ijlpr 2022; doi 10.22376/ijpbs/lpr.2022.12.2. P45-53

International Journal of Life science and Pharma Research

ISSN 2250-0480

Research Article

Pharmacognosy for efficient natural therapy

Pharmacognostic And Phytochemical Evaluation Of Cordia Obliqua Willd Fruits

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Abstract: Cordia (family Boraginaceae) is a genus of deciduous flowering trees or shrubs comprising more than 300 species distributed widely in the tropical regions. The aim of this research was to provide scientific information on phramacognostical nature and presence of various phytochemicals in the fruits of Cordia obliqua. The present study helped to evaluate anatomical, histochemical, physico chemical analysis and chemical tests of various extracts of Cordia obliqua willd fruits. Common names of plants are "Naruvili, Ali and Namaviri (Tamil), Lassura Viri, Cheruvari (Kerala). China-nakeru (Andhra and Telangana)". It is considered as one of the most important medicinal plants and various plant parts like leaves, fruits, bark and seed were found to possess abundant ethno medicinal value. Most frequently they are used to treat many ailments such as respiratory disorders, stomach pain, wound, inflammation, myalgia, cough, dysentery and diarrhoea. The fruits are widely used for the treatment of spleen and hepatic disorders. The anatomical and histochemical characteristics of the fruits of Cordia obliqua have been studied which helped in authentication and diagnose the various anatomical characters like presence of epidermis, mucilage, xylem and phloem fibers and calcium oxalate crystals. Proximate analyses of fruits were performed which included moisture content, extractive values and ash values helped to identify the purity and solubility of fruits in different solvents which was revealed by extractive values. Chemical tests helped to know the various phytoconstituents present in the fruits of Cordia obliqua when tested with various chemical reagents. Phytochemical tests revealed the presence of steroids, glycosides, alkaloids, tannins, phenolic and flavonoid compounds and carbohydrates.

Keywords: *Cordia Obliqua* (Boraginaceae), anatomical, histochemical, fruit, proximate analysis, Phytochemicals.

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Received On 16 November, 2021
Revised On 20 February, 2022
Accepted On 22 February, 2022
Published On 3 March, 2022

Funding This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

Citation G Tharun, S Sivakrishnan, JVC Sharma , Pharmacognostic And Phytochemical Evaluation Of Cordia Obliqua Willd Fruits.(2022).Int. J. Life Sci. Pharma Res.12(2), P45-53 http://dx.doi.org/10.22376/ijpbs/lpr.2022.12.2. P45-53

1. INTRODUCTION

The Boraginaceae family plant has about 2700 species, which are widely distributed in tropical, subtropical and temperate areas around the world. It has more than 130 species and with 6 subfamilies, of which the Cordiaceae is one. It consists of the Cordia species, which are evergreen trees and shrubs. About 300 species of the Cordia genus have been identified. There are 13 species which are widely present in India[1,2] and *Cordia Obliqua* Willd is one of them. It is a tropical tree, located within the center of the Himalayas up to 4000 ft [1,470 m]. It shows sturdy growth. There are two types of *Cordia Obliqua* Willd, found in Himachal Pradesh and the main difference between the 2 types is the size of their fruit, one with much less fruit than the opposite. This low-yielding plant is extensively available[3]. Clammy cherry is a medium sized deciduous tree, 10.5 meters excessive, the girth of trunk of tree being 75.5 cm, branchlets glabrous, wood gentle, moderate grey, no heartwood.[4] Fruits are available in the state of Tamil Nādu and are called as *Cordia Obliqua* Willd. Var. obliqua and var. Tomentosa (known through the neighborhood name Virusu and Kal virusu respectively). Some species of Boraginaceae, is also used as food.[5] The fruits are sweet in taste and exhibits various activities when taken. It fruits known for its cooling nature, in the treatment of intestinal worms, constipation, exhibits diuretic action, relieves cough, and sicknesses of the chest, urethra, in liver disorders and in high temperature[6]. It decreases thirst and scalding of urine, gets rid of inflammation in joints, prevents imbalance in humors, irritation of the throat and in toning of spleen (As in step with unani device). The bark juice is used in gripes and in conjunction along with coconut oil. Tonic is prepared from the bark and unripe fruits. The kernels are used in treatment of ringworm and the leaves are beneficial to treat ulcers and headaches. Raw fruits are used as a vegetable and a top-notch pickle. Pieces of fruit are used as a glue to attach sheets of paper and cardboard.[2]

