

## Pharmacognostical Evaluation and Phytochemical Standardization of *Abrus precatorius* L. Seeds

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**Abstract** – The seeds of *Abrus precatorius* L. (Family- Fabaceae) constitute the drugs Abrus, Gunja, or Ratti in commerce. 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 and therefore are used after mitigation. The protein abrin is responsible for the highly toxic properties of seeds. Quantitative HPTLC analysis of the methanolic extract of seeds determined the presence of 0.4018% gallic acid and 0.4009% glycyrrhizin. The present study was undertaken to develop an HPTLC method, as well as ascertain the physico-chemical, morphological and histological parameters to establish the authenticity of *A. precatorius* seeds.

**Keywords** – *Abrus precatorius*, Pharmacognosy, Standardization, HPTLC

### Introduction

*Abrus precatorius* L. commonly known as “Indian liquorice, rosary pea, crab’s eye, and jequirity” is an important medicinal plant belonging to family Fabaceae. Plant is a climber found in all tropical or sub-tropical areas. Seeds are used internally in the affections of the nervous system and their paste is applied locally in sciatica, stiffness of shoulder joint, paralysis and eye diseases (kirtikar & Basu, 1933). These are said to be useful in diarrhoea, dysentery and possess antidiabetic (Monago & Alumanah, 2005), antitumor and proapoptotic effect (Bhutia *et al.*, 2008), antibacterial, anthelmintic activity (Asolkar *et al.*, 2000; Adelowotan *et al.*, 2008) and protective effect against alcohol induced renal damage (Ligha *et al.*, 2009). Poultice of the seeds is used as suppository to bring about abortion. Seeds are however poisonous but after mitigation are said to have very powerful and aphrodisiac property (Anonymous, 1985; Chopra *et al.*, 1958; Duke, 2001; Singh *et al.*, 2000). Seeds contain toxic protein Abrin and non-toxic protein Agglutinin; the two alkaloids viz. Methyl ester of N, N-dimethyl tryptophan methocation and precatorine along with apron, hypaphronine, choline, and trygonelline have been isolated from defatted seeds (Ghosal & Dutta, 1971). Abrin exerts its toxic action by attaching itself to

the cell membranes. Abrin’s toxic effect is due to its direct action on the parenchymal cells (e.g. liver and kidney cells) and red blood cells (Hart, 1963).

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). Seeds of another plant *Rhynchosia phaseoloides* are also sold in market as “Ratti” or “Gunja” and appear identical as both are red and have jet black seed coat regions. However, their exomorphological features i.e. shape, hilum, and testa are quite distinct from each other. Although pharmacognostic studies of *A. precatorius* L. seeds, white variety (creamish white in colour with a brownish scar at hilum) has been carried out (Issar & Israili, 1978), 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 for *A. precatorius* L. A simple, rapid, and precise HPTLC method was also developed for the determination of two active marker components, namely gallic acid and glycyrrhizin found in this plant.

### Experimental

**Plant material** – Plant material was collected from Sitapur (U.P.), India (latitudes 27°34’N and longitude 80°41’E). It was authenticated by Dr. A.K.S. Rawat, Head

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**Microscopic analysis** – Seeds were soaked in water and then preserved in 70% ethyl alcohol for histological studies. Transverse sections were cut using YSI-118 Yorco Cryostat Microtome Automatic Deluxe Model (Yorco Scientific Industries Pvt. Ltd. Delhi, India). Sections were stained with safranin and counterstained with fast green. For study of powdered elements fine powder of seeds was treated with chloral hydrate for about 10 min, followed by a gentle heating and then finally mounted in glycerine for observation (Anonymous, 1998). All preparations were observed under Olympus CX-31 microscope. Photomicrographs were taken using Olympus digital camera Model No. E-420 (Johansen, 1940).

**Fluorescence analysis** – Behaviour of powdered drug with different chemical reagents was studied as per methods described by Chase and Pratt, and Kokoski *et al* (Chase & Pratt, 1949; Kokoski *et al.*, 1958).

