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Pharmacognostic, Phytochemical Analysis and Anti Diabetic Activity of Dried Leaves of *Abrus precatorius* – an *in vivo* Approach

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ABSTRACT

Diabetes mellitus is among the most common disorder in developed and developing countries, and the disease is increasing rapidly in most parts of the world. It has been estimated that up to one-third of patients with diabetes mellitus use some form of complementary and alternative medicine. The medicinal plants refer to the plants extracts of different parts (leaves, seeds, roots, fruits etc.) which are used in the treatment of various diseases of humans, animals and plants. So, the study was carried out to ascertain the anti diabetic properties present in different extracts of dried scale leaves of *Abrus precatorius*. The present study is an attempt to highlight the anti diabetic activity as well as phytochemical and pharmacological reports on *Abrus precatorius* and calls for better-designed clinical trials to elucidate possible therapeutic effects on diabetes. The *in-vivo* studies on the chloroform leaves extract of *Abrus precatorius* revealed the presence of significant anti diabetic activity. Therefore, it can be used as a source of natural anti diabetic and used in drug formulations for treatment of diseases.

Keywords: Anti-diabetic activity, diabetes, dexamethasone, *in-vivo* studies, clinical trials, *Abrus precatorius*.

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INTRODUCTION

Medicinal plants are being widely used, either as a single drug or in combination in health care delivery system. Medicinal plants can be important source of

with

potential therapeutic effects. *Abrus precatorius* L. is commonly known as Gunja or Jequirity and abundantly found all throughout the plains of India,

from Himalaya down to Southern India and Ceylon. The plants have been utilized for basic and curative health care since time immemorial. The use of plants as food and medicines started ever since man started life on the planet. [1] The plant kingdom is a virtual goldmine of potential drug targets and other active drug molecules waiting to be discovered. During the last decade, use of traditional medicine has expanded globally and gained popularity. Plant based drugs are having a revived interest now-a-days because of awareness of deleterious effects of modern synthetic drugs. Natural products can play a very crucial role in pharmaceutical industry as drug them or as drug carrier or bio-enhancers or excipients. The importance of herbal/plant medicines is well documented in Vedas, which proved to be the ancient literature. The properties of the plants and their remedies are given in detail and in fact Ayurveda is the very principle root for the emergence of Ancient medical science in India that gave origin to branches like Sushruta and Charka Samhita. [2]

According to WHO, today more than one billion people relied on herbal medicines to some extent. The WHO has listed 21,000 plants have reported medicinal uses around the world. India has a rich medicinal plant flora of some 2500 species, of these 2000 to 3000 atleast 150 species are used commercially on a fairly large scale. Foreign researchers have always appreciated the traditional Indian healers. The healing powers of traditional herbal medicines have been realized since antiquities. [3-4] About 34 percent of all pharmaceutical preparations come from higher plants and it goes to 60 percent when bacteria and fungi origins are taken into account. It is estimated that the country exports about 550 cores worth of herbal drugs but with the rich diverse botanical resource in our country, this is not an impressive export performance considering the world wide herbal market worth US 60 billion dollars. Due to the need and emphasis on quality control of drugs, chemical assay, stability, safety assessment, pharmacokinetic studies etc. have been necessary to establish the products and its efficacy in the global markets. [5]

In order to set up quality in production and products, research documentation is mandatory to supply to international requirements. By referring global standards and international pharmacopoeia like Herbal B.P, China, Japanese Herbal, Ayurvedic Formulary of India, WHO Guidelines on Herbal Medicines, this could be met with. It is also necessary to integrate modern knowledge with traditional knowledge. The drugs and products of the industry are working on the scientifically defined techniques and explained with modern biological and chemical definitions and tools, and that alone will give a therapeutically active herbal original drug available for health care worldwide. [6-7]