2. MATERIALS AND METHODS

2.1 Pharmacognostic Studies

2.1.1 Collection, *Identification and Authentication of plant*

The fruits of *Cordia Obliqua* Willd were amassed from Chidambaram district, Tamil Nadu, India. The fruits were identified in the department of Botany, Palamuru University, Mahabubnagar, Telangana and authentication of fruits was done by Prof.P. Jayaraman, Director, Plant Anatomy Research Center (PARC), West Tambaram, Chennai and Tamilnadu. The herbaria of Specimens were prepared and submitted in Dept. of Botany, Palamuru University and in PARC. The specimen voucher number is HPU:102/2019 and PARC/2021/4509.

2.1.2 Collection of Specimen

The plant specimens for the proposed study were collected from the district of Chidambaram; enough precautions were taken to select fruits from healthy plants. The required samples were collected from the fruits and fixed in Formalin 5ml, 5ml of Acetic acid and 70% Ethanol 90 ml for 24h. After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary-Butyl alcohol as per the method used by Saas, 1940[7]. Infiltration of the specimens was carried by gradual addition of paraffin wax (melting point 58-60°C) until TBA solution obtained super saturation. The specimens were cast into paraffin blocks.

2.1.3 Sectioning of fruits

The paraffin embedded specimens were sectioned with the help of Rotary Microtome. The thicknesses of sections were 10-12 µm. Dewaxing of the sections was done by customary procedure of Johansen 1940[8]. The sections were stained with Toluidine blue as per the procedure specified by O'Brien et.al 1964[9] Since Toluidine blue is a polychromatic stain. The staining results were good along with cytochemical reactions. The dye rendered pink colour to the walls of cellulose, blue colour to lignified cells, dark green colour to suberin, violet colour to mucilage and blue colour to protein bodies. The other staining reagents were also used as per the requirement like safranin, fast green and Iodine-Potassium Iodide. Glycerin mounted preparations were made from cleared materials. For powder materials of different parts, the samples were cleared with 5% Sodium hydroxide and mounted the medium after staining. Different cell components were measured and studied.

2.1.4 Photomicrographs

Microscopic descriptions of tissues were supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon lab photo type 2 microscopic units. For normal observations, a bright field microscope was used. Polarized light was used to study various starch grains, calcium oxalate crystals and lignified cells. Since these structures have a birefringent property, under polarized light, they appear bright against dark background. Magnifications of the figures are indicated by scale-bars. Descriptive terms of the anatomical features are as given in the standard Anatomy books.[10,11]

2.2 Physico-Chemical Analysis

The powdered fruits had been subjected to various parameters which are mentioned in "Pharmacopoeia of India". All the observations were performed in triplicate and the values had been recorded.

2.2.1 Moisture Content

An accurately weighed amount of powdered fruit material was taken in a tared dish and dried at temperature of 105^0 C in an oven till a constant weight is obtained. The difference in the weight of the drug before and after drying was noted down and the moisture content in the sample was calculated[12].

2.2.2 Total Ash

An accurately weighed amount of powdered drug was taken in a tared silica crucible and incinerated by gradually increasing the heat, not exceeding dull red heat until it was free from carbon[14]. It was then allowed to cool in a desiccator. Weighed and the percentage of total ash was calculated with reference to the air-dried drug[12].

i Acid- Insoluble Ash

After obtaining the total ash of the fruits as above, the total

as was subjected for estimation of acid insoluble ash as follows:
The total ash was boiled for 5 minutes with 25ml of 0.2N HCL. The insoluble matter was collected on an ash-less filter paper, washed with hot water, dried at 110^{0}C and weighed. The weight of Insoluble matter was subtracted from the weight of ash. The difference in weight represents the acid insoluble ash. The percentage of acid insoluble ash was calculated with reference to the air-dried powder[13].

ii Water Soluble Ash

The total ash was subjected for estimation of water-soluble ash as follows: -
The total ash was boiled for 5 minutes with 25ml of distilled water. The insoluble matter was collected on an ash-less filter paper, washed with hot water, dried at 110^{0}C and weighed. The weight of Insoluble matter was subtracted from the weight of ash. The difference in weight represents the water-soluble ash. The percentage of water-soluble ash was calculated with reference to the air-dried powder[13].

