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PHARMACOGNOSTICAL STUDIES AND CHROMATOGRAPHIC EVALUATION OF THE DIFFERENT EXTRACTS OF *ABRUS PRECATORIOUS* LINN.

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ABSTRACT
Abrus precatorius L. is the common folk medicinal plant found widely in the West Bengal. To maintain the quality of the herbal products, proper control of starting material is almost essential. The first step to ensuring quality of starting material is authentication. Despite the modern techniques, identification of plant drugs by pharmacognostic studies is more reliable. In the Indian System of Medicine, the seeds are used for sciatica, paralysis, headache, dysentery, diarrhoea, leprosy, ulcer, nervous disorders, alopecia, as well as anti inflammatory, antidiabetic, antibacterial, antitumor, sexual stimulant and abortifacient. Seeds are poisonous which contain the protein abrin and therefore are used after mitigation. Abrus precatorius Linn. (Family-Papilionoideae), commonly known as Indian Liquorice. In present study, fluorescence analysis, behavior of the powder with various chemical reagents, chromatographic & phytochemical studies of alcohol-water mixture and petroleum ether, chloroform, ethanol extract of Abrus precatorius Linn seed. These studies will provide referential information for identification of this important crude drug.

Keywords *Abrus precatorious Linn. Seeds, Pharmacognosy, Phytochemical evaluation, Fluorescence analysis.*

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INTRODUCTION

India is one of the largest producers of herbal products. Due to increasing demand in the field of herbal medicine it has become necessary and pertinent to probe into the area of systematic knowledge about herbal drugs[1]. Herbal medicines are prepared from variety of plant parts like leaves, stem, roots, barks, seeds, flowers and so on. *Abrus precatorious* Linn. is a woody twinning plant of the Papilionoideae family with characteristic red seeds

with black mark at the base[2]. The seeds are highly toxic such that they are often ingested as a means of suicide in India[3]. They are sometimes made into necklaces. *Abrus precatorius* Linn., a highly medicinal plant, commonly known as "Indian Liquorice, rosary pea, crab's eye, and jequirity", in English *Gunchi, Ratti*- Hindi; Kannada- *Gulganji*; Malayalam- *Kumi*; Sanskrit- *Rektika Gunja* and in Tamil as *Kuntumani.* The seed pod curls back when it opens and reveals the seeds. The seeds are flat and truncate shaped, 1.5 – 2 cm long, with attractive scarlet colour. It is a medicinal herb used for various diseases. The roots, leaves and seeds are used medicinally. The roots and leaves contain glycyrrhizin, the principle constituent of liquorice, and are used as a substitute for liquorice in coughs and catarrhal infections hence the plant known as Indian Liquorice. In India, hot water extract of dried leaves and roots are applied to the eye for eye diseases. In Brazil, water extract of dried leaves and root is taken orally as a nerve tonic. Seeds are acrid, bitter, astringent, purgative, emetic, tonic, antiopthalmic, and antiphlogistic, aphrodisiac, toxic, abortificient. Seeds paste applied locally for sciatica, stiffness of shoulder joints, paralysis, leucoderma, ulcers, skin diseases, wounds, alopecia, asthma, tubercular glands, stomatitis, hyperdipsia and fever. Seeds contain "poisonous protein"- a fat splitting enzyme, glucoside abrussic acid, haemagglutinin, a quantity of urease and an albuminous substance named Abrin, which is a highly toxic protein present to an extent of 0.15% in seed[4]. Medicinal plants are believed to be an important source of new chemical entities with potential therapeutic effects[5]. Plants extracts as well as their primary & secondary metabolites have important therapeutic role in the treatment of many human diseases[6, 7]. The seeds are deadly poisonous but it has been reported that the toxic form of abrin gets converted to mitogenic form, upon long refrigerated storage[8, 9]. However, cooking destroys the poison, so that the seeds may be eaten[10]. In crude drug market two types of seeds of *A. precatorius* are reported to be sold under the name of "Ratti" (Scarlet red in colour having black scar) and Ghongachi Safed (creamish white in colour with a brown scar at hilum). Although pharmacognostic studies of *A. precatorius* L. seeds, white variety (creamish white in colour with a brownish scar at hilum) has been carried out, but there is no detailed pharmacognostical work reported for *Abrus precatorius* L. seeds red variety (scarlet red in colour having black scar). The purpose of the present study was to develop pharmacognostical parameters & Chromatographic evaluation for *A. precatorius* L.