The efficacy of some herbal products is beyond doubt, the most recent example being *Artemisia annua*, *Taxus*

brevifolia, and *Silybium marianum*, *Hypericum perforatum*, *Allium sativum*, *Ginkgo biloba* are popularly used herbal remedies among people. All these herbals are standardized for active constituents. Standardization means adjusting the herbal drug preparation to a defined content of active constituent. Extract refers to a concentrated preparation of active constituent of a medicinal herb. [8] The concept of standardization extracts definitely provides a solid platform for scientific validation of herbals. Photochemistry deals with the determination of chemical constituents in plant material. Most of the herbal extracts are made from crude herb they can vary in percentage of active constituents, which further influences the therapeutic activity of herbs depending upon the source. These medicinal herbs become popular due to single active constituents. Keeping in mind the overall scenario, only selected medicinal herbs have been used in standardized form and market analysis clearly favors them. [9] The present research was focused to evaluate the pharmacognostical screening and anti diabetic activity of different leaf extracts of *Abrus precatorius* in dexamethasone induced diabetic rats.



Fig. 1: External features of the plant *Abrus precatorius*

MATERIALS AND METHODS

Plant profile

In the range of India to South china, Malaysia, Philippines, Java, Taiwan, wide spread throughout island. [10]

Classification

Kingdom	-	Plantae
Sub Kingdom	-	Tracheobionta - Vascular plants
Phylum	-	Magnoliophyta - Flowering plants
Sub Phylum	-	Spermatophyta
Class	-	Magnoliopsida
Subclass	-	Rosidae
Order	-	fabeles
Family	-	fabaceae
Genus	-	<i>Abrus</i>
Species	-	<i>A. precatorius</i>
Botanical name	-	<i>Abrus precatorius</i>

Physical characters

It is a deciduous shrub growing to 1 m. The flowers are hermaphrodite. The plant prefers light (sandy) medium

(loamy) and heavy (clay) soils. The plant prefers acid, neutral, basic (alkaline) soils. It can grow in semi shade or no shade. It requires moist soil. [11]

External features of the plant

Abrus precatorius is a severely invasive plant in warm temperate to tropical regions, so much so that it has become effectively pantropical in distribution. It had been widely introduced by humans, and the brightly coloured and hard-shelled seeds had been spread by birds. By the end of the twentieth century, it had been proclaimed as an invasive weed in many regions including some in Belize, Caribbean Islands, Hawaii, Polynesia and parts of the mainland United States. In Florida in particular, the plant has invaded undisturbed pinelands and hammocks, including the vulnerable pine rocklands. Once *Abrus precatorius* plants have grown to maturity under favourable conditions, their deep roots are extremely difficult to remove, and the plants aggressive growth, hard-shelled seeds, and ability to sucker, renders an infestation extremely difficult to eradicate and makes it very difficult to prevent re-infestation. [12-13]

Physicochemical standards

Ash value

The ash content of the crude drug is generally taken to be the residue remaining after incineration. It usually represents the inorganic salts naturally occurring in the drug and adhering to it, but it may include inorganic matter added for the purpose of adulteration. Ash value varies with narrow limits in case of the individual drug but varies considerably in case of different drugs.

Determination of Total ash

About 2 g of powdered drug was accurately weighed in a silica crucible, which was previously ignited and weighed. The powdered drug was spread as a fine layer on the bottom of the crucible. The crucible was incinerated at a temperature not exceeding 450°C until free from carbon. The crucible was cooled and weighed for constant weight. The percentage of total ash was calculated with reference to the air-dried drug.

Determination of water soluble ash

The ash obtained in the determination of total ash was boiled for 5 minutes with 25 ml of water. The insoluble matter was collected on an ash less filter paper and washed with hot water. The insoluble ash was transferred into a tarred silica crucible and ignited for 15 minutes at a temperature not exceeding 450°C. The weight of the insoluble matter was subtracted from the weight of total ash. The difference in weight was considered as the water soluble ash. The percentage of water soluble ash was calculated with reference to the air-dried drug.

