 g of drug was extracted with diethyl ether. The residue was transferred to a digestion flask containing 200ml of 0.225N sulphuric acid fitted with the condenser and heated. After 30 min the contents were filtered, washed ith boiling water until the washings are basic. The residue was transferred to a flask with 200 ml of sodium hydroxide solution (0.1N). The flask was connected with the reflux condenser and boiled for 30 min, then filtered through ash less filter paper (Whatmann No. 41) followed by washing with water until free from alkali, it was washed with 15 ml of alcohol. The filter paper was transferred to a crucible and ignited at 450°C. It was cooled in desiccators and weighed. The loss in weight represents the crude fiber content.

Crude fibre content (% w/w) = (Initial Wt. of the sample-Final Wt.of the sample) X 100
Wt. of the sample

2.5. Preparation of plant extracts for phyto chemical analysis [23]
For the present study, the extracts were obtained by coarsely powdered crude drug is placed in a stoppered container with the water,70% hydroalcohol and methanol and allowed to stand at room temperature for a period of at least 3 days with frequent agitation until the soluble matter has dissolved. The mixture then is strained, the marc (the damp solid material) was pressed and filtered. The filtrate was concentrated in rotary evaporator at 40ºC and kept in the desiccators for futher use. The extracts were calculated for their extractive value in the respective solvents

The various extracts of the powdered leaves of *Abrus precatrius* and *Cordia wallichi* analysed for the presence of various phyto constituents like steroids, triterpenoidal, saponins, alkaloids, carbohydrates, flavonoids, glycosides and phenolic compounds by using standard phytochemical procedures as described by Harborne.

3. Results and Discussion
Microscopic examination of transverse section and powdered drug aided by stains help in distinction of anatomy in adulterants. Lamina showed common dicotyledonous characters. The mesophyll differentiated with palisade and spongy parenchyma cells. There is a single layer epidermis covered externally with prominent cuticle in upper or lower surface. Palisade layer made up of one to two layers of columnar cells which occupied a little less than half of the width of the mesophyll. The spongy mesophyll covered of irregular cells. The midrib was one or less circular Following the epidermis, it was a zone of cortex composed of one to two layers of collenchyma surrounding the central portion. The shape of the vascular bundle was ovoid. Sclerenchyma sheath was lignified thick walled cells cover vascular bundles. The xylem was lignified and phloem was non-lignified. Xylem consists of large lumen vessels and small tracheids.

Diagnostic characters of powder include Epidermis, Anomocytic stomata, Reticulate thickening xylem vessels, Warty covering trichomes,Prismatic shapped calcium oxalate crystels and starch grains.

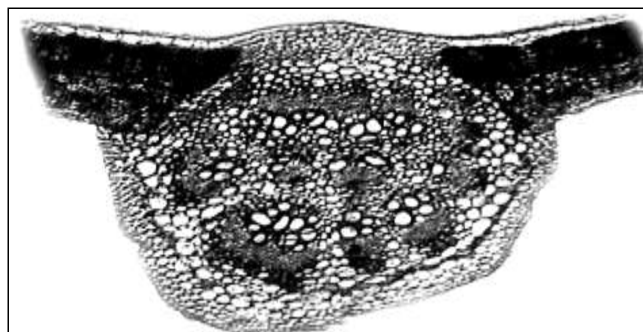

Fig 1: Transverse section of the leaf of *Cordia wallichi* (Don.)

Fig 2: Powder Microscopy of the leaf of *Cordia wallichi*. (D.). A: Epidermis in surface view; B: Anomocytic stomata; C: Xylem vessels with reticulate thickening D: Dagger-shaped warty covering trichome; E: Calcium oxalate prism crystals; F: Starch grains

The transverse section of the leaf of *Abrus precatorius (*L.), dorsiventral leaf (dicot leaf) shows three main parts called 1. Epidermis 2. Mesophyl, 3. Vascular bundles, as shown in the figure 3.A thin T.S of young dicot leaf, when examined under the microscope, shows the following regions from outside to inside.