MATERIALS AND METHODS

Plant materials collection and identification

Plant material was collected from the district of Bankura, West Bengal, India. It was authenticated by Botanical Garden, Howrah- 711103 (West Bengal) field voucher no.-CNH/69/2011/Tech. II/ which has been deposited in herbarium, Botanical garden, Kolkata. Among variety colonel seed types, red forms were selected for this study. The collected seeds were claimed to remove dust by using water then dried under shade. Dried seeds were firstly crushed, then it is pulverized by a Panasonic mechanical grinder (Model No – MX – AC300S) and passed through 44 mesh sieve, 22 mesh sieve & powdered were stored in air-tight container at dark Condition.

Microscopic analysis − Some seeds were soaked in distilled water overnight after that preserved in 70% ethanol for histological studies. Transverse sections were cut using Labotech Microtome, Model No – Bo – 91, Sl. No - 32212 (B.D.Instrumentation, Ambala cant, Haryana, India). Sections were stained with light green and counterstained with safranin. For the study of powdered elements fine powder of seeds was treated with chloral hydrate for about 10 min, followed by a gentle heating, filtered and were stained with weak solution of Iodine, Phlorogucinol with HCl (1:1) & then finally mounted in glycerine for observation (Anonymous, 1998). All preparations were observed under Olympus OIC microscope, Model No - 607124. Photomicrographs were taken using Sony digital camera Model No. DSC-S650.

Solvents

Petroleum ether (60°–80°C) (Merck India Pvt Ltd, Mumbai, India); Chloroform (Pure Chemicals Works, Kolkata - 06) and Methanol (Bengal Chemical & pharmaceutical Limited, Kolkata), all of LR grade, under normal atmospheric pressure were employed for extraction of the plant material.

Recovery of solvents

Solvents from extracts were recovered under Distillation, and the dried extracts were preserved in a desicator containing fused calcium chloride (S.D. Fine Chemicals).

EXTRACTION OF PLANT MATERIAL

A- Water-alcoholic (30:70) extracts (Percolation): Coarsely powdered seed of *Abrus precatorius L.* were extracted with water-ethanol mixture (30:70) by cold extraction process (Percolator). A suspension of (100 gm) of dried course seed in (250 ml) of distilled water-ethanol mixture was kept one week at room temperature. The mixture was filtrated and the filtrate was taken to dryness and weighted. The crude extracts were then filtered and the solvent were removed until solid/semisolid mass were produced on a water bath[11].

B- Pet. Ether extracts: 100 grams of dried course powder was heated (20°C) with (500 ml) of Pet. Ether (60°–80°C) for 3 days (15 cycles), using Soxhlet Apparatus. The hot suspension extract was concentrated up to 10 ml by water bath[11]& the extracts were taken to dryness and weighed (4.4 gm).

C- Chloroform extracts: After complete extraction of Pet. Ether, the powder was again heated (20°C) with (500 ml) of Chloroform for 3 days (15 cycles), using same Soxhlet Apparatus. The hot suspension extract was concentrated up to 10 ml by water bath[11] & the extracts were taken to dryness and weighed (1.84 gm).

D- Alcohol (Methanol) extract: After complete extraction of Pet. Ether, the powder was again heated (20°C) with (500 ml) of Alcohol for 3 days (15 cycles), using same Soxhlet Apparatus. The hot suspension extract was concentrated up to 10 ml by water bath[11] & the extracts were taken to dryness and weighed (16.4 gm).