Determination of acid insoluble ash

The ash obtained as described in the determination of total ash was boiled with 25 ml of hydrochloric acid for 5 minutes. The insoluble ash was collected on an ash less filter paper and washed with hot water; the insoluble ash was transferred into pre-weighed silica

crucible. The percentage of acid insoluble ash was calculated with reference to the air-dried drug.

Loss on drying

Five grams of the powdered crude drug was accurately weighed in a tarred dish and dried in an oven at 100-105°C. It was cooled in desiccators and again weighed. The loss on drying was calculated with reference to the amount of the dried powder taken.

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 with 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.

Solubility value

Solubility values of crude drugs are useful for their evaluation especially when the constituents of a drug cannot be readily estimated by any other means. Further, these values indicate the nature of the constituents present in a crude drug.

Ethanol soluble extractive

5 g of air-dried coarsely powdered drug was macerated with 100 ml of 90% ethanol in a closed flask for 24 hours, shaking frequently during the first 6 hours and allow standing for 18 hours. It was then 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 and weighed. The percentage of ethanol soluble extractive with reference to the air-dried drug was calculated.

Water soluble extractive

5 g of air-dried coarsely powdered drug was macerated with 100 ml of Chloroform water (95 ml of water + 5 ml of Chloroform) in a closed flask for 24 hours, shaking frequently during the first 6 hours and allowed to stand for 18 hours. It was then 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 and weighed. The percentage of water-soluble extractive with reference to the air-dried drug was calculated.

Extractive values

The solvents obtained commercially (LR – Grade Extra pure) were purified by distillation methods prior to use for extraction and for Phytochemical investigation. 1kg coarse powder was subjected to maceration for 72 hours, followed by exhaustive maceration for 48 hours by various solvents of increasing polarity (pet ether, chloroform, ethyl acetate, alcohol, water) by decanting

and drying the marc after each extraction. The solvents were recovered by distillation of the extracts at 75°C to 80°C. The extracts were dried under desiccator and percentage yield was calculated.

Fluorescence Analysis

Fluorescence analysis of the powder was observed in day/visible light and UV light (Long wavelength – 365 nm and Short wave length – 245 nm). The drug powder was treated with various solvents like benzene, chloroform, ethyl acetate and 50% aqueous-ethanol and acids like 1M Hydrochloric acid and alkaline solutions like 1N sodium hydroxide. They were subjected to fluorescence analysis in daylight and in UV-light.

Qualitative phytochemical evaluation ^[14-15]

The different chemical tests were performed for establishing profile of the extract for its chemical composition; the following chemical tests for various phytoconstituents in the petroleum ether, chloroform, ethyl acetate, alcohol and water extracts were carried out as described below.

Test for alkaloids

Dragendroff's Test: In a test tube containing 1ml of extract, few drops of Dragendroff's reagent was added and the colour developed was noticed. Appearance of orange colour indicates the presence of alkaloids.

Wagner's Test: To the extract, 2 ml of Wagner's reagent was added; the formation of a reddish brown precipitate indicates the presence of alkaloids.

Mayer's Test: To the extract, 2 ml of Mayer's reagent was added, a dull white precipitate revealed the presence of alkaloids.

Hager's Test: To the extract, 2 ml of Hager's reagent was added; the formation of yellow precipitate confirmed the presence of alkaloids.

Test for terpenoids

Salkowski test: To 1 ml of extract, tin (one bit) and thionyl chloride were added. Appearance of pink colour indicates the presence of terpenoids.

Hirshonn reaction: When the substance was heated with trichloroacetic acid, red to purple colour was observed.

Test for steroids

Liebermann Burchard Test: To 1 ml of extract, 1 ml of glacial acetic acid and 1 ml of acetic anhydride and two drops of concentrated sulphuric acid were added. The solution become red, then blue and finally bluish green indicates the presence of steroids.

Salkowski test: To 1 ml of extract, salkowski reagent was added. Appearance of red colour indicates the presence of steroids.