2.3 Alcohol Soluble Extractive

5gm of the dried powdered fruit material was macerated with 100 ml of 95% ethyl alcohol in a stopper flask, shaking frequently. After 24hrs, it was filtered rapidly taking precautions against loss of alcohol. An aliquot of 25ml from the filtrate was taken and evaporated to dryness in a tared flat-bottomed shallow dish at 105^0 C and the weight of residue was noted. The % of alcohol soluble extractive was calculated with reference to the air- dried drug[13].

2.4 Water Soluble Extractive

5 g of the air powdered material of the fruit was subjected to water soluble extractive value determination in the same manner as described in case of alcohol soluble extractive using chloroform water instead of 95% alcohol[13].

2.5 Successive Solvent Extraction

The fruits were grinded to fine powder and sieved to get uniform particle size which is used for extraction with various solvents in increasing order of polarity viz. Pet.ether, benzene, chloroform, acetone, ethanol and chloroform water[14].

2.6 Qualitative Chemical Tests

The individual extracts after successive solvent extraction were then subjected to a qualitative chemical test to find out the presence of various phytoconstituents. Steroidal glycosides were detected by Legal's test, carbohydrates by Molischs test, and sterols by Liebermann Burchard test, alkaloids by Mayer's reagent, Wagners and Dragendorff's reagent, phenols by lead acetate test, flavonoids by Shinoda test and saponins by Foam test[15,16]

3. RESULTS

3.1 Pharmacognostic Studies

3.1.1 Macroscopic studies

Fruit: The *Cordia Obliqua* fruit is round, clean, sweetish and reddish in colour (Table 1). The fruit is spherical, smooth and reddish. The fruit has a brief, small conical continual base facet. The sepal is round, extensive, and a lobed cup formed. The fruit is 1cm in diameter. (Figure 1) and 3-5 cm in length. After ripening of fruit, it turns from yellowish to brown or red or nearly too black colour after complete repining. The fruits are shiny with minutely rogues, endocarp is very tough, and with sweetish viscid and apparent pulpy.[3] Epicarp of the fruit has thick mesocarp and is mucilaginous. St:Stigma, Pe:Pedicle, CC:Capsularcaly. The seed is tough and stony and its miles embedded in the viscous mucilage. The stone is observed in the fruit, and its miles are 8.5 × 7 mm. During this period, the quantity is 298 micro liters and the weight is 375 mg. Each stone is composed of two seeds, which can be separated by a stone diaphragm. The seeds have a slight sweet taste.

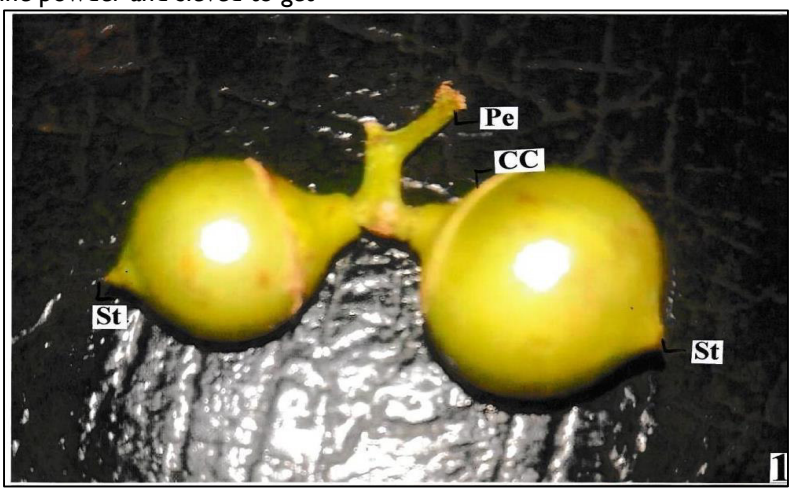

Fig 1: Fruit of *Cordia Obliqua*

Table 1: Morphological observation of *Cordia* fruit:

Sr. no	Colour	Taste	Odour
1.	Reddish	Sweet	Characteristic

3.1.2. Anatomy of Fruit

The seed consists of thick tough epicarp and mucilaginous mesocarp[17]. The epicarp includes a thick wavy epidermal layer and thick cuticle (Figure 2). The epidermis is a zone consisting of big elliptical sclereids. The sclereids are elongated, thick walled with canal-like easy pits and huge lumen. (Figure 3). Below the region of sclereids, arise many small clusters of fibers. Innermost component occurs as a horizontal elongated thick segment of xylem elements. (Figure 4). The internal maximum part of the fruit takes place as a dense mass of incredibly viscous sticky mucilage and fibers (Figure 5). Inside the mucilage, many massive spherical calcium oxalate crystals were present. (Figure 6).

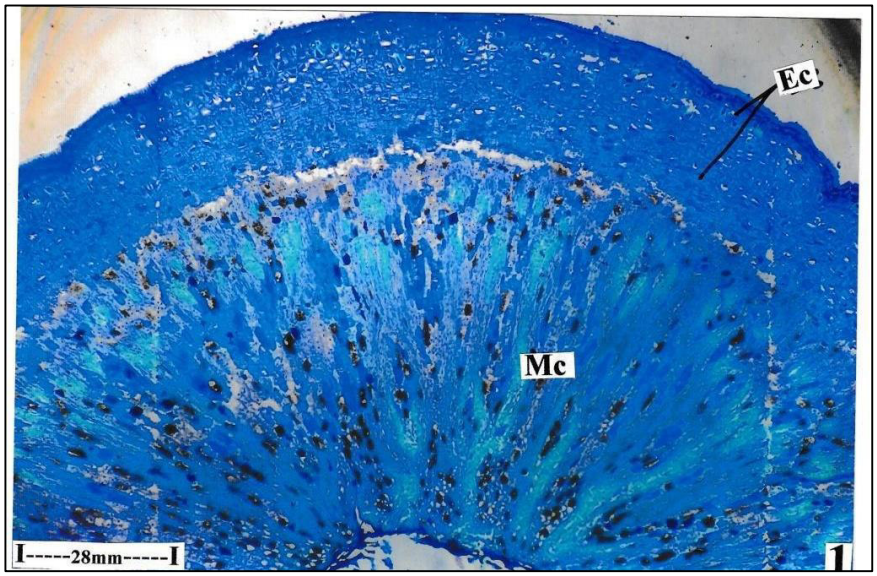

Fig 2: Cross sectional enlarged view of epicarp (Ec) and mucilaginous mesocarp (Mc)

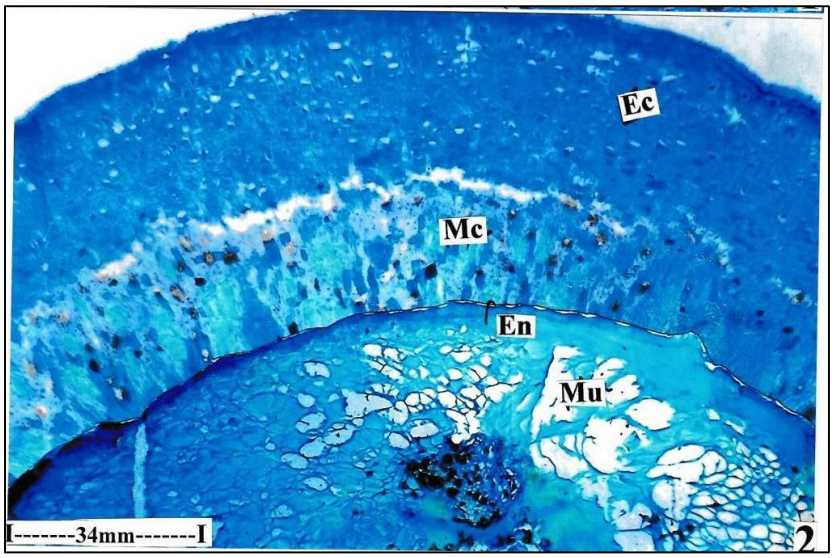

Fig 3: Cross sectional enlarged view seed coat is displaying epicarp(Ec), mesocarp (Mc), endodermis(En) and mucilage(Mu).