Phytochemical investigations

Morphological characteristics of seed[12], fluorescent analysis[13], histochemical color reaction[12], behavior of drug powder with different chemical reagents[14] & phytochemical analysis were carried out as per the standard methods[15, 16]. The qualitative and TLC study were carried out for the all the extracts and fractions. The chemical tests and TLC study were performed with different chemical reagents and a TLC study of extracts was carried out using silica gel G as stationary phase and ethyl acetate, formic acid, acetic acid, water (100:11:11:27, v/v) and chloroform, benzene, diethylether (2:2:0.5, v/v) as mobile phase for petroleum ether and chloroform extract and alcohol, alcohol-water fraction respectively. Spots were observed under visible light & UV – Chamber by using dragendroff's reagent, antimony pentachloride, ninhydrin etc as a detecting reagent.

The qualitative evaluation of the plate was done by determining the migrating behavior of the separated substances given in the form of R_f values[17].

PRELIMINARY PHYTOCHEMICAL GROUP TEST

The preliminary phytochemical group test of the Water-alcoholic (30:70), Pet. Ether, Chloroform & Alcohol (Methanol) extract of dried leaves of *Abrus precatorius L.* was performed by the standard methods[18, 19, 20].

A. Test for Alkaloids

Mayer's test:

Alkaloids give cream color precipitate with Mayer's reagent [Potassium mercuric iodide solution].

Dragendorff's test:

Alkaloids give reddish brown precipitate with Dragendorff's reagent [Potassium bismuth iodide solution].

Wagner's test:

Alkaloids give a reddish brown precipitate with Wagner's reagent [Solution of iodine in potassium iodide].

Hager's test:

Alkaloids give yellow color precipitate with Hager's reagent [saturated solution of Picric acid].

B. Test for Cardiac Glycoside

Keller killiani test [Test for Deoxy sugars]:

Extract the drug with chloroform and evaporate it to dryness. Add 0.4ml of glacial acetic acid containing a trace amount of ferric chloride. Transfer to a small test tube; add carefully 0.5ml of concentrated sulphuric acid by the side of the test tube, blue color appears in the acetic acid layer.

C. Test for Tannins & Phenolic Compounds

Gelatin test:

Test solution with 1 % gelatin solution containing 10% sodium Chloride gives white precipitate.

Ferric chloride test:

Test solution gives blue green color with ferric chloride.

D. Test for Flavonoids

Alkaline reagent test:

To the test solution add few drops of sodium hydroxide solution; formation of an intense yellow color, which turns to Colorless on addition of few drops of diluted acid, indicates presence of Flavonoids.

E. Test for Proteins & Amino Acids

Ninhydrin test:

Amino acids and Proteins when boiled with 0.2% solution of Ninhydrin (Indane 1, 2, 3 trione hydrate), Violet color appears.

F. Test for Steroids & Triterpenoids

Salkowski test:

Treat extract in Chloroform with few drops of concentrated Sulfuric acid, shake well and allow standing for some time, red color appears at the lower layer indicates the presence of Steroids and formation of yellow colored lower layer indicates the presence of Triterpenoids.

G. Test for Carbohydrates

Molisch's test:

Treat the test solution with few drops of alcoholic alpha napthol. Add 0.2ml of concentrated Sulfuric acid slowly through the sides of the test tube, a purple to violet color ring appears at the junction.

Fehling's test:

Equal volume of Fehling's A (Copper sulfate in distilled water) and Fehling's B (Potassium tartarate and Sodium hydroxide in distilled water) reagents are mixed and few drops of sample is added and boiled, a brick red precipitate of cuprous oxide forms, if reducing sugars are present.

H. Test for saponin glycoside

Haemolysis test:

Add 0.2ml solution of saponin (prepared in 1% normal saline) to 0.2ml of v/v blood in normal saline and mix well, centrifuge and note the red supernatant compare with control tube containing 0.2ml of 10% blood in normal saline diluted with 0.2ml of normal saline.