Powder microscopy was done according to the standard procedure mentioned. Anomocytic stomata, Phloem fibers, trichomes, xylem vessels, calcium oxalates, Epidermis and starch grains were observed.

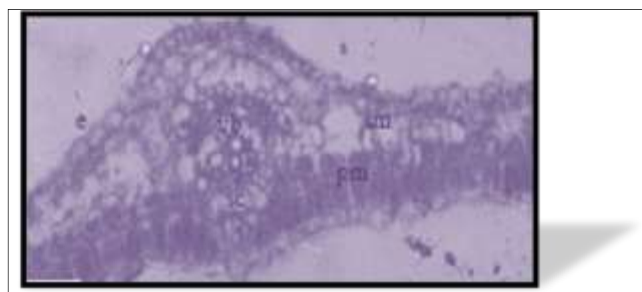

Fig 3: Transverse section of the leaf of *Abrus precatorius (*Linn.)

Fig 4: Powder Microscopy of the leaf of *Abrus precatorius (*Linn).). A. Anomocytic stomata. B. Phloem fbres. C. Unicellular covering trichomes, D. Xylem vessels, E. Calcim oxalate crystals.

Table 1: Summary of results of physico-chemical parameters

Parameters	Results (n= 3, Mean ± SD)	
Ash value	**%yield (Abrus)**	**% yield (Cordia)**
Total ash% w/w	2.46±0.04 NMT3%	2.87±0.05
Acid insoluble ash% w/w	0.36±0.04 NMT 0.5%	0.823±0.02
Water soluble ash% w/w	24.66±0.05 NLT 15%	1.24±0.02
Extractive values		
Alcohol soluble extractive% w/w	9.33±0.06 NLT 3%	6.09±0.08
Hydro-alcoholic extractives (% w/w)	11.33±0.06	7.23±0.25
Water soluble extractive	12.33±0.28	8.16±0.25
Loss on drying	5.76±0.05	6.5±0.24
Foreign organic matter	1.16±0.06NMT 2%	5.83±0.28
Crude fiber content	1.5±0.06	1.33±0.06
Foaming index	<100	<100
pH values of aqueous solution		
pH of 1 % aqueous solution	5.66±0.05	5.13±0.06
pH of 10% aqueous solution	7.6±0.02	7.16±0.15

NMT= Not more than, NLT= Not less than as per Ayurvedic Pharmacopoeia.

Table 2: Fluorescence analysis [23, 24]

	Abrus. precatorius Cordia wallichi					
Reagent	**Long (365nm)**	**Short (256nm)**	**Day**	**Long (365nm)**	**Short (256nm)**	**Day**
50% H_2SO_4	Light brown	Light Green	Brown	Light brown	Green	Brown
50% HNO_3	Green Light	Green	Green	Green Light	Green	Green
5% NaOH	Green	Green	Green	Green	Green	Green
1N NaOH	Green	Dark green	Green	Green	Dark green	Green
1N KOH	ThickGreen	Green	Green	ThickGreen	Green	Green
5% KOH	Green	Green	Green	Green	Green	Green
5% $FeCl_3$	Light Green	Black green	Green	Light Green	Black green	Green
Methanol	Green	Green	Green	Green	Green	Green

Conc. HCl	ThickGreen	Light Green	Light Green	ThickGreen	Light Green	Light Green
Conc. H_2SO_4	Light Black	Black	Brown	Light Black	Black	Brown
Ammonia	ThickGreen	Light Green	Green	ThickGreen	Green	Green
Conc HNO_3	ThickGreen	Green	Brown	ThickGreen	Green	Brown

Table 3: Extractive values in different solvents.

