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Investigation & study of Pharmacognostical and phytochemical features of leaves of *Abrus precatorius. Linn* (Leguminosae) An unexplored medicinal plant of India

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ABSTRACT

Abrus precatorius.Linn is a small diaceous creeper which belongs to the family Leguminosae and is distributed in through India. It is usually found on surroundings. The whole plant is medicinally important and many reports on claims to cure several diseases in traditional system of medicine folklore in particularly. Therefore in this background meticulous pharmacognostic study of leaves has been carried to establish the pharmacognostical standards. The parameters selected were microscopical studies, proximate analysis, fluorescence analysis, behavior of powder drug with different chemical reagents and preliminary phytochemical screening. In physico-chemical evaluation the ash values and extractive values were studied. Fluorescence analysis performed showed the wide range of fluorescence colors for the crude powder as well as the extracts. Behavior of powder drug with different chemical reagents showed the different colors. The powder of Abrus precatorius.Linn was successively extracted with petroleum ether, benzene, chloroform, ethanol and water. In the ethanol was the identification of best solvent. Preliminary phytochemical screening was carried out for ethanol extract give maximum chemical constituents and percentage yield were observed. Phytochemical test like preliminary phytochemical analysis and different chemical behavior analysis were performed. Through chemical test presence Alkaloids, flavonoids, steroids, cardiac glycosides, phenols and tannins were revealed. From the chemical behavior analysis presence of Quercetin, Gallic acid and its derivatives were identified.

Keywords: *Abrus precatorius*, Fluorescence analysis, chemical behavior analysis, Flavonoids, phenols.

INTRODUCTION

Abrus precatorius.L is a twining herb with delicate feathery leaves, climbing shrub, with greenish yellow branches. Leaves 5-17 compounds, leaflets obovate or oblong; Flowers are crowded racemes, sub sessile, pale purple to yellowish growing at the end of a stalk. Fruits are short pods containing hard, shiny, scarlet and black seeds. The seeds are slightly smaller than ordinary peas; ovoid scarlet with a black spot round the hilum. The root is woody, tortuous and much branched, with a sweet taste, rather like liquorice. Abrus precatorius is a slender, perennial, much branched, perennial climber that twines around trees, shrubs, deciduous, woody, prickly twinning herbaceous. [1-12]

The fruit, which is a pod, is flat, oblong and truncate-shaped with a sharp deflexed beak is about 3 to 4.5 cm long, 1.2 cm wide, and silky-textured. The pod curls back when opened to reveal pendulous seeds. Each fruit contains

from 3 to 5 oval-shaped seeds, about 0.6 cm. They are usually bright scarlet in colour with a smooth, glossy texture, and a black patch on top.

Distribution:
It grows in tropical climates such as India, Sri Lanka, Thailand, the Philippine Islands, South China, tropical Africa and the West Indies. It also grows in all tropical or subtropical areas.

Habitat:
Abrus precatorius is a wild plant that grows best in fairly dry regions at low elevations. Abrus precatorius commonly known as Crabs eye, Abrus seed, Indian bead and lucky bean.

Diagnosis:
Diagnosis is made by the presence of the typical manifestations following ingestion: gastroenteritis with risk of dehydration, haematemesis and melaena. Drowsiness and convulsions may occur.

Figure:1 Abrus precatorius.Linn

CHEMICAL CONSTITUENTS:
Reported chemical constituents are *Abrus precatorius Abrine: Leaf, Abrasine: Root, Abrectorin, Abridin, Abrin, Abrin A, Abrin B, Abrin C, Abrin D.*

USES:[10-13]
The bright red seeds of A. precatorius are strung as jewellery. The seeds of Abrus precatorius are much valued in native jewelry for their bright coloration. Most beans are black and red, suggesting a ladybug, though other colors are available. Jewelry-making with jequirity seeds is dangerous, and there have been cases of death by a finger-prick while boring the seeds for beadwork.

