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### RESEARCH ARTICLE

#### ESTABLISHMENT OF QUALITY STANDARDS OF *ABRUS PRECATORIUS* L. (ROOT) BY PHYSICOCHEMICAL AND HPTLC METHOD

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#### Abstract

Standardization of herbal drugs is an essential aspect while considering the quality of the herbal drug, consistency in bioactive content and therapeutic efficacy. The roots, seeds and leaves of *Abrus precatorius* L. possesses a number of biological activities such as anti-bacterial, anti-cancer, anti-diabetic, anti-fertility, nephroprotective and anti-seratnergic activity. The root of *Abrus precatorius* L. contains a special compound glycyrrhizin, which is an important bioactive compound widely used against cough in the Siddha, Unani and Ayurvedic system of medicines. In view of its medicinal importance, the present study was focused to standardize the *Abrus precatorius* L. root according to pharmacopoeial standard methods. Qualitative physicochemical studies and High Performance Thin Layer Chromatography (HPTLC) analysis were performed to identify and to assure the quality of the herbal drug.

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#### Introduction:-

In recent days, peoples are showing great interest towards the traditional medicine in preventing various diseases, because of its cheap, safe and effective effects [1]. The traditional medicinal plants are major raw materials for pharmaceutical industry and gaining importance in worldwide drug market. The unavailability of rigid quality control profiles for herbal materials are facing major problem in herbal industry [2]. Hence, the proper standardization and identification of herbal materials is important in order to contribute the assurance of safety, quality, efficacy and reproducibility of the herbals [3]. HPTLC is one of the most ideal, flexible, reliable and cost effective separation techniques for the analysis of botanicals and herbal drugs. This technique is also used for the identification of chemical constituents, determination of impurities and quantitative determination of active substances. It also serves as a vital technique in the routine identification of complex finger prints of plant extracts, pharmaceutical products and guarantees reproducible results [4].

*Abrus precatorius* L. belongs to Fabaceae (Leguminosae) - pea family, abundantly found throughout India mainly Himalaya to southern India and Ceylon [5]. The leaf of *Abrus precatorius* L. contain bioactive compounds like abrine, inositol, abruslactone, abrusoside A, B, C, D, etc. which is used to cure various diseases like aphrodisiac, leucoderma, itching, skin diseases, eye diseases, wounds and removes biliousness [6]. Seeds contain abrine, abrin A, B, C, I, II, III, abrus agglutinin, saponin, lectin, flavonoids, abrectorin, arecatorin, campestanol etc and are considered for abortifacient, anodyne, aphrodisiac, antimicrobial, diuretic, emetic, hemostat, laxative, purgative,

refrigerant, sedative, expectorant, emollient, febrifuge, vermifuge and antidote. Hence, it is used in various ailments such as headache, snakebite, blennorrhagia, diarrhea, fever, gastritis, gonorrhea, jaundice, malaria, boil, cancer, cold, colic, conjunctivitis, convulsion, cough, night-blindness, ophthalmia, rheumatism, diabetes and chronic nephritis [7]. Root of this plant contains abrol, abrasine, precasine, precol and some proteins like abraline, abricin, delphinidin, gallic-acid, picatorine etc. It also contains triterpenoids, saponins, glycyrrhizin, oleanolic acid and abrusosides a, b, c, d [8]. The root is considered as emetic, diuretic, and alexiteric. The watery root extract is useful to treat diarrhoea, gonorrhea, jaundice, insomnia, bronchitis, hepatitis, cancer, gastritis, heart diseases, kidney diseases, CNS sedative and to relieve obstinate coughs [9, 10]. Chewing of root can be used as a remedy from snake bite [11]. It is also used as an anti-malarial and anticonvulsant drug [12]. Glycyrrhizin is an important bioactive component is widely used in the pharmaceutical and food industry [13].

The present work is concentrated on standardization of *Abrus precatorius* L. root. The development of chromatogram serves as a template for identification and authentication of *Abrus precatorius* L. root.

## Materials and Methods:-

### Plant material

*Abrus precatorius* L. root were collected around Chennai and were identified at Drug Standardization Research Unit, Regional Research Institute of Unani Medicine, Chennai, Tamil Nadu, India. The collected root was washed, dried, pulverized and stored in an air tight container for further studies.

### Standardization Parameters

The powdered *Abrus precatorius* L. root were subjected to qualitative physico-chemical parameters such as foreign matter (%), moisture content (%), total ash (%), acid-insoluble ash (%), pH and extractive values (%) were carried out as per IPC approved standard methods [14].

### Development of HPTLC Finger print profile

The HPTLC analyses were performed on aluminum plates pre-coated with silica gel 60 F254 (Merck, Germany). 8 µl of root extract were applied on the plate of 5 x 10 cm by HPTLC as bands of 8 mm of each with help of CAMAG ATS4 sample applicator. The plates were developed in a CAMAG twin-trough chamber previously equilibrated with a mobile phase for 30 mins. The solvent system of Toluene: Ethyl acetate: Formic acid (7.2: 2.8: 0.01) were used to develop HPTLC finger print profile. The plates were developed upto 8 cm, air dried and scanned at wavelength of 254 and 366 nm using CAMAG TLC Scanner. After the chromatogram recorded the plates were derivatized with Vanillin Sulphuric acid and heated at 105°C on hot plate till the development of color of bands and observed under UV and white light.