**Physicochemical analysis** – Chemicals used for physico-chemical and phytochemical analysis were procured from Loba Chemie laboratory reagents and fine chemicals Mumbai, India. Physico-chemical and phytochemical studies like total ash, water soluble ash, acid insoluble ash, extractive values, sugar, starch, total phenolics and tannins were calculated from the shade dried and powdered (60 mesh) plant material (Peach & Tracy, 1955; Anonymous, 2007; Anonymous, 1984; Anonymous, 2004).

#### HPTLC analysis

**Reagents and chemicals** – Standards: Gallic acid and Glycyrrhizin were procured from Sigma-Aldrich (Steinheim, Germany). Solvents used to prepare mobile phase solutions were of HPLC grade and purchased from Merck (Germany).

**Sample preparation** – Air dried (35 - 40 °C) powdered seeds (100 mesh) of *A. precatorius* (5.0 g) were soaked in methanol (3 × 20 ml, for three times, three days). Extracts were combined, filtered and evaporated to dryness through rotary evaporator and then lyophilized. Accurately weighted methanol extract (10 mg) was dissolved in 1 ml of methanol to prepare 10 mg mL<sup>-1</sup> of solution.

**Standard preparation** – A stock solution of standard compounds (1 mg mL<sup>-1</sup>) was prepared by dissolving 1 mg of each accurately weighted standards (Gallic acid and Glycyrrhizin) in 1 ml of methanol and further working solution of 100 ng mL<sup>-1</sup> was prepared by adding 900 µl HPLC grade methanol in to 100 µl of stock solution.

**Chromatography conditions** – Chromatography was

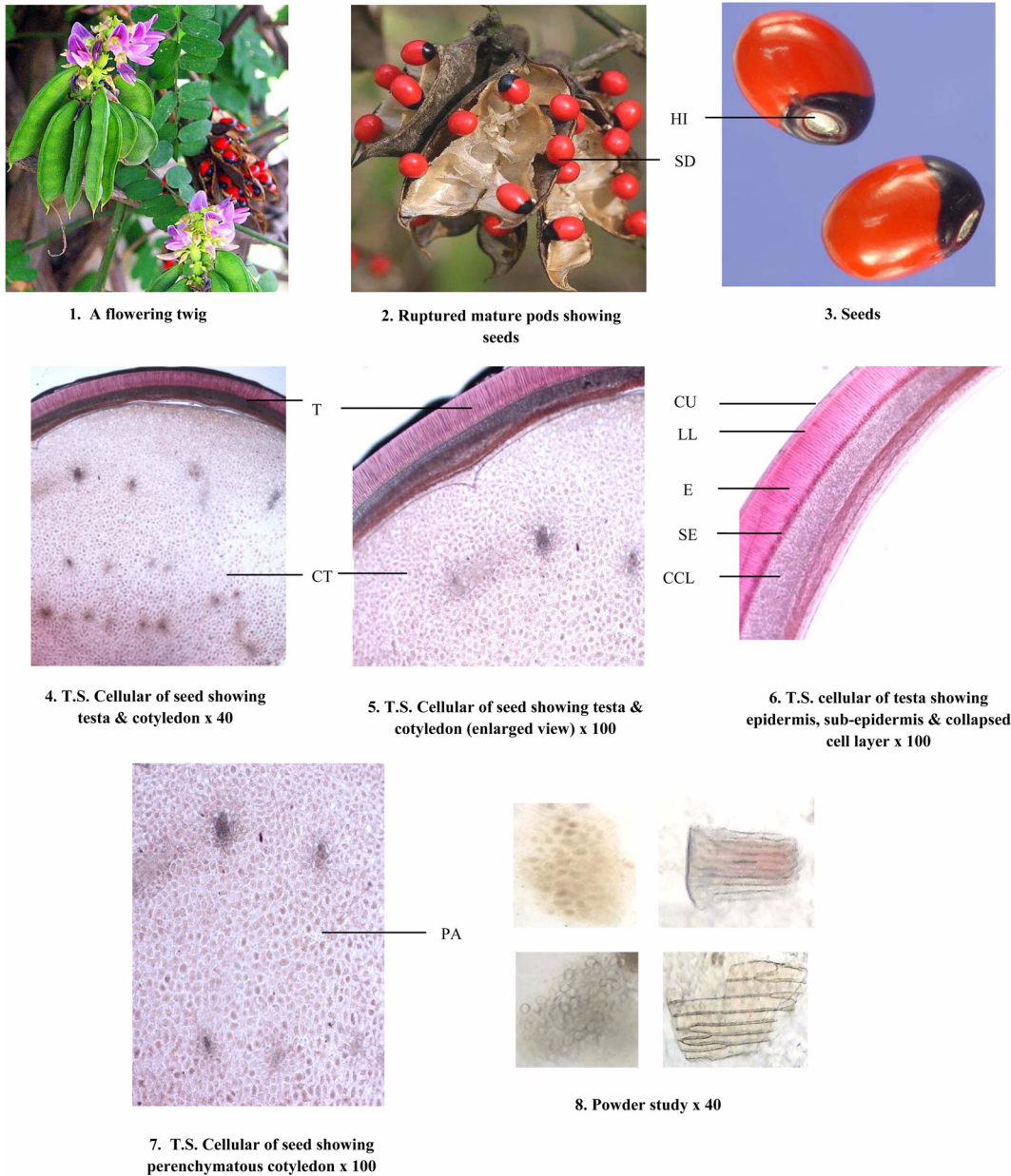
performed on Merck HPTLC percoated silica gel 60 GF<sub>254</sub> (20 × 10 cm) plates. Methanolic solutions of samples and standard compounds (Gallic acid & Glycyrrhizin,) of known concentrations were applied to layers as 6 mm wide bands positioned 15 mm from bottom and 10 mm from side of plate, using Camag Linomat 5 automated TLC applicator with nitrogen flow providing a delivery speed of 150 nl/s from application syringe. These conditions were kept constant throughout analysis of samples.