Antipruritic, Anti-inflammatory, Analgesic, Antitumour, Antipyretic, Antiplasmodia, Abortificient, Anti helmintic activity, Anti bacterial activity, Anti fungal activity, Anti viral activity, CNS depressant activity, Diuretic activity, Anti Convulsant activity, Anti diarrheal activity, Anti estrogenic activity, Anti fertility activity and Hypoglycemic activity.

MATERIALS AND METHODS

Collection and authentication:
Plant was collected in the Kothapally (Havali) regions of Karimnagar Distict, Telangana. By final year students of CVM College of Pharmacy.

It was authenticated by Dr.Thirunahsari Ugendhar (B.ED, M.Sc, Ph.D, Assistant professor Depart of BOTANY. SRR Govt. DEGREE & PG COLLEGE, KARIMNAGAR. The plant herbarium was prepared and deposited in the Department of Pharmacognosy in CVM College of pharmacy for further reference. The plant was identified as *Abrus precatorius.Linn* (Leguminosae) and was certified under Voucher No: CVMP-KMNR/ph'cog/2014-15/04.

Chemicals and reagents: [3, 7]
All the chemicals and reagents like Phloroglucinol, hydrochloric acid, nitric acid, potassium hydroxide, picric acid, lead acetate etc used were of analytical grade.

Instruments used:
Silica crucible, Stage micrometer, Eye piece micrometer & Soxhlate apparatus.

MICROSCOPICAL STUDIES: [3-6]
Transverse section of leaf: [12]
Section cutting done by with help of blade for fresh leaf 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.

Transverse section of Stem: [12]
Section cutting done by with help of blade for fresh leaf 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.

Determination of physico-chemical properties: [6,7]
Total ash, acid insoluble ash, water soluble ash and sulphated ash of *Abrus precatorius.Linn*., was determined by standard method and the results are tabulated in table. The crude fibre content, moisture content, alcohol soluble extractive value, water soluble extractive values were determined by standard method and the results obtained are tabulated in table.

Powder Microscopy [8, 17]
Shade dried leafs 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 leafs, was taken in watch glass and stained with quantities of phloroglucinol and hydrochloric acid was taken in the watch glass. Slide was prepared with help of a brush. Focused under microscope.

Determination of Fluorescence analysis[7, 8]
Powdered leaf was subjected to analysis under ultra violet light after treatment with various chemical and organic reagents.

Successive Solvent Extraction: [7]
The powdered leaf material was subjected to soxhlet extraction using petroleum ether, benzene chloroform, ethanol and water by successive solvent extraction method based on the increasing order of polarity of solvent. All the extracts were subjected to preliminary chemical tests.

Preliminary chemical screening: [7]
The extract obtained was subjected to various chemical tests as per the procedure mentioned in the standard reference books.

RESULTS

TRANSVERSE SECTION OF LEAF:[10 – 12, 19]
The transverse section of dorsiventral leaf (dicot leaf) shows three main parts called 1. Epidermis 2. Mesophyl, 3. Vascular bundles, as shown in the figure 2.

A thin T.S of young dicot leaf, when examined under the microscope, shows the following regions from outside to inside.