Extracts	Percentage Yield (Abrus)	Percentage Yield (Cordia)
Aqueous	6.7%	9.5%
70% hydroalcoholic	9.6%	5.2%
Methanolic	12.8%	5.2%

Table 4: Qualitative phytochemical secreening of Aqueous, Hydroalcoholic and Methanolic extracts of *Abrus precatorius* and *Cordia wallichi* leaves

Sr. No	Secondary Metabolites	Test Name	Aqueous Extract		70% hydroalcoholic extracts		Methanolic extracts	
			Ap	Cw	Ap	Cw	Ap	Cw
1	Triterpenes	1. Salkowski Test	-ve	-ve	-ve	-ve	++ve	+ve
		2. Lieberman-Buchard's	-ve	-ve	-ve	-ve	+ve	+ve
2	Steroids	1. Salkowski test	+ve	-ve	-ve	-ve	+ve	++ve
		2. Lieberman-Buchard's	-ve	-ve	-ve	-ve	+ve	+ve
3	Alkaloids	1. Mayer's test	+ve	+ve	+ve	+ve	+ve	+ve
		2. Dragendroff's Test	+ ve	+ ve	+ ve	+ ve	++ ve	+ ve
		3. Wanger'sTes	+ve	+ve	-ve	+ ve	++ ve	++ ve
4	Flavonoids	1. Sinoda test	+ ve	+ve	+ ve	-ve	++ ve	+++ ve
		2. Alkaline Reagent Test	+ve	--ve	+ve	+ ve	++ve	++ ve
5	Phenolic compounds	1. Ferric cyanide test	+ ve	+ ve	+ ve	+ ve	++ ve	++ ve
		2. Gelatin test	+ve	-ve	-ve	+ ve	++ ve	+ ve
6	Tannins	1. Ferric chloride Test	+ ve	+ve	+ ve	+ ve	+++ ve	+ ve
		2. Lead acetate Test	-ve	+ ve	++ ve	++ve	+++ v	++ ve
7	Saponins	1. Foam Test	--ve	-ve	-ve	-ve	+ve	+ve
		2. Hemolytic Test	-ve	-ve	-ve	-ve	+ve	+ve
8	Carbohydrates	1. Fehilings test	-ve	-ve	-ve	-ve	+ve	+ve
		2. Benedict's Test	-ve	-ve	-ve	-ve	+ve	-ve
9	Protein and amino acid	1. Biuret test	-ve	-ve	-ve	-ve	-ve	-ve
		2. Millon's test	-ve	+ve	-ve	-ve	+ve	+ve
10	Glycosides	1. Brontrager's test	-ve	+ve	-ve	-ve	+ve	+ve
		2. Killer killani test	-ve	-ve	-ve	+ve	-ve	++ve
11	Coumarins	1. Sodium hydroxide test	+ve	-ve	-ve	-ve	+ve	-ve
+++Strong coloration, ++Moderate coloration, +Weak coloration, - Absent								

4. Conclusion

With the tremendous development and expansion in the use of traditional medicine worldwide, authenticity as well as qualitycontrol of herbal medicines has become a matter of most importance. According to the World Health Organization, the macroscopic and microscopic description of a medicinal plant is the first step towards establishing the identity and degree of purity and should be carried out before any other tests are undertaken. In this context the study was undertaken to set the standardized parameters for the establishment of standard quality of the leaves of *Abrus precatorius* (L.) and *Cordia wallichi* (D.).

Evaluation of physicochemical parameters is an important part in the preparation of modern monograph. The Ash Values, Exractive Values, Loss on Drying & Foreign Organic Matter content in *Abrus precatorius* are found within the specified limits as mentioned in the Ayurvedic Pharmacopoeia Part-1 Vol-3. Hence, the collected plant is authentic.

Cordia wallichi also screened for all physicochemical parameters and found similar as its other specis, however there is no reference standard for *Cordia wallichi* is given in any official monographs.

Fluorescence analysis of powdered plants was studied in U.V. light at wavelengths of 254nm and 365nm. The powders of both plants showed brown fluorescence with methanol in UV light at 254nm which indicates the presence of chromophore in the drug.

Preliminary phytochemical screening results reveals that, among all three solvents used for extraction in a series, methanolic extract in both the plants showed positive results for many numbers of chemical compounds. It contains Steroids, Saponins, Alkaloids, Carbohydrates, flavonoids, tannins and phenolic compounds.

The pharmacognostical, microscopical and physicochemical parameters of leaves of *Abrus precatorius* (L.) and *Cordia wallichi* (D.). The leaves were successively extracted and the methanolic extracts were further studied for HPLC and HPTLC quantification.

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