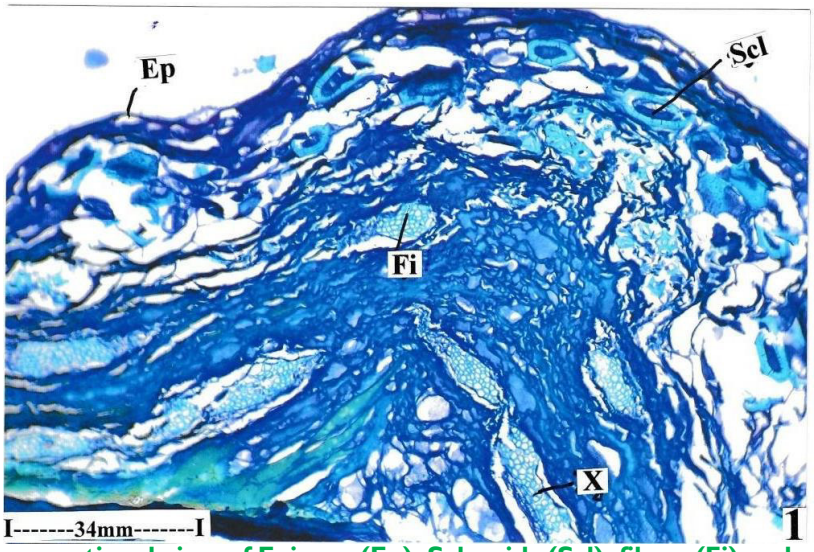

Fig 4: Cross sectional view of Epicarp (Ep), Sclereids (Scl), fibers (Fi) and xylem (X)

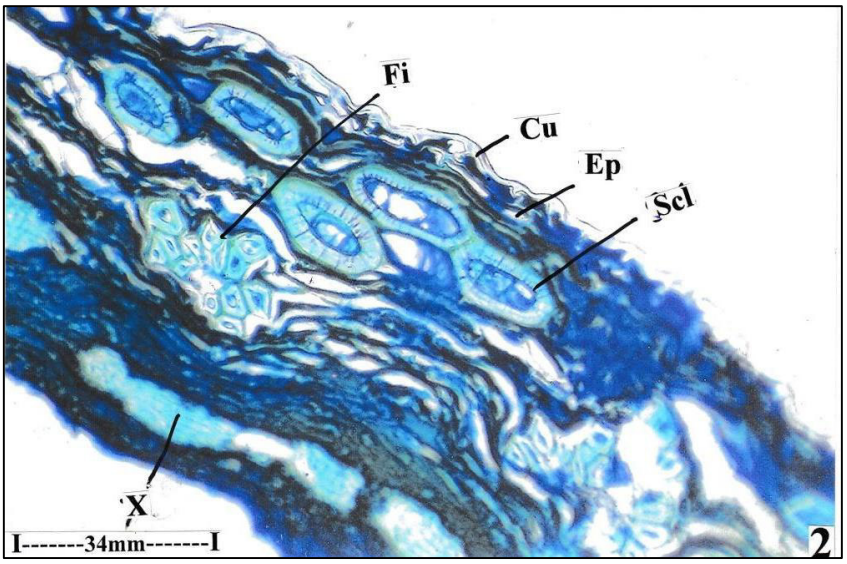

Fig 5: View of Mucilage (Mu) and fibers(Fi), Sclereids (Scl) and xylem (X)