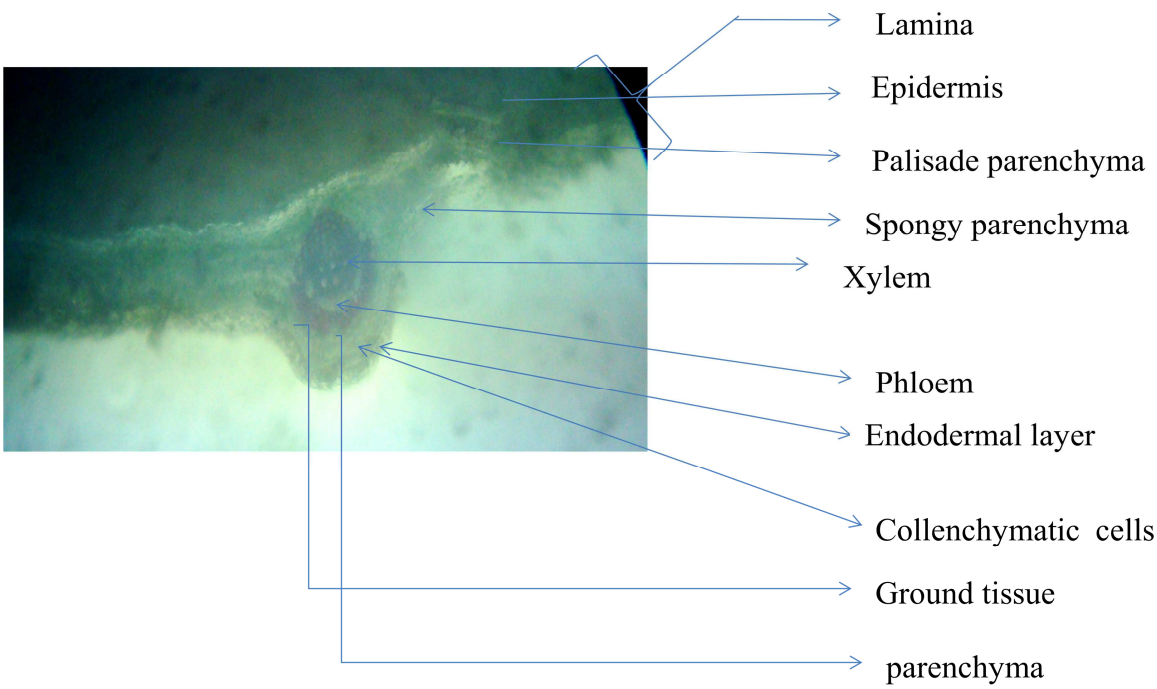

Figure: 2: T.S of Leaf

1. **EPIDERMIS:** It is the outer most layer. It was present on both sides of the leaf, that is upper epidermis and lower epidermis.

The upper epidermis covers the upper surface of a dorsiventral leaf. it consist of closely arranged single layer of cells , and was covered externally by a layer of cuticle. The upper epidermis is a continuous layer and lacks stomata. Trichomes are absent in both upper and lower epidermis.

2. **MESOPHYLL:** The region between the upper and lower epidermis of leaf blade constituted by mesophyll. Beanth the upper epidermis palisade parenchymatous cells were present in a single layer and loosely arranged. The lower part of the mesophyll consist of chlorenchymatous, spangeparenchymatous cells mid rib portion occupied by the ground tissue.

3. **VASCULAR BUNDLE:** This is consisting of xylem and phloem and they were found to be open type and cork shape. Xylem is triach type. In mid rib upper surface, the palisade are interrupted by collenchymatous cells. The collenchymatous cells in 3-4 rows are present above the lower epidermis in mid rib region.

TRANSVERSE SECTION OF STEM: [09]
A thin T.S of young dicot stem, when examined under the microscope, shows the following regions from inside to outside.

