

Establishment of Quality Standards of *Abrus precatorius* Linn. Seed

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Tabasum et al.: Quality parameters of *Abrus precatorius* Linn. Seeds

Standardization of herbal formulations is an important aspect while considering the quality of the herbal drug, consistency in active principle content and therapeutic efficacy. The seeds of *Abrus precatorius* Linnaeus, a well-established medicinal plant belonging to the family Fabaceae is commonly known as *Gunja* or *Rati*. In view of its medicinal importance the present study was conducted to standardize the crude drug according to the standard laboratory methods and to define pharmacopoeial standards for the seeds of this species. Qualitative phytochemical analysis and physiochemical studies of the powdered drug using various methods including organoleptic analysis, ash value, extractive value, thin-layer chromatography, loss on drying, and fluorescence analysis were performed to establish quality standards of the herb, which would help in identification as well as develop quality assurance parameters for herbal formulations containing this herbal product as an active component.

Key words: *Abrus precatorius*, standardization, quality, phytochemical analysis

Medicinal plants have been known for millennia and are highly appreciated all over the world as a rich source of therapeutic agents for curing various diseases and disorders^[1]. Today, people are much aware of medicinal plant resources and are showing great interest towards the herbal drugs and products by making their use in health maintenance and prevention of various diseases and ailments^[2,3]. This rising interest towards the herbal medicines is because of the traditional belief that these are cheap, safe and natural without harmful effects^[4]. Medicinal plants are also major raw materials for pharmaceutical industry and thus have gained importance in global drug market. However, the unavailability of documentation and rigorous quality control acts as a key hindrance in the approval of herbal products as alternative medicine in the developed countries. Thus, it is very important to make significant efforts towards standardization of the plant materials along with documentation for those to be used as medicines^[3].

Standardization of herbal medicines is the process to lay down a set of technical standard parameters for the assurance of quality, safety, efficacy and reproducibility for each herb. Such constant parameters are developed by observation and experimentation to confirm a set of

characteristics exhibited by a particular medicinal herb or its products. Hence the process of standardization is a basic tool in the quality assurance of herbal medicine^[5] that can be attained by stepwise phytochemical and pharmacognostic investigations. These studies help in correct identification, classification and standardization of the plant material. Proper identification and quality assurance of the starting plant materials is a fundamental requirement to establish the reproducible quality of herbal medicinal products that will contribute to their safety and efficacy^[6,7].

Abrus precatorius L. commonly known as Rosary pea, Indian liquorice, Jequirity and Crab's eye is an important medicinal plant belonging to the family Fabaceae. It is known as *Ratti* in *Hindi* and *Gunja* in *Sanskrit*. Plant is native to India, but now found almost in all tropical and sub-tropical areas throughout the world^[8]. *A. precatorius* is a twinning climber with herbaceous branches and pinnate leaves. The flowers

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are purple pink or reddish in colour and are borne in clusters. Fruits are pods bearing characteristic red seeds with a black spot round the hilum^[9]. The seeds have remarkably uniform weight of 1/10th of a gram, therefore were used by goldsmiths as standard weight for weighing gold and silver in previous time^[10]. It was first recognized and mentioned as a homoeopathic medicine by William Boericke in his Homoeopathic *Materia Medica* under the heading of Jequirity^[11]. The plant is used in some traditional medicine to treat scratches, sores and wounds caused by dogs, cats and mice; and are also used with other ingredients to treat leucoderma, tetanus and rabies^[10]. Seeds are useful in diarrhoea, dysentery and have antidiabetic^[12], antitumor and proapoptotic^[13], antibacterial, anthelmintic^[14,15] and protective effect against alcohol-induced renal damage^[16]. Seeds are considered to be purgative, emetic, tonic, antiphlogistic, aphrodisiac, antiophthalmic^[17] and abortifacient^[18]. Seeds are also poisonous but after mitigation are said to have very powerful aphrodisiac property. These are also used in veterinary medicine for the treatment of fracture^[19-22]. It is said that cooking destroys the poison of seeds^[23,24]. A protein called abrin is responsible for their toxic properties^[8]. In view of its medicinal importance, present study was performed to standardize *A. precatorius* seeds on the basis of phytochemical and physicochemical parameters.

MATERIALS AND METHODS

Seed collection and authentication:

Firstly the mature seeds of *A. precatorius* (fig. 1A) were purchased from the local market of Bhopal, Madhya Pradesh, India and were identified and authenticated in the Department of Botany, Safia Science College, Bhopal, Madhya Pradesh, India. A sample voucher specimen No. 520/Bot/Saifia/2015 was deposited there for further reference.