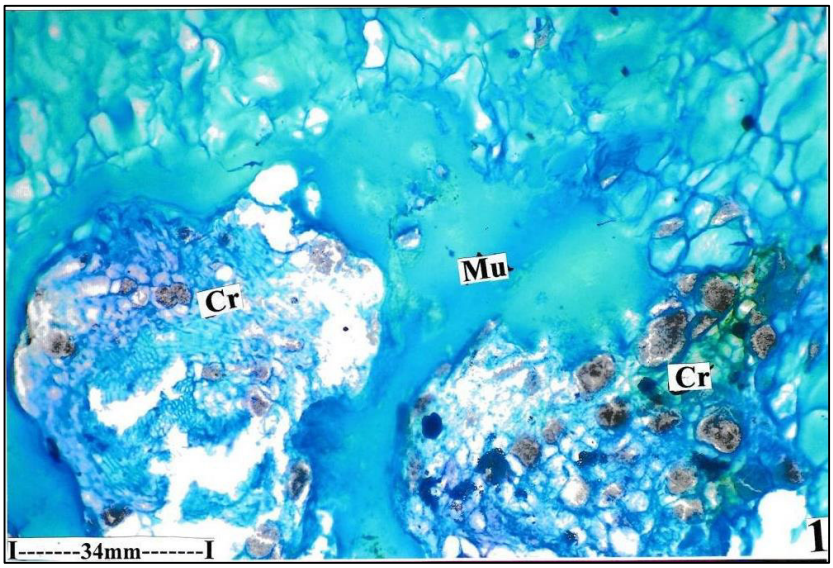

Fig 6: View of massive spherical calcium oxalate crystals (Cr)

3.1.3. *Powder Microscopy*

The powder analysis suggests the presence of elongated or long slim fibers along with abundant mucilage. The mucilage content determines the weight of the total fruit content. The fibers are very thick lignified cells with slender lumen. The fibers are 280µm lengthy and 10µm thick. (Figure 7 & 7A). The mucilage occurs as a dense, quite viscous substance. Sometimes mucilage takes place as numerous parallel bands or present as lumpy masses distributed throughout the fruits and appears as pink to red masses.

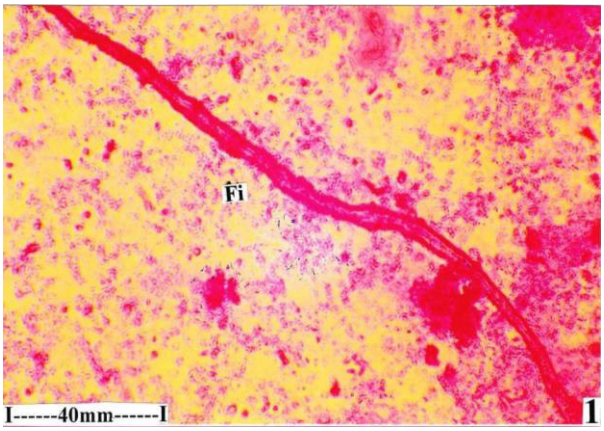

Fig 7: Fibers (Fi) in powder analysis

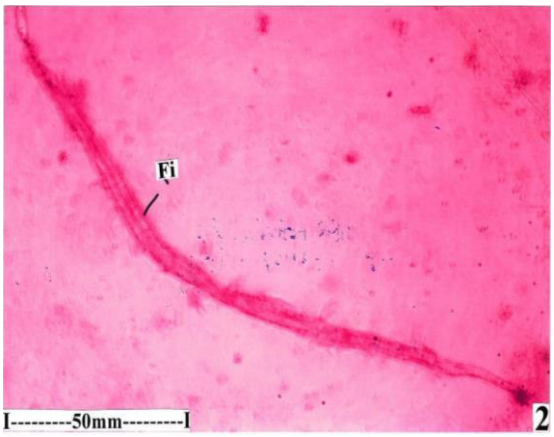

Fig 7A: Fibers (Fi) in powder analysis

3.2 Epidermal cells

In surface view, the epidermal cells of the epicarp and mesocarp seems as horizontally elongated with square compact and thick-walled cells Figure 8 and 9. The cells are compact and lumen is empty. The vessel elements are frequently visible in powder. The element is cylindrical, alongside wide indirect give up wall perforation. The lateral walls have dense round bordered pits. The vessel factors are 70µm long and 8um wide.