**Detection and Quantification of standards** – Following sample application, layers were developed in a Camag glass twin trough chamber (20 cm × 10 cm) that had been presaturated with mobile phase of Toluene: Ethyl acetate: Formic acid (50 : 40 : 10v/v) for Gallic acid and Chloroform: Glacial acetic acid: Methanol: Water (60 : 32 : 12 : 8) for Glycyrrhizin till proper separation of bands up to 80 mm height from point of application. After development, layers were dried with dryer and Gallic acid & Glycyrrhizin were quantified by using Camag TLC scanner model 3 equipped with Camag winCATS (version 3.2.1) software. Following scan conditions were applied: slit width, 4 mm × 0.45 mm; wavelength 254 nm for Gallic acid and Glycyrrhizin and absorption-reflection scan mode. In order to prepare calibration curves, stock solution of standards (100 ng spot<sup>-1</sup>) was prepared and various volumes of these solutions were analyzed through HPTLC, calibration curves were also prepared by plotting peak area vs. concentration. Calibration curve range (200 - 1200 ng spot<sup>-1</sup>) for Gallic acid and Glycyrrhizin were found to be linear.

**Determination of *In-vitro* anti oxidant activity (DPPH radical scavenging activity)** – Effect of extract on DPPH radical was estimated using method of Liyana-Pathirana and Shahidi, 2005. A solution of 0.135 mM DPPH (2, 2-Diphenyl-2-picrylhydrazyl) in methanol was prepared and 1.0 ml of this solution was mixed with 1.0 ml of extract in methanol containing 0.02 - 0.1 mg of extract. Reaction mixture was vortexed thoroughly and left in dark at room temperature for 30 min. Absorbance of mixture was measured at 517 nm using Double beam UV-Vis Spectrophotometer (Thermo Electron Corporation, Cambridge, England). Ascorbic acid and Gallic acid were used as references. Ability to scavenge DPPH radical was calculated by the following equation.

$$\text{DPPH radical scavenging activity (\%)} = \left[ \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \right] \times 100$$

Where Abs<sub>control</sub> is absorbance of DPPH radical + methanol; Abs<sub>sample</sub> is absorbance of DPPH radical + sample extract/standard.



**Plate 1.** Macro and Microscopic characters of the seeds of *Abrus precatorius* L. CCL, collapsed cells layer; CT, cotyledon; CU, cuticle; E, epidermis; HI, hilum; LL, linea lucida; PA, parenchymatous cells of cotyledon; SD, seed; SE, Sub-epidermis & T, testa.