Extraction^[25]:

The seeds were washed with distilled water to remove residual material and were shade-dried. After drying, seeds were crushed into a coarse powder (fig. 1B) using an electrical grinder. A part of this dried coarse powder of *A. precatorius* was filled and stored in air tight containers for physicochemical and other studies in future. The remaining powder was extracted with petroleum ether and the marc remaining was air-dried and extracted successively by 70 % methanol through maceration process. Filtrates obtained from both the solvents were evaporated in a rotary evaporator under



Fig. 1: *Abrus precatorius* L. mature seeds (A) and powder (B)
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reduced pressure, vacuum-dried and were packed in air tight containers, labelled and stored in a refrigerator (2-4°) until needed for the experimental purpose. Percent yield of the extracts were calculated with reference to air-dried powder as: percent yield = weight of extract/weight of plant material × 100.

Standardization parameters:

Organoleptic evaluation^[26] is a method of qualitative evaluation based on study of morphological and sensory profiles of crude drugs. Organoleptic characteristics including colour, odour, taste and texture of seed powder. Petroleum ether extract and methanol extracts were evaluated using reported methods. Loss on drying^[27] gives an estimate of the moisture present in a crude drug. The moisture content of the powdered drug was determined by the loss on drying method as per standard procedure.

Determination of ash value^[25,27] allows for detecting low-grade drugs, exhausted drugs and sandy or earthy matter. The ash value was determined by four different methods, which measure total ash, acid-insoluble ash, water-soluble ash and sulphated ash, using standard methods. Extractive values^[25,27] of the crude drug in different solvents such as distilled water, chloroform, ethanol, ethyl acetate, methanol and petroleum ether were determined as per standard methods.

The fluorescence characteristics^[3] of the fine seed powder as such and with different reagents was examined in both visible and UV light (254 and 366 nm) using reported methods. Changes in colour of the sample when treated with different reagents were recorded under visible and UV light (254 and 366 nm).

Qualitative phytochemical analysis^[25] is based on the property of selective reactivity of phytochemicals present in extracts formed the basis of chemical tests for identification of various compounds. Phytochemical screening was performed for analysing secondary metabolites, which were believed to be responsible for useful pharmacological effects with therapeutic application. The preliminary phytochemical screening for secondary metabolites was carried out on petroleum ether and methanol (70 %) seed extracts of *A. precatorius* using standard procedures. The phytochemicals tested for were alkaloids, flavonoids, carbohydrates, tannins, steroids, proteins, triterpenes, glycosides, saponins, and fat and oils. Presence and absence of phytochemical constituents were noted.

Chromatographic analysis^[26-28] was carried out using thin-layer chromatography (TLC), which is one of the most useful techniques employed for separation, identification and estimation of single or mixture of components present in different plant extracts. In the present study, TLC was carried out for 70 % methanol and petroleum ether extracts of *A. precatorius* seeds using various solvent systems as per the standard methods to find out the probable number of compounds present in these extracts. The extracts were spotted on activated silica gel plates. After development TLC plates were observed under visible and UV light (short- 254 nm and long- 365 nm). The retention factor (Rf) for each spot observed was calculated using the formula as: $Rf = \frac{\text{spot distance from origin}}{\text{solvent front distance from origin}}$.

RESULTS AND DISCUSSION

It is very important and in fact an essential prerequisite to establish the standard specifications of medicinal plants used in the preparation of various drugs. The study of natural drugs mostly of plant origin is known as pharmacognosy. It mainly deals with identification, authentication and quality control of herbal medicines. Most of the research in pharmacognosy has been done to identify controversial species of plants, authentication of commonly used traditional medicinal plants through morphological, phytochemical and physicochemical analysis^[29]. Hence, in recent years there has been increased emphasis on standardization of medicinal plants, of which evaluation of plant drugs using pharmacognostic studies appears to be more reliable, accurate and economical. Pharmacognostic parameters and standards must be established for all the crude drugs prior to their incorporation in herbal

pharmacopoeia. The present study deals with the physicochemical and phytochemical evaluation of the seeds of *A. precatorius*, a well-known medicinally important plant.

Percent yield of the extracts in different solvents indicate the quantity and nature of various phytochemicals present in the crude drug. The percent yields of petroleum ether and 70 % methanol extracts obtained by successive maceration method were 1.146 and 3.292 % w/w, respectively.

Organoleptic evaluation is the first step of establishing the identity and purity of the medicinal plant materials before further tests are performed. Organoleptic characters are very simple and quicker means to establish the quality and purity of a particular crude drug. Organoleptic evaluation indicated the brown colour, characteristic odour, bitter taste and sticky texture of the 70 % methanol extract; and yellowish colour, pungent odour and oily texture of the petroleum ether extract. The value of loss on drying, expressed as a percent of air-dried material was 8.2 % w/w.