Test for coumarins

To 1 ml of extract, 1 ml of 10% sodium hydroxide was added. The presence of coumarins is indicated by the formation of yellow colour.

Test for tannins

i) To few mg of extract, ferric chloride was added, formation of a dark blue or greenish black colour showed the presence of tannins.

ii) The extract was mixed with basic lead acetate solution; formation of white precipitate indicated the presence of tannins.

Test for saponins

To 1 ml of the extract, 5 ml of water was added and the tube was shaken vigorously. Copious lather formation indicates the presence of saponins.

Test for flavones

i) **Shinoda Test:** To the extract, a few magnesium turnings and 2 drops of concentrated hydrochloric acid were added, formation of red colour showed the presence of flavones.

ii) To the extract, 10% sodium hydroxide or ammonia was added; dark yellow colour shows the presence of flavones.

Test for quinones

To 1 ml of the extract 1 ml of concentrated sulphuric acid was added. Formation of red colour shows the presence of quinones.

Test for flavanones

i) To the extract, 10% sodium hydroxide was added and the colour changes from yellow to orange, which indicates the presence of flavanones.

ii) To the extract, conc. sulphuric acid was added, and the colour changes from orange to crimson red, which indicates the presence of flavanones.

Test for anthocyanins

i) To the extract, 10% sodium hydroxide was added, and the blue color shows the presence of anthocyanins.

ii) To the extract, conc. sulphuric acid was added, and the yellowish orange colour confirms the presence of anthocyanins.

Test for anthraquinones

Borntrager's test: The extract was macerated with ether and after filtration; aqueous ammonia or caustic soda was added. Pink red or violet colour in the aqueous layer after shaking indicates the presence of anthraquinones.

Test for phenols

Ferric chloride test: To the extract, few drops of 10% aqueous ferric chloride were added. Appearance of blue or green colour indicates the presence of phenols.

Test for proteins

Biuret Test: To the extract, 1 ml of 40% sodium hydroxide solution and two drops of one percent copper sulphate solution were added. Formation of violet colour indicates the presence of proteins.

Xanthoprotein Test: To the extract, 1 ml of concentrated nitric acid was added. A white precipitate was formed; it is then boiled and cooled. Then 20% sodium hydroxide or ammonia was added. Orange colour indicates the presence of aromatic amino acids.

Tannic Acid Test: To the extract, 10% tannic acid was added. Formation of white precipitate indicates the presence of proteins.

Test for carbohydrates

Molisch's Test: To the extract, 1 ml of alpha-naphthol solution, and concentrated sulphuric acid through the sides of test tube were added. Purple or reddish violet

colour at the junction of the two liquids revealed the presence of carbohydrates.

Fehling's Test: To the extract, equal quantities of fehling's solution A and B were added and on heating, formation of a brick red precipitate indicates the presence of carbohydrates.

Benedict's Test: To 5 ml of Benedict's reagent, extract was added and boiled for two minutes and cooled. Formation of red precipitate showed the presence of carbohydrates.

Test for amino acids

Ninhydrin test: Two drops of ninhydrin solution were added to the extract, a characteristic purple colour indicates the presence of amino acids.

Test for Fixed Oils and Fats

Spot Test: A small quantity of extract was pressed between two filter papers. Oil stains on the paper indicates the presence of fixed oils and fats.

In vivo Anti Diabetic Study

Dexamethasone-induced insulin resistance in mice [16-17]

The albino mice of 20-22 g of either sex animals (24 numbers) were used for the study. All the mice were weighed before treatment, group I (Normal Control) received equivalent amount of 0.9% w/v saline (1 mL/kg, *p.o.*), and 18 mice were rendered hyperglycemic by daily administration of a prestandardised dose of dexamethasone (1 mg/kg, *i.m.*) for consecutive seven days and then divided into three groups of six each. Group II (Dexa-control) continued to receive only dexamethasone for next fifteen days, group III Animals treated with *Abrus precatorius* leaves extract (100 mg/kg *p.o.*) Groups IV received glibenclamide (5 mg/kg, *p.o.*) along with dexamethasone respectively for twenty one days (CPCSEA-NNRG/SOP/2017/21b).