## Results

**Macroscopic Characters of the seed** – Seeds are about 5 - 7 mm long, 3 - 5 mm broad, ovoid, or sub-globular in shape. It is usually bright scarlet and has a jet black spot surrounding the hilum which is the point of attachment. Seed coat or testa is smooth and glossy and becomes hard when seed matures (Plate. 1)

**Microscopic Characters of the seed** – Transverse section of seed is circular in outline, shows testa 75 - 80  $\mu$

thick. Outer most layer of testa is cuticle, greater parts being formed by epidermis, composed of radially, much elongated palisade like cells, arranged irregularly and measure 45 - 50  $\mu$  in length, inner region of thin testa consists of collapsed cells forming a hyaline layer about 25  $\mu$  thick, endodermis composed of thick-walled cellulosic parenchyma, isodiametric cells larger towards inside, walls mainly of hemicellulose and swell considerably in water, outer one or two layers of cells of endodermis (pseudoeperidermis) formed of rather smaller cells, walls of

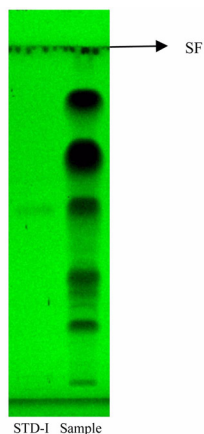


Fig.1a. HPTLC profile of *A. precatorius* seeds along with Glycyrrhizin (STD-I) under 254 nm.

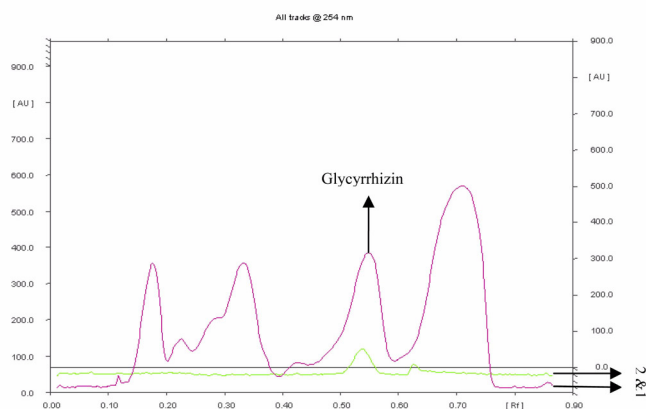


Fig.1b. HPTLC Chromatogram of *A. precatorius* seeds sample (1) along with STD-I (2)

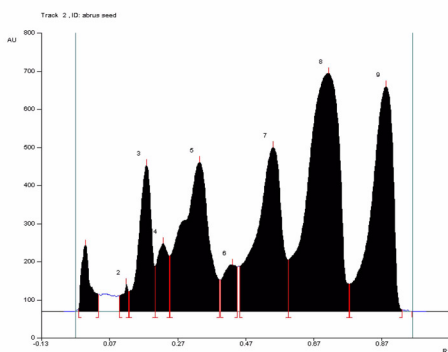


Fig.1c. HPTLC densitometric scan (at 254 nm) of methanolic extract of *A. precatorius* seeds.

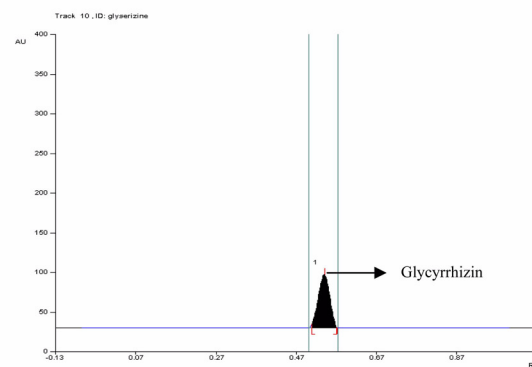


Fig.1d. HPTLC densitometric scan (at 254 nm) of Glycyrrhizin (STD-I)

Fig. 1. Detailed HPTLC study of *A. precatorius* seeds with Glycyrrhizin as reference compound.