The ash value is particularly helpful in determination of purity and quality of drugs. A high ash value is indicative of adulteration, contamination, substitution or carelessness in preparing the crude drug for marketing. Low ash values indicate the purity of plant drugs. The values are expressed as percent of air-dried material. The values of total ash, acid-insoluble ash, water-soluble ash and sulphated ash were found to be 2.091, 0.102, 0.067 and 1.019 % w/w, respectively.

Extractive values are valuable to evaluate the chemical constituents present in the crude drug and assist in estimation of specific components soluble in particular solvent. These are mainly helpful for the determination of exhausted and adulterated drugs. The extractive values of powdered drug in distilled water, methanol, ethanol, petroleum ether, chloroform and ethyl acetate extracts were 51.78, 9.10, 4.94, 3.20, 2.09 and 1.96 % w/w, respectively. The extractive value of powdered sample was thus found maximum in distilled water and least in ethyl acetate, which implied that most of the phytoconstituents of the seed were soluble in water. The values are expressed as percent of air-dried material.

Fluorescence study is an essential parameter for first line standardization of crude drug. Light rich in short wavelengths is very active in producing fluorescence and for this reason ultraviolet light produces fluorescence in many substances, which do not

visibly fluoresce in day light. The fluorescence colour is specific for every compound. The fluorescence analysis of seed powder displayed their characteristic fluorescent colour in different chemicals used. The results of fluorescence analysis were shown in Table 1.

Qualitative phytochemical screening gave valuable information about the various phytoconstituents present in the extracts, which could facilitate future investigators to select of a particular extract for further analysis of isolating the active principle. From preliminary phytochemical studies, 70 % methanol

extract was found to be rich in phytoconstituents than the petroleum ether extract. This investigation confirmed the presence of carbohydrates, proteins, amino acids, glycosides, saponins, alkaloids, terpenoids, steroids, flavonoids, tannins and phenolic compounds in 70 % methanol extract; and carbohydrates, terpenoids, steroids, tannins and fats and oils in the petroleum ether extract. Presence of different phytochemicals in both the extracts revealed their therapeutic potential and offer specific distinctiveness and properties to the plant. The results of this investigation are compiled in Table 2.

TABLE 1: FLUORESCENCE ANALYSIS OF POWDERED *ABRUS PRECATORIUS* L. SEEDS

Powdered crude drug+reagent	Visible Light	UV (short) 254 nm	UV (long) 366 nm
Powder drug as such	Creamish-yellow	Fluorescent green	Light brown
Drug+methanol	Creamish-yellow	Fluorescent green	Brownish-green
Drug+ethanol	Yellowish-cream	Fluorescent green	Brownish-green
Drug+ethyl acetate	Yellowish-cream	Fluorescent green	Brownish-green
Drug+chloroform	Creamish-yellow	Fluorescent green	Brownish-green
Drug+concentrated H ₂ SO ₄	Brownish-red	Dark brown	Dark green
Drug+dilute H ₂ SO ₄	Reddish-grey	Greyish-yellow	Dark brown
Drug+concentrated HCl	Reddish-brown	Brownish-green	Dark brown
Drug+dilute HCl	Greyish-red	Fluorescent green	Brownish-black
Drug+n-hexane	Creamish-yellow	Fluorescent green	Greyish-brown
Drug+10 % NaOH	Brown yellowish	Brown greenish	Black
Drug+5 % FeCl ₃	Brown yellowish	Brown greenish	Black
Drug+distilled water	Creamish-yellow	Greyish-green	Brownish-green
Drug+ammonia solution	Creamish-yellow	Brownish-green	Dark green
Drug+concentrated acetic acid	Creamish-grey	Greyish-yellow	Greyish-brown
Drug+dilute acetic acid	Creamish-red	Brownish-green	Black
Drug+50 % KOH solution	Brownish-yellow	Fluorescent green	Brownish-black
Drug+acetone	Creamish-yellow	Creamish-green	Greenish-brown