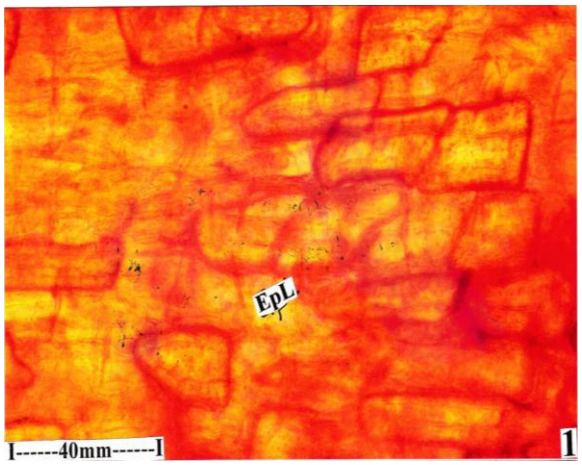

Fig 8: Epidermal cells (EpL) of the epicarp

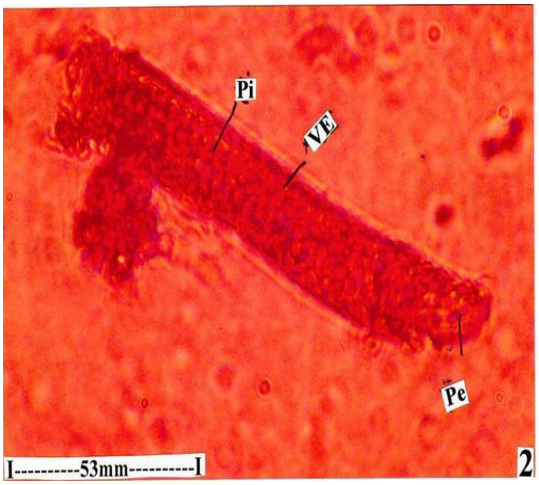

Fig 9: Pits (Pi), Vessel Elements (VE), Perforation (Pe) in isolated Vessel element

3.3 Physico chemical analysis:

The total ash values of the fruit are determined and given in percentages. Water soluble ash values, which indicates the presence of cellulosic substances. Acid insoluble ash values for fruit indicates the presence of silicacious substances. Similarly, air-dried material of fruit showed moderate values of loss on dying (LOD). Therefore, care should be taken while storing the fruits. Various solvents like ethanol and chloroform water are used to determine the extractive values, which are important quality control parameters for herbal drugs. As expected, the extractive values were more with polar solvents. Different values received from the physico chemical analysis of the fruits are recorded in Table 2.

Table-2: Physico chemical analysis of Fruits of *Cordia Obliqua*.

Sr. no	Determination	Percentage w/w
1.	Total ash	8.6%
2.	Acid insoluble ash	1.5%
3.	Water soluble ash	3.6%
4.	Alcohol soluble extractive	5.8%
5.	Water soluble extractive	6.2%
6.	Loss on drying	3.9%

3.4 Preliminary Phyto profile of Fruits of *Cordia Obliqua*

The fruit extracts of *Cordia Obliqua* received after the successive solvent extraction were identified for their percentage of yield, colour and consistency which are enumerated in Table 3. The extractive values were more with polar solvents e.g., ethanol and water and extractive value decreased as the polarity decreases. In the non polar solvents Petroleum ether has shown moderate extractive value when compared with benzene, acetone and chloroform.

Table 3: Preliminary Phyto profile of Fruits of *Cordia Obliqua*

Sr. no	Solvent	Colour and consistency	Average extractive value % w/w
1.	Petroleum ether	Yellowish green solid	2.12%
2.	Benzene	Green and solid	0.36%
3.	Acetone	Light yellow and solid	0.15%
4.	Chloroform	Greenish and sticky	0.10%
5.	Ethanol	Greenish and solid	5.17%
6.	Chloroform water	Brownish and viscous	27.1%

3.5 Qualitative Chemical Tests

The extracts after successive solvent extraction were subjected to qualitative chemical tests to identify the presence of diverse phytoconstituents. More than one test was employed in the case of alkaloids, tannins, saponins and steroids. The results from table 4 shows that the fruit extracts contain steroidal glycosides, carbohydrates, gums, mucilage, proteins, alkaloids, tannins, saponins, flavanoids and phenols.