Table 1. Fluorescence of the powder prepared from *A. precatorius* seeds

S.No.	Treatment	Day light	UV- light	
			UV-254 nm	UV-366 nm
1.	Powder (P) alone	Creamish yellow	Light brown	Brown
3.	P + 1N NaOH in water	Yellowish green	Greenish brown	Dull brown
4.	P + 1N NaOH in methanol	Fluorescent green	Yellowish cream	Brown
5.	P + 50% KOH	Yellowish green	Green	Brownish black
6.	P + 1 N HCl	Creamish red	Creamish brown	Greyish brown
7.	P + 50% H <sub>2</sub> SO <sub>4</sub>	Yellowish brown	Dark green	Dark brown
8.	P + 50% HNO <sub>3</sub>	Yellowish brown	Greenish yellow	Dark brown
9.	P + Glacial acetic acid	Creamish grey	Creamish grey	Greyish brown
10.	P + Conc. H <sub>2</sub> SO <sub>4</sub>	Brownish red	Dark green	Brown
11.	P + I <sub>2</sub> water	Brownish red	Greenish brown	Dark brown
12.	P + Methanol	Creamish yellow	Creamish grey	Creamish brown
13.	P + Acetone	Yellowish cream	Creamish	Greenish brown
14.	P + Ethanol (95%)	Creamish grey	Creamish grey	Greenish brown

which swell to less extent in water. Cotyledons are composed of wide radially elongated parenchymatous

mesophyll tissues (Esau, 1960; Trease & Evans, 2002) (Plate. 1).

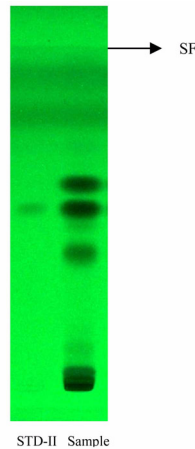


Fig.2a. HPTLC profile of *A. precatorius* seeds along with Gallic acid (STD-II) under 254 nm.

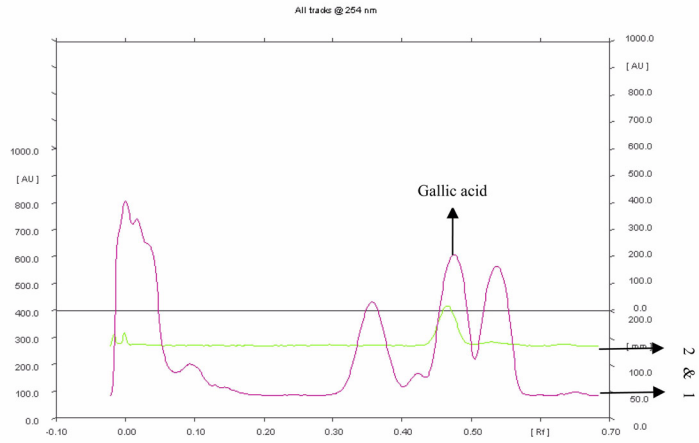


Fig.2b. HPTLC Chromatogram of *A. precatorius* seeds sample (1) along with STD-II (2)

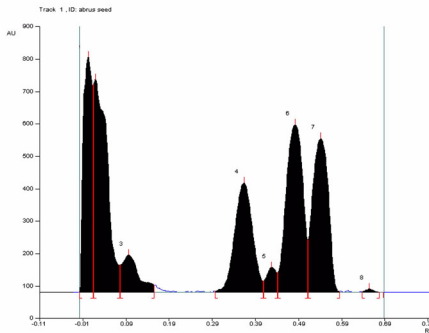


Fig.2c. HPTLC densitometric scan (at 254 nm) of methanolic extract of *A. precatorius* seeds.

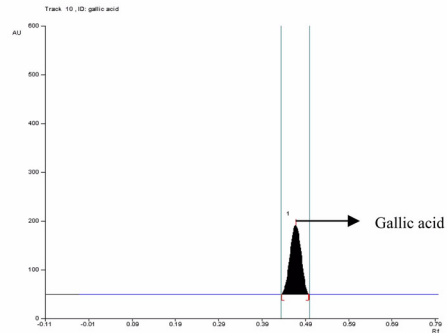


Fig.2d. HPTLC densitometric scan (at 254 nm) of Gallic acid (STD-II)

Fig. 2. Detailed HPTLC study of *A. precatorius* seeds with Gallic acid as reference compound.