TABLE 2: PRELIMINARY PHYTOCHEMICAL ANALYSIS FOR CRUDE EXTRACTS OF EXTRACTS OF *ABRUS PRECATORIUS* L. SEEDS

Phytochemicals	Tests	Observations	
		Methanol (70 %) extract	Petroleum ether extract
Carbohydrates	Molisch's test	+	-
	Benedict's test	+	+
Proteins	Biuret's test	+	-
	Million's test	+	-
Amino acids	Ninhydrin test	+	-
Glycosides	Keller-Killiani test	+	-
		+	-
Saponins	Froth test	+	-
	Mayer's test	+	-
	Wagner's test	+	-
Alkaloids	Dragendorff's test	+	-
	Hager's test	-	-
	Salkowski's test	+	+
Terpenoids and steroids	Liebermann-Burchard's test	+	+
	Lead acetate test	+	-
Flavonoids	Alkaline reagent test	+	-
	Shinoda test	+	-
Tannins and Phenolics	Ferric chloride test	+	+
	Lead acetate test	+	-

'+' indicates presence, '-' indicates absence

TLC is particularly important for the preliminary separation and determination of plant constituents. It is a competent tool to standardize plant preparations. It is relatively simple, handy, easy, convenient, efficient and inexpensive technique for quick evaluation of the quality of herbal preparations. From qualitative chromatographic analysis of the petroleum ether and 70 % methanol extracts, a number of spots were observed in different solvent systems used. It was found that the petroleum ether extract showed maximum separated components under longer wavelength UV light in a solvent system of chloroform:methanol (15:1). On the other hand, in case of the methanol extract, highest density of components were separated under visible light in solvent system composed of ethyl acetate:formic acid:water (8:1:1). The Rf values of

the spots detected on TLC analysis of petroleum ether and methanol extracts developed in different solvent systems are presented in Table 3 and Table 4. The TLC profile of the seed extracts of the *A. precatorius* furnished a preliminary view of the phytochemicals present in them.

In order to ensure the quality of herbal products, appropriate quality and purity of starting material is very important. Standardization is an essential measure for establishing quality, purity and sample identification. It will act as a tool to detect adulterants and substituents and will help in maintaining the quality, reproducibility and efficacy of natural drugs. Information obtained from these studies can be used as reference information for correct identification of particular plant and in distinguishing it from its

TABLE 3: TLC ANALYSES OF METHANOL (70 %) EXTRACT OF *ABRUS PRECATORIUS* SEEDS

Solvent system	Ratio	Condition	No. of spots	Rf value
n-butanol:glacial acetic acid:water	4:1:5	UV long	1	0.683
		UV short	2	0.7, 0.85
		Visible	2	0.916, 0.716
		UV long	2	0.242, 0.314
Ethyl acetate:formic acid:water	8:1:1	UV short	1	0.857
		Visible	3	0.408, 0.281, 0.225
		UV long	-	-
Chloroform:methanol	15:1	UV short	1	0.140
		Visible	1	0.140
		UV long	1	0.231
Ethyl acetate:formic acid:glacial acetic acid:water	100:11:11:26	UV short	1	0.246
		Visible	1	0.144
		UV long	2	0.213, 0.053
Toulene:ethyl acetate:acetic acid	31:14:5	UV short	1	0.213
		Visible	1	0.213
		UV long	1	0.708
		UV short	2	0.680, 0.25
Ethyl acetate:formic acid:glacial acetic acid	36:12:5	Visible	2	0.972, 0.666

TABLE 4: TLC ANALYSES OF PETROLEUM ETHER EXTRACT OF *ABRUS PRECATORIUS* SEEDS

Solvent system	Ratio	Condition	No. of spots	Rf value
n-butanol:glacial acetic acid:water	4:1:5	UV long	1	0.885
		UV short	1	0.3
		Visible	-	-
Ethyl acetate:formic acid:water	8:1:1	UV long	1	1.00
		UV short	1	0.972
		Visible	1	1.00
Chloroform:methanol	15:1	UV long	3	0.94, 0.838, 0.750
		UV short	1	0.941
		Visible	-	-
Ethyl acetate:formic acid:glacial acetic acid:water	100:11:11:26	UV long	1	0.955
		UV short	1	0.955
		Visible	1	0.955
Toulene:ethyl acetate:acetic acid	31:14:5	UV long	2	0.957, 0.628
		UV short	1	0.957
		Visible	-	-
Ethyl acetate:formic acid:glacial acetic acid	36:12:5	UV long	1	0.953
		UV short	1	0.953
		Visible	1	0.953

closely related species. Such studies are also helpful in authentication of the plant material, standardization and establishing the plant as a herbal remedy and also will be useful in making a monograph of the plant for the pharmacopoeia. By following these simple but reliable standards, a person could be very sure of using the crude drug as a home remedy. Physicochemical and phytochemical analysis of seeds confirms the quality and purity of plant and its identification. The study thus tends to scientifically authenticate the use of this plant in traditional medicine.

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Conflict of interest:

The authors declare no conflict of interest.

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