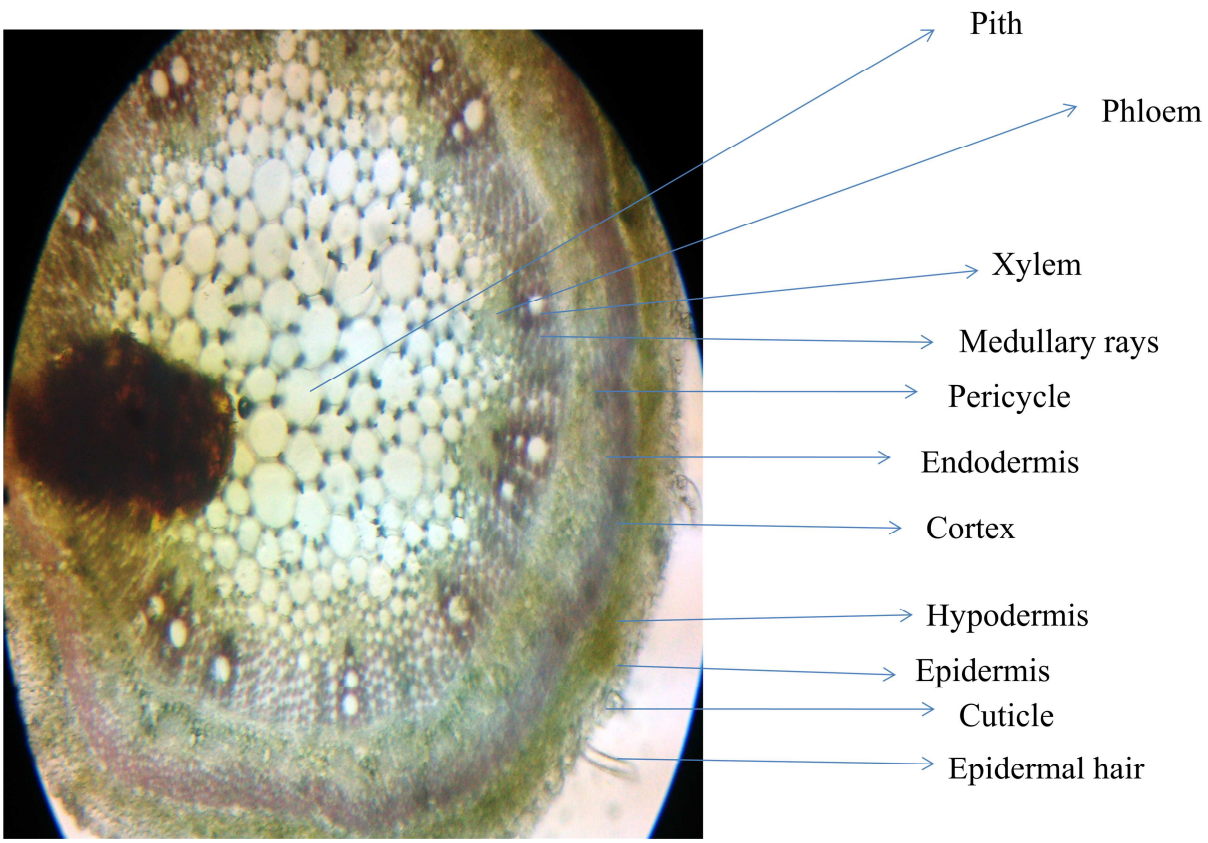

Figure:3: T.S of Stem

1. EPIDERMIS: It is the outermost region of the stem and is formed of a single layer of rectangular cells. The outer surface of the epidermis is covered by a layer of cuticle. The epidermis may produce a few unicellular stem hairs.

2. HYPODERMIS:- The hypodermis lies just below the epidermis and consist of a few layers of collenchymatous cells. In young stem, the collenchyma contains chloroplast.

3. CORTEX:- It is the region next to the hypodermis and is formed of thin walled parenchymatous cells arranged in single layer

4. ENDODERMIS:- It is a wavy layer of barrel shaped cells end is the innermost layer of the cortex.The cells of endodermis are thickened at their radial walls.

5. PERICYCLE:- It lies inside the endodermis and is formed of several layers of cells.The pericycle is distinguished in to alternately occurring sclerenchymatous and parenchymatous region, the former situated outside the vascular bundles and the latter in between them. The sclerenchymatous regions of the pericycle provide mechanical support to the vascular region.

6. VASCULAR BUNDLES: The vascular bundles in stem are wedge shaped in T.S they are arranged in a ring just inside the pericycle. Each bundle consists of phloem on the outside and xylem on the inner side, both lying on the same radius. such vascular bundles are called conjoint and collateral. Present between the xylem and phloem is a meristematic strip called intrafaciular cambium. It consists of 4-5 layers of cells.The vascular bundles having cambium between xylem and phloem are called “open”.

The protoxylem that is the xylem formed earlier lies towards the centre while the metaxylem i.e, the later formed xylem is towards the periphery. The condition is called endarch. Pholem consists of phloem parenchyma and phloem fibres. Xylem consists of xylem parenchyma, vessels, and xylem fibers. All elements are lignified.

7. **MEDULLARY RAYS:** The region between the vascular bundles is called medullary rays. They are formed of radially arranged thin walled parenchymatous cells. The medullary rays are concered with radial conduction of food and water.