I. Test for fats and fixed oils

Copper sulphate test:

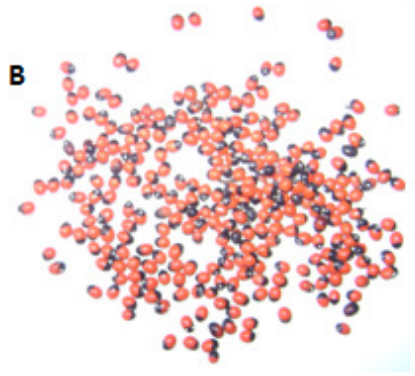
Treat 5 drops of sample with 1 ml of 1%copper sulphate solution, and then add 10% NaOH solution. A clear blue solution is obtained, which shows glycerin is present in the sample.

RESULTS & DISCUSSION

Botanical Information

The seeds are bitter in taste and posses characteristic odour. The weight of single seed of red variety is 0.098 gm.

Pic 1 – A – Twig with inflorescence in p*recatorius* red form of A. *precatorius*



B – Seeds of red form of A.

Organoleptically seeds yield course powder and color of red seeds is yellowish brown with red and black spots. The fluorescence analysis can be presented in Table 1. Behavior of the powder with different chemical reagents showed the presence of alkaloids, steroids and proteins (Table 2). Qualitative phytochemical analysis of the different extract will showed the Alkaloid, Cardiac glycoside, Tannin, Flavonoid, Proteins and amino acids, Steroids and terpenoids, carbohydrate, Fats and fixed oil are present (Table 3). These parameters will help to identify the correct species of the plant since no such data is available for the same. Adulterants, if any, can easily be identified.

TABLE AND FIGURES:

Table1 - Fluorescence analysis of seed extracts of *Abrus precatorious L.*

Extract	Day light	Ultraviolet light	
		365 nm	254 nm
Water-alcoholic (30:70) extract	Light yellow	Shiney blackish yellow	Light shiney yellow
Petroleum Ether extract	Deep yellow thick	Milkey white	Yellow(light)
Chloroform extract	White and bright yellow mixture	Colourless	Very light yellow
Alcohol extract	Brown(deep)sticky mass	blackish	Very shiney yellow brown sticky mass

Table 2 – Behaviour of seed powder with different chemical reagents of *Abrus precatorious L*

Sl. No.	**Test**	**Observation**	**Inferences**
1	Powder + Picric acid	Deep Yellow color	Presence of Alkaloids
2.	Powder + Conc. H_2SO_4	Brown Color	Presence of Steroids
3.	Powder + Aq. $FeCl_3$	Light green fluorescence	Presence of flavonoids
4.	Powder + Iodine Solution	No blue Color	Absence of Starch
5.	Powder + Ammonia Solution	No Pink Color	Absence of Anthraquinone glycoside
6.	Powder + Aqueous 5% KOH	No Blood Red Color	Absence of Anthraquinone glycoside
7.	Powder + Aqueous NaOH	No Yellow Color	Absence of Flavonoids
8.	Powder + Aqueous $AgNO_3$	White Precipitate	Presence of Proteins

Table 3 – The result of preliminary phytochemical screening of the plant extract.

Plant Constituents		Water-alcoholic (30:70) extract	Petroleum Ether extract	Chloroform extract	Alcohol extract
Alkaloid	Mayer's test	+ ve	- ve	+ ve	+ ve
	Wagner test	+ ve	- ve	+ ve	+ ve
	Dragendroff's test	+ ve	- ve	+ ve	- ve
	Hagar's test	- ve	- ve	- ve	- ve
Cardiac Glycoside	Kellar killiani test	+ ve	- ve	- ve	- ve
	Gelatin test	- ve	- ve	+ ve	- ve

Tannin	Ferric chloride test	+ ve	- ve	+ ve	- ve
Flavonoid	Alkaline reagent test	+ ve	+ ve	+ ve	- ve
Protein & Amino acids	Ninhydrine test	- ve	- ve	- ve	- ve
Steroids & terpenoids	Salkowski test	+ ve	+ ve	- ve	- ve
Carbohydrate	Molish test	+ ve	+ ve	- ve	- ve
	Fellings test	+ ve	+ ve	- ve	- ve
Fats & Fixed Oil	Copper sulphate test	- ve	+ ve	- ve	- ve
Saponin Glycoside	Haemolysis test	- ve	- ve	- ve	- ve

+ Ve indicate **Present** and – Ve indicate **Absent**

Figure 1. Chromatogram of *Tylophora indica* by GC-MS.