Table 2. Phytochemical analysis of various extracts of *A. precatorius* seeds

Phytochemicals	Hexane extract	Chloroform extract	Acetone extract	Methanol extract	Water extract
Steroids	+	+	-	-	-
Triterpenoids	-	-	-	-	-
Sugars	-	-	-	+	+
Alkaloids	-	-	-	+	-
Tannins	-	-	-	+	+
Flavonoids	-	-	+	-	-
Proteins	-	-	-	+	+
Glycosides	-	-	-	-	+
Saponins	-	-	-	+	+

(+: present; - : absent)

Powder creamish yellow coloured, possess a characteristic bitter odour and taste. Powder shows fragments of palisade like cells of epidermis, parenchymatous cells of

cotyledons, collapsed cells of hyaline layer and isodiametric endodermal cells.

**Fluorescence analysis** – The powder when treated

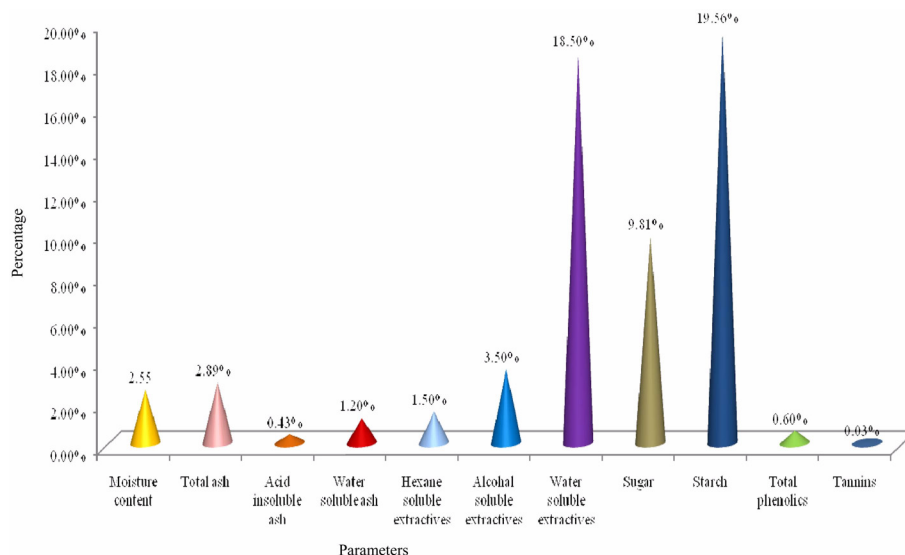


Fig. A. Physicochemical Values of *A. precatorius* seeds.

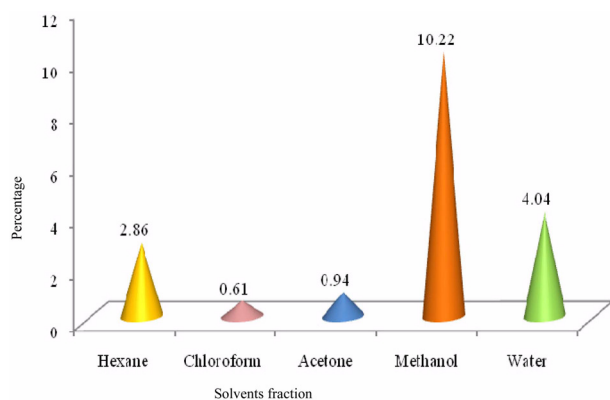


Fig. B. Successive Soxhlet extractive values of *A. precatorius* seeds.

with 1N NaOH in methanol, it showed fluorescent green colour under day light (Table 1).

**Preliminary phytochemical screening** – 5 gm of dried seeds powder was extracted in Soxhlet with hexane, chloroform, acetone, Methanol, and water successively and percentage was calculated (Fig. 2) and tested for different constituents namely steroids and triterpenoids (Liebermann-Burchard's test), flavonoids (Shinodas test), alkaloids (Mayer's reagent), tannin (Ferric chloride test), sugar (Fehling solution test), saponins (foam test) and protein (Ninhydrin test). Study revealed that steroids are present in hexane soluble parts, proteins, reducing sugars, tannins and saponins in methanol and water soluble part while glycosides only in water soluble part, alkaloid in methanol soluble part and flavonoids only in acetone soluble part (Table 2).

**Physico-Chemical Studies** – Physico-chemical studies

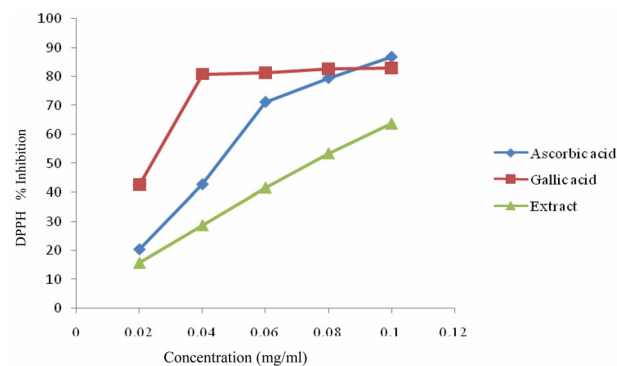


Fig. C. DPPH radical scavenging activity of the methanolic seed extract of *A. precatorius*.

of air dried plant material gave following values: moisture 2.55% total ash 2.89%, acid insoluble ash 0.43%, water soluble ash 1.20%, hexane soluble extractives 1.50%, alcohol soluble extractives 3.50%, water soluble extractives 18.50%, sugar 9.81%, starch 19.56%, total phenolics 0.60 % and tannins 0.03% (Fig. A). Successive Soxhlet extractives were found as follows hexane 2.86%, chloroform 0.61%, acetone 0.94%, methanol 10.22%, and water 4.04 % (Fig. B).

**HPTLC Studies** – On quantitative HPTLC analysis methanolic extract of *A. precatorius* seeds gave: Gallic acid - 0.4018% and Glycyrrhizin - 0.4009%.  $R_f$  values for standards were as follows Gallic acid  $R_f$   $0.47 \pm 0.02$  and Glycyrrhizin  $R_f$   $0.57 \pm 0.02$  (Fig. 1-2).

**DPPH radical scavenging activity** – Figure C shows dose-response curve of DPPH radical scavenging activity of the seeds of *A. precatorius* with gallic acid and

ascorbic acid. It was observed that at concentration of 0.1 mg/ml the scavenging activity of seeds found 63.70%.

### Discussion

Pharmacognostic characters and 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 easily identified using these parameters. Effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability. Though the DPPH radical scavenging abilities of the extracts were significantly less than those of ascorbic acid (86.8%) and gallic acid (78.8%), study showed that extract have proton donating ability and could serve as free radical inhibitor or scavengers, acting possibly as primary antioxidants. Plant extract showed 63.70% radical scavenging ability at 0.1 mg/ml concentration. Polyphenols are major plant compounds with antioxidant activity. This activity is believed to be mainly due to their redox properties, which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen or decomposing peroxides. Result strongly suggests that phenolics are one of the components of this plant part, and some of its pharmacological effects could be attributed to the presence of these valuable constituents. Besides, in the present study quantitative determination of four active marker components has been carried out which will be helpful in the effective use of this medicinal plant.

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### References

- Adelowotan, O., Aibinu, I., Adenipekun, E., and Odugbemi, T., The in-vitro antimicrobial activity of *Abrus precatorius* L. Fabaceae extract on some clinical pathogens. *Niger Postgrad Med J.* **15**(1), 32-37 (2008).
- Anonymous, *Ayurvedic Pharmacopoeia of India*, Part I, vol I. Department of Health, Ministry of Health and Family Welfare, Government of India, New Delhi, pp. 152-165, 2004.
- Anonymous, *Indian Pharmacopoeia*, Govt. of India, Ministry of Health and Welfare, Controller of Publications, New Delhi, pp. 191, 2007.
- Anonymous, *International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use: Harmonised Tripartite Guideline on Validation of Analytical Procedures: Methodology, Recommended for Adoption at Step 4 of the ICH Process by the ICH Steering Committee*, IFPMA, Switzerland, 1996.
- Anonymous, *Official methods of Analysis* (AOAC), 4<sup>th</sup> edn. Association of Official Chemists, Inc. U.S.A., pp. 187-188, 1984.
- Anonymous, *Quality control methods for medicinal plant materials*, World Health Organization, Geneva, pp. 4-21, 1998.
- Anonymous, *Wealth of India - A Dictionary of Indian Raw Materials and Industrial Products*, vol. I, National Institute of Science Communication, CSIR, New Delhi, pp. 2-3, 1985.
- Asolkar, L.V., Kakkar, K.K., and Chakre, O.J. *Second Supplement to Glossary of Indian Medicinal plants with Active principles*, Part-I National Institute of Science Communication, CSIR, New Delhi, pp. 3-6, 2000.
- Bhutia, S.K., Mallick, S.K., Maiti, S., and Maiti, T.K., Antitumor and proapoptotic effect of *Abrus* agglutinin derived peptide in Dalton's lymphoma tumor model. *Chemico-Biological Interactions.* **174**, 11-18 (2008).
- Chase, C.R. and Pratt, R.J., Fluorescence of powdered vegetable drugs with particular reference to development of a system of identification. *J. Am. Pharm Assoc.* **38**, 324-333 (1949).
- Chopra, R.N., Chopra, I.C., Handa, K.L., and Kapur, L.D., *Indigenous drugs of India*, 2<sup>nd</sup> ed. U. N. Dhur and Sons Pvt. Ltd. Calcutta, pp. 260-262, 1958.
- Duke, A.K., *Handbook of Medicinal Herbs*, CRC Press Boca Raton London New York Washington, D.C., pp. 3-4, 2001.
- Esau, K., *Anatomy of seed plants*, John Wiley and Sons, New York, pp. 326-334, 1960.
- Ghosal, S. and Dutta, S.K., Alkaloids of *Abrus precatorius*. *Phytochemistry.* **10**, 195-198 (1971).
- Hart, M., Jequirity bean Poisoning. *N Engl J Med.* **268**, 885-886 (1963).
- Hooker, J.D., *The Flora of British India*, vol.II, pp. 175, 1961.
- Issar, R.K. and Israili, A.H., Pharmacognostic studies on the Unani drug "Ghongchi-Safaid" (*Abrus precatorius* L. seed). *JRIMYH.* Vol. XIII, **4**, 34-44 (1978).
- Johansen, D.A., *Plant Microtechnique*, 1st edn. MC Graw Hill Book Co. Inc., New York and London, pp. 15-93, 1940.
- Kirtikar, K.R. and Basu, B.D., *Indian Medicinal Plants*, vol. I, L.M. Basu Pub. Allahabad, pp. 764-765, 1933.
- Kokoski, J., Kokoski, R., and Slama, F.J., Fluorescence of powdered vegetable drugs under ultraviolet radiation. *J. Amer. Pharmacol. Assoc.* **47**, 75 (1958).
- Ligha, Ae., Jaja, Bnr1. and Numbere, N.f., Protective Effect of *Abrus precatorius* Seed Extract following Alcohol Induced Renal Damage. *European Journal of Scientific Research.* **25**, 428-436 (2009).
- Liyana-Pathiranan, C.M. and Shahidi, F., Antioxidant activity of commercial soft and hard wheat (*Triticum aestivum*) as affected by gastric pH conditions. *Agr Food Chem* **53**, 2433-2444 (2005).
- Monago, C.C. and Alumanah, E.O., Antidiabetic Effect of Chloroform-Methanol Extract of *Abrus Precatorius* Linn Seed in Alloxan Diabetic Rabbit. *Journal of Applied Sciences & Environmental Management.* **9**, 85-88 (2005).
- Peach, K. and Tracy, M.V., *Modern Methods of Plant Analysis*. Vol III and IV Springer, Heidelberg, pp. 258-261, 1955.
- Singh, S., Pandey, P., and Kumar, S., *Traditional knowledge on the medicinal plants of Ayurveda*, Central Institute of Medicinal and Aromatic Plants, Lucknow, pp. 290-296, 2000.
- Trease, G.E. and Evans, W.C., *Pharmacognosy*, 10<sup>th</sup> edn. Berilliee, Tindal, London, pp. 31-32, 2002.
- Wagner, H. and Bladt, S., *A thin layer chromatography Atlas, Plant Drug Analysis*, 2<sup>nd</sup> ed. Springer, pp. 305-314, 1996.

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