Biochemical analysis

On the last day all the animals were weighed. Blood samples were collected and plasma and serum separated for estimation of glucose and triglyceride, respectively. Biochemical estimation of plasma glucose and serum triglyceride was done by glucose oxidase (GOD)/POD and glycerol-3-phosphate oxidase (GOD)/PAD methods, respectively using standard diagnostic kits.

Statistical analysis

The results were expressed as mean $\pm$ standard error of mean (SEM) and statistically analyzed by ANOVA followed by Dennett test, with level of significance set at $p < 0.05$ and $p < 0.01$.

RESULTS

The physicochemical analysis proves the stability, purity and firmness of the plant drug for use and is helpful to standardize them to be used as potential drug. Fluorescence analysis reveals the presence of fluorescence compounds in the leaves of *Abrus precatorius*. The physicochemical standard values,

fluorescence analysis of *Abrus precatorius* leaves and phytochemical investigation have been summarized.

Table 1: Physicochemical analysis of *Abrus precatorius* leaves

S. No.	Parameters	% Value
1	Total ash	13.2
2	Acid insoluble ash	2.0
3	Water soluble ash	7.02
4	Crude fiber content	1.5
5	Loss on drying	8.6
6	Solubility	
	a) Alcohol	7.01
	b) Water	8.5
7	Extractive value	
	a) Petroleum ether	0.20
	b) Chloroform	0.1
	c) Ethyl acetate	0.32
	d) Alcohol	2.62
	e) Water	1.64

Table 2: Fluorescence analysis of *Abrus precatorius* leaves

S. No.	Reagent	Visible/day light	UV-245 nm	UV-365 nm
1	Drug power	Light green	Green	Brown
2	Powder + 1N NaOH (aq.)	Light green	Light green	Brown
3	Powder + 1N NaOH (alc.)	Light green	Green	Light green
4	Powder + 1N HCl	Light green	Light green	Brown
5	Powder + 50% H ₂ SO ₄	Light green	Light green	Brown
6	Powder + 50% HNO ₃	Light brown	Light green	Dark Brown
7	Powder + picric acid	Light yellow	Light green	Pale Brown
8	Powder + acetic acid	Light brown	Light green	Brown
9	Powder + FeCl ₃	Pale green	Light green	Brown
10	Powder + HNO ₃ + NH ₃	Light brown	Light green	Brown

Table 3: Preliminary phytochemical screening of *Abrus precatorius* leaves

Constituents	Pet ether Extract	Chloroform extract	Ethyl acetate extract	Alcohol extract	Water extract
Terpenoids	-	-	-	-	-
Saponins	+	+	-	+	-
Steroids	-	+	+	-	-
Phenols	-	-	+	-	-
Flavonoids	-	-	-	+	-
Coumarins	-	-	+	+	+
Reducing sugars	-	+	-	-	-
Alkaloids	-	-	+	+	-
Quinones	-	+	+	+	+
Tannins	-	+	+	-	-
Proteins	-	-	-	-	-
Amino acids	-	-	-	-	-
Anthraquinones	-	+	+	+	-

+ Present, - Absent

DISCUSSION

The pharmacognostic, phytochemical and *in vivo* pharmacological activities of *Abrus precatorius* were been reported. The present study revealed the presence of flavonoids, alkaloids, glycosides, steroids, phenols, saponins and tannins as the chemical class present in the leaf extracts of *Abrus precatorius*. The *in vivo* studies