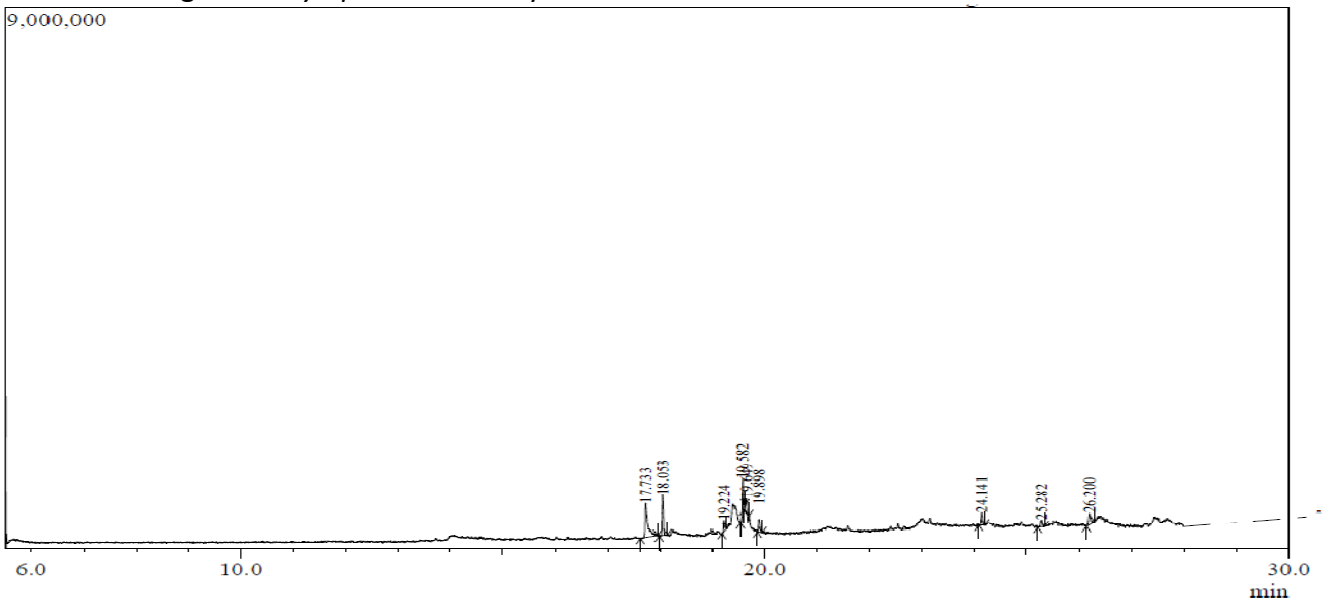


Table 1- Components identified in *Tylophora indica* plant extract.

Sr. No.	Retention time	Name of compound	Molecular formula	Molecular weight
1.	17.73	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256
2.	18.05	Nonadecanoic acid, ethyl ester	$C_{21}H_{42}O_2$	326
3.	18.05	Ethyl tridecanoate	$C_{15}H_{30}O_2$	242
4.	19.22	Phytol	$C_{20}H_{40}O$	296
5.	19.58	9,12-Octadecadien-1-ol	$C_{18}H_{34}O$	266
6.	19.65	Oleic Acid	$C_{18}H_{34}O_2$	282
7.	19.90	Ethyl tridecanoate	$C_{15}H_{30}O_2$	242
8.	24.14	2,9-Dimethyldecane	$C_{12}H_{26}$	170
9.	25.28	6,11-Dimethyl-2,6,10-dodecatrien-1-ol	$C_{14}H_{24}O$	208
10.	26.20	Pentacosane	$C_{25}H_{52}$	352