8. PITH: - It constitutes the central region of the stem and is composed of loosely arranged thin walled parenchymatous cells. Pith stores food in its cells.

POWDER MICROSCOPY: [8]
Powder microscopy was done according to the standard procedure mentioned.

Table 1: quantitative microscopy of leaf

Parameter	Length	Width
Phloem fibres	100- 175-250 µ	12.5-25-37.5 µ
Trichomes	75-125-175 µ	12.5-18.75-25µ
Xylem vessels	25-50-87.5 µ	12.5-25-37.5 µ
Starch grains	50-100-175 µ	12.5-18.75-25 µ

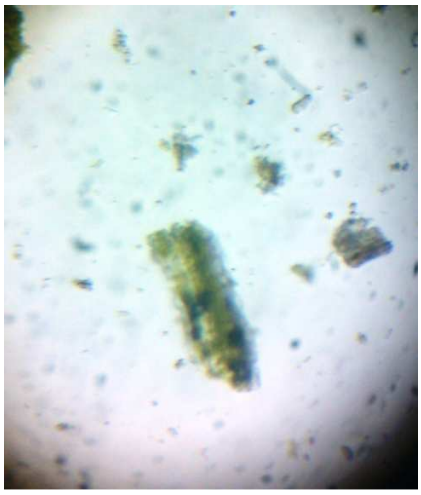

Figure: 4: EPIDERMIS

Figure: 5: XYLEM VESSELS

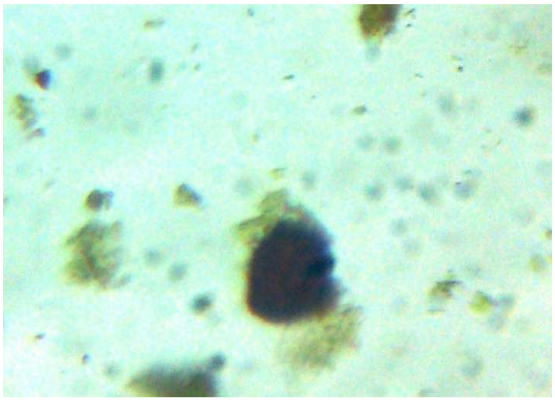

Figure:6:STARCH GRAINS

Figure:7:UNICELLULAR COVERING TRICHOMES

Figure: 8: PHLOEM FIBERS

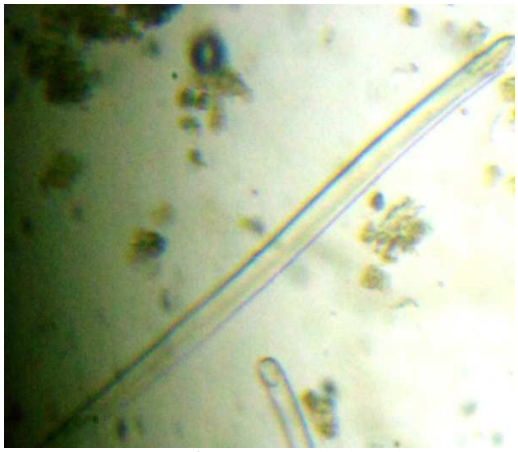

Figure: 9: TRICHOMES

Powder microscopy was done according to the standard procedure mentioned.

DETERMINATION OF LEAF CONSTANTS: [14,15]
Leaf constants like stomatal index, vein islet number, vein islet termination and palisade ratio were determined according to the standard procedure.

Leaf constants aid to determine the adulteration and substitution of the drug, because these parameters were fixed to the particular plant.

Table 2 measurements of different leaf cellular components:

Parameter	**value**
Stomatal number Upper surface Lower surface	 7-8 11- 13
Stomatal index upper lower	 17.5-19.4-21.5 11.5-13.5-15.5
Vein islet number	12-14-16
Vein islet termination	21-26-28
Palisade ratio	20-22-23

Leaf Constants

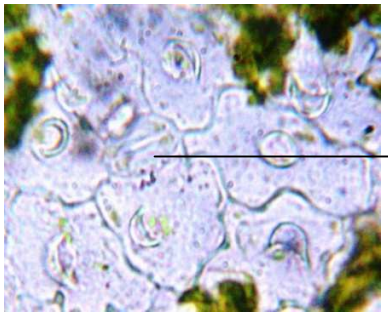

Figure 10 Stomata (x45)

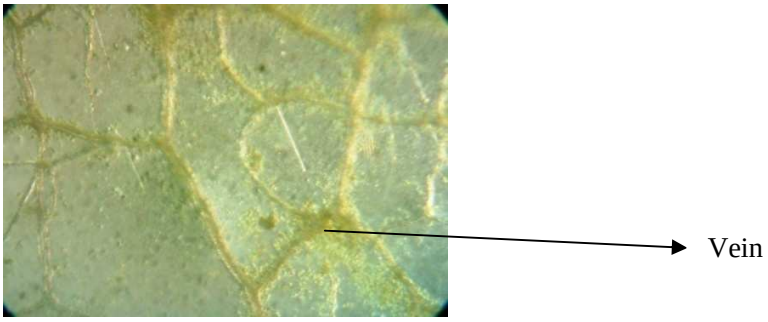

Figure 11: Vein islet

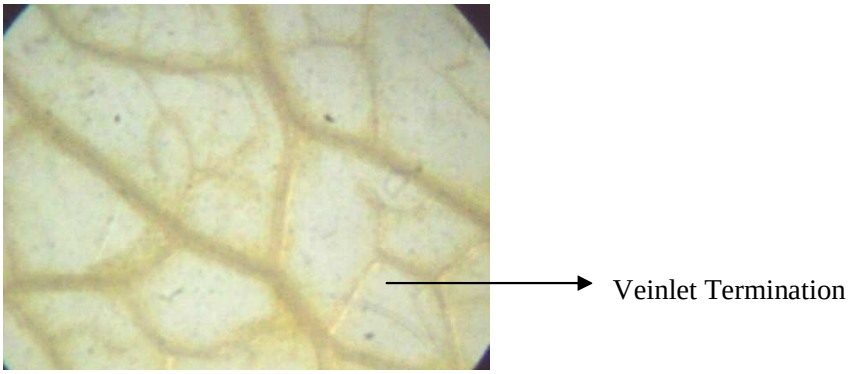

Figure 12: Veinlet Termination

PROXIMATE ANALYSIS:

Proximate Analysis of Leaf: [17, 18]

Proximate analysis of *Abrus precatorius.Linn leaves* were determined by standard method and the results were tabulated in

Table 3: Measurements of different proximate values of leaf:

S.NO	Parameters	% yield
1	Total ash	25 % w/w
2	Acid insoluble ash	64 % w/w
3	Water soluble ash	43% w/w
4	Sulphated ash	71 % w/w
5	Loss on drying	9.8 % w/w
6	Crude fiber content	25 % w/w
7	Foreign Organic Matter	4 % w/w
EXTRACTIVE VALUES		
8	Water soluble	4 % w/w
9	Alcohol soluble	8.5% w/w
10	Moisture content	1.5% w/w

Table No:4: Extractive values

S.NO	Parameters	% yield
1	Alcohol soluble extractive	15.2% w/w
2	Water soluble extractive	40.8% w/w

MAJOR EXTRACTION:

The 50 gms of powdered plant material was extracted with ethanol and water in maceration and a soxhlet apparatus for 2 4 hrs. The extract was evaporated with help of vacuum rotary evaporator to obtain a thick mass.

Alcoholic extract by Soxhlation

Figure: 13: Soxhlation apparatus

Table 5: Flourescence study

Reagent	Long (365nm)	Short (256nm)	Day
50% H_2SO_4	Light brown	Light Green	brown
50%HNO_3	Green	Light Green	Green
5% NaOH	Green	Green	Green
1N Me NaOH	Green	Dark Green	Green
1N KOH	Thick Green	Green	Green
5% KOH	Green	Green	Green
5%$FeCl_3$	Light black	Green	Green
Methanol	Green	Green	Green
Conc HCl	Thick Green	Light Green	Light green
Conc H_2SO_4	Black	Light green	Black/Brown
Ammonia	Thick Green	Light green	Green
Conc HNO_3	Thick green	Green	Brown

FLUORESCENCE ANALYSIS:
Powdered leaves were subjected to analysis under ultra violet light after treatment with various chemical and organic reagents. The findings were tabulated in the Table No:5.

Fluorescence Analysis of Leaf with different chemical reagents:

SUCCESSIVE SOLVENT EXTRACTION:
The leaf powdered material was subjected to soxhlation using alcoholic (ethanol) by successive solvent extraction method.

Initially 25gm of crude powder was taken and packed in a packing paper. This pack was placed in a soxhlet extractor for 24 hrs (approx) with different solvents i.e. . (Alcoholic - ethanol) and the temperature was adjusted as per the solvent been used in the extraction. The percentage yield and physical characteristics of extracts obtained was calculated and reported in the following.

Table: 6 successive solvent extraction, consistency, colour, fluorescence character and percentage yields of different solvents

Parameters	Extracts				
	Pet.Ether	Benzene	Chloroform	Ethanol	Water
Consistency		sticky	viscous	Viscous	Waxy
Colour (Day light)	Green	Thick green	Green	Reddish black	Cream
Percentage yield	2.4%	1.8%	3.2%	4.28%	3.24%

PRELIMINARY PHYTOCHEMICAL ANALYSIS:

Powdered drug was subjected to successive solvent extraction with different solvents. The obtained extracts were subjected to preliminary phytochemical screening according to the standard procedures mentioned. Findings were tabulated below.

Table 7 Preliminary Phytochemical Analysis of petroleum ether, benzene, chloroform, ethanol & water extracts: [16]

Phyto constituents	Pet. Ether	Benzene	chlChloroform	Ethanol	Water
Carbohydrates	-	+	-	+	+
Amino acids	-	-	-	-	-
Proteins	-	-	-	-	-
Alkaloids	+	+	+	+	-
Phenol's & Tannins	+	+	+	+	+
Steroids	-	-	-	+	+
Volatile oils	-	-	-	-	-
Flavonoids	-	-	-	+	+
Saponins	-	-	-	-	-
Cardiac glycosides	-	-	-	+	-

BEHAVIOR OF POWDERED DRUG WITH DIFFERENT CHEMICAL REAGENTS:

Behavior of Leaf Powder with Different Chemical Reagents: (15,16)

Powdered leaf was subjected to behavioral analysis with different reagents. The findings were tabulated in the Table: 8

Table: 8 Behavior of Leaf Powder with Different Chemical Reagents:

Reagent	Observation	Inference
powder+ iodine	Black color observed	Presence of starch
powder+ $HgCl_2$	Bule color observed (Black color)	Presence of Alkaloids
powder+ Ammonia	No pink color observed	Absence Of Cardiac Glycosides
powder+ $AgNO_3$	No ppt formed	Absence of Proteins
powder+ Picric Acid	Colour change (bluish black)	Presence of Alkaloids
powder+ Water (shaking)	Foam not appeared	Absence of Saponins
powder+ conc H_2SO_4	Black	Presence of starch
powder+$FeCl_3$	Bluish black	Presence of tannins
powder+ Conc HNO_3	Orange yellow (orange brown)	Presence of tannins

CONCLUSION

In this current study a complete pharmacognostic study was done along with the preliminary phytochemical studies of its traditional claims.

The main Tissue of Diagnostic Importance (TDI) of *Abrus precatorius* was found to be Unicellular covering trichomes. The determination of leaf constants, qualitative physical and chemical analysis was done.

In conclusion, the present study on pharmacognostical characters of *Abrus precatorius Linn* leaf will be providing useful information in regard to its correct identity and help to differentiate from the closely related other species of *Abrus*. The other parameters observed may be useful for the future identification of the plant and animal work carried to which chemical constituents was responsible for screening activity.

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