Table 4: Phytochemical analysis summary

Sl.No	Phytochemical Tests	Petroleum Ether	Benzene	Chloroform	Acetone	Ethanol	Water
1	Carbohydrates	-	-	+	+	+	+
2	Gums	-	-	-	-	-	+

3	Mucilage	-	-	-	-	-	+
4	Proteins	-	-	-	-	-	-
5	Amino acids	-	-	-	-	-	+
6	Alkaloid	-	-	-	-	+	-
7	Steroids	+	+	+	+	-	-
8	Cardiac Glycosides	+	+	+	+	-	-
9	Saponins	-	-	-	-	+	+
10	Flavonoids	-	-	-	-	+	+
11	Phenolic	-	-	-	-	+	+
12	Tannins	-	-	-	-	+	+

*+ =*Presence* *- = *Absence*

4. DISCUSSION

This study is an attempt to establish, the diagnostic characteristics of *Cordia obliqua* fruits. These results can be employed as suitable quality control measures to ensure the quality, safety and efficacy of this herbal drug material. The parameters studied here are useful to identify and authenticate the traditionally important medicinal plant *Cordia obliqua willd* fruits and this will be helpful in the preparation of herbal monographs and pharmacopoeial standards as emphasized by WHO[18,19]. Because the safety and efficacy are the ultimate goals, to ensure the reproducible quality of the herbal drugs, the exact identification and quality assurance of the raw material is essential[20]. The application of pharmacognostic protocols such as macro morphology, micro morphology, organoleptic tests, ash value and histochemical studies will help in identifying genuine drugs because these tests result in specific results for a particular drug. The macroscopic and microscopical studies along with preliminary chemical tests of Cordia obliqua fruits helped to establish standards for the identification & authentication[21]. Microscopy also plays an important role in drug identification. The microscopic examination helped to identify the various diagnostic features present in the plant either as a whole or in powder form. Microscopic characteristics discovered the presence of epicarp, mesocarp, endodermis, mucilage, vascular bundles, form of calcium oxalate crystals and presence of elliptical sclereids. The importance of epidermal characters, vascular bundles and calcium oxalate crystals in general, are widely recognized in taxonomic considerations and in many cases these are successfully used in the identification of taxa at genus as well as species levels[22,23]. The physical parameters are almost constant for a plant therefore these are helpful in setting standards for a crude drug. Various physicochemical parameters were evaluated for the fruit parts as mentioned in WHO guidelines. These parameters are important for detection of drug adulteration or improper handling of raw materials[24,25]. Physico chemical analysis was performed which gave statistics concerning the Ash values, moisture content, extractive values. Ash value suggests the presence of inorganic salts which offers a concept about the fine and purity of a drug. The total ash value is also important for detection of metal, salts, and silica[26].Moisture content material was used to know the presence of water or moisture which shows the drying value of the plant. Moisture content influences the physical and chemical properties of the crude drug which includes weight, density, viscosity and chances of microbial contamination of crude drugs. The moisture content of crude drug is directly related to its stability and consequently with the shelf life of crude drug[27]. Extractive values give the records at the solubility of the plant part; this solubility represents the active constituents of plant material, that's critical for the pharmacological activity[28]. Extractive Value determines the quality and purity of the crude drugs and the quality and purity of the crude drugs determine the pharmacological action. Successive solvent extraction of the Fruit part of the plant helped to identify the solubility of various phytoconstituents in different solvents and all the extracts were tested for the presence of various plant constituents[29,30]. The quantity of extracts determines the solubility pattern of phytochemicals in solvents which helps in choosing the specific solvents for future extraction in large scale for isolation of phytoconstituents. Identification of the different classes of phytochemical constituents of the plant is an important parameter, which gives an indication of the pharmacological active metabolites present in the plant[31,32]. The qualitative chemical assessments of various extracts display the presence of carbohydrates, steroidal glycosides, alkaloids, tannins, phenols and saponins. The pharmacognostic study of the *Cordia obliqua* fruits has been carried out for the first time.

5. ACKNOWLEDGMENTS

The authors acknowledge Prof.P. Jayaraman, Director, Plant Anatomy Research Center (PARC), West Tambaram, Chennai and Tamilnadu for providing research work.

6. AUTHOR CONTRIBUTION STATEMENT

Dr.S.Sivakrishnan has conceptualized and designed the study which helped in gathering the necessary data. All authors read and discussed the methodology and results and contributed their valuable suggestions in final version of manuscript

7. CONFLICT OF INTEREST

Conflict of interest declared none.

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