on the leaves of *Abrus precatorius* revealed the presence of significant anti diabetic activity. The chloroform extract shows good anti diabetic activity when compared to other extracts. *Abrus precatorius* leaf as a useful anti-diabetic agent in diabetic complications. The present study show that anti diabetic effect of *Abrus precatorius* extract is significant when compare with diabetic control group. In dexamethasone control group there was significant increase in blood glucose levels ($p < 0.05$) when compared to the vehicle control. Chloroform extract of *Abrus precatorius* leaves (100 mg/kg *p.o*) and Glibenclamide (5mg/lg *p.o*) decreased blood glucose level as compared with normal control. Plant extract of *Abrus precatorius* leaves significantly reducing blood glucose level. Further study is needed for the molecular information of the extract behind this anti-diabetic activity. Furthermore, a detailed and systematic approach can be done in exploiting and identifying the phytopharmacology to explore in knowing the maximum potentiality of the plant which will be useful to mankind. Thus, it is clearly evident that the active compound of this selected plant *Abrus precatorius* is a secondary metabolite and shows bioactivities.

Table 4: Effect of administration of *Abrus precatorius* leaves extract (100 mg/kg, *p.o*) + glibenclamide (5 mg/kg, *p.o*) for 21 days on blood glucose levels in diabetic rats

Group	Treatment	Glucose level at 11 th day	Glucose level at 16 th day	Glucose level at 21 st day
Group - 1	Control (Normal control)	106 ± 1.61	99.86 ± 1.56	95.58 ± 1.32
Group - 2	Diabetic control	287.53 ± 5.51*	274.93 ± 9.31**	270.6 ± 9.16**
Group - 3	<i>Abrus precatorius</i> leaves extract (100 mg/kg, <i>p.o</i>)	294.13 ± 15.41*	199.73 ± 36.50**	150.06 ± 4.48**
Group - 4	Glibenclamide (5 mg/kg, <i>p.o</i>) (reference standard)	285.43 ± 5.48*	189.65 ± 34.65**	145.58 ± 5.49**

Value expressed in MEAN ± SEM, n=6 Experimental groups statically compared with control groups where significant * $p < 0.05$, moderately significant ** $p < 0.01$. All the values are compared with the control group animals.

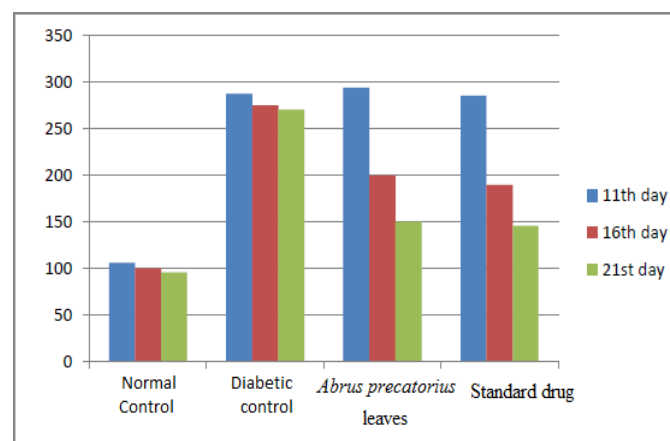


Fig. 2: Effect of administration of *Abrus precatorius* leaves extract (100 mg/kg, *p.o*) Glibenclamide (5 mg/kg, *p.o*), for 21 days on serum blood glucose levels in dexamethasone induced diabetic rats

The pharmacognostic, phytochemical and *in vivo* pharmacological activities of *Abrus precatorius* were been reported. The *in vivo* studies on the leaves of *Abrus precatorius* revealed the presence of significant anti diabetic activity. The chloroform extract shows good anti diabetic activity when compared to other extracts. *Abrus precatorius* leaf as a useful anti-diabetic agent in diabetic complications. Further study is needed for the molecular information of the extract behind this anti-diabetic activity. We can therefore conclude from this study that the presence of the phytochemicals in these plants might be the reason for the activity and that the plants may essentially contain herbal bioactive compounds which require further structural elucidation and characterization methodologies to identify the bioactive constituents. Further *ex vivo* investigations should be done for confirming the anti diabetic activity of the plant. The plant extracts understudy can serve as therapeutic agents and can be used as potential sources of novel bioactive compounds for treating Diabetes mellitus.

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