+ Ve indicate **Present** and – Ve indicate **Absent**

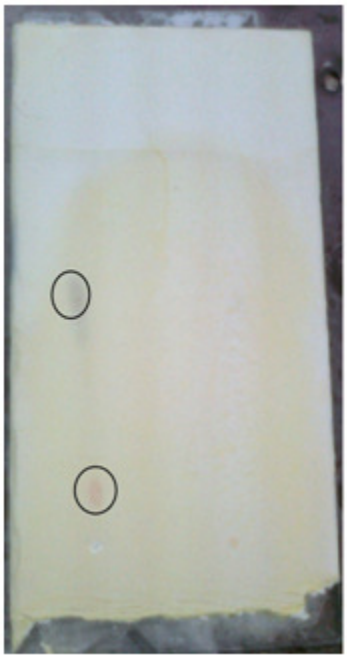


Fig 1: TLC plate of alcohol extract at day light

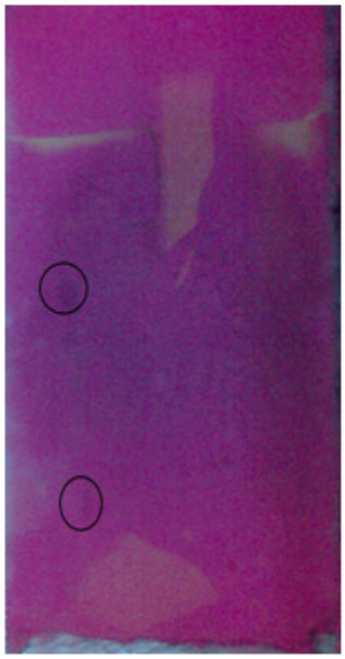


Fig 2: TLC plate of alcohol extract at 254 nm UV light

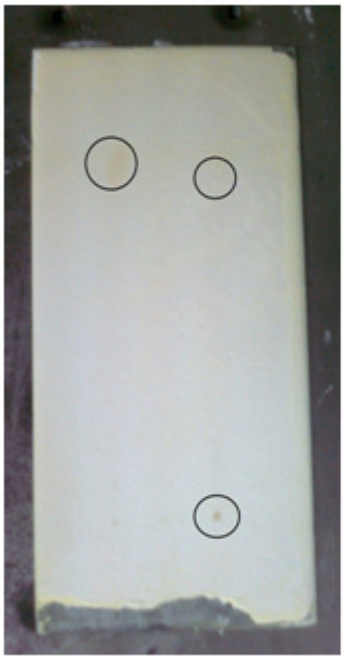


Fig 3: TLC plate of petroleum ether ether extract at day light

Fig 4: TLC plate of petroleum extract at 254 nm UV light

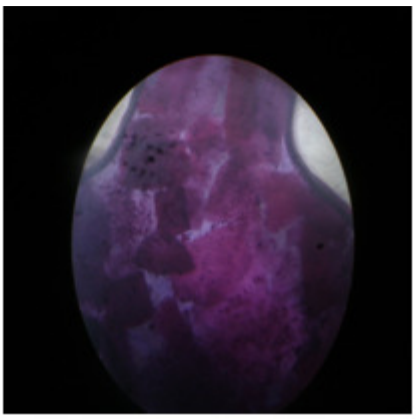


Fig 5: - Study of fine powder of seeds

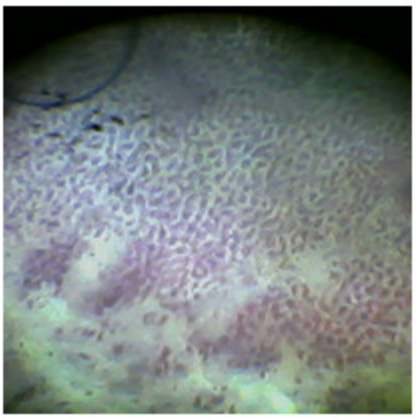


Fig 6: - Transverse sections of Seed

CONCLUSION

The pharmacognostic characters & phytochemical values reported in this paper could be used as the diagnostic tool for the standardization of this medicinal plant. Adulterants if any can be identified morphologically as well as on the basis of other parameters studied in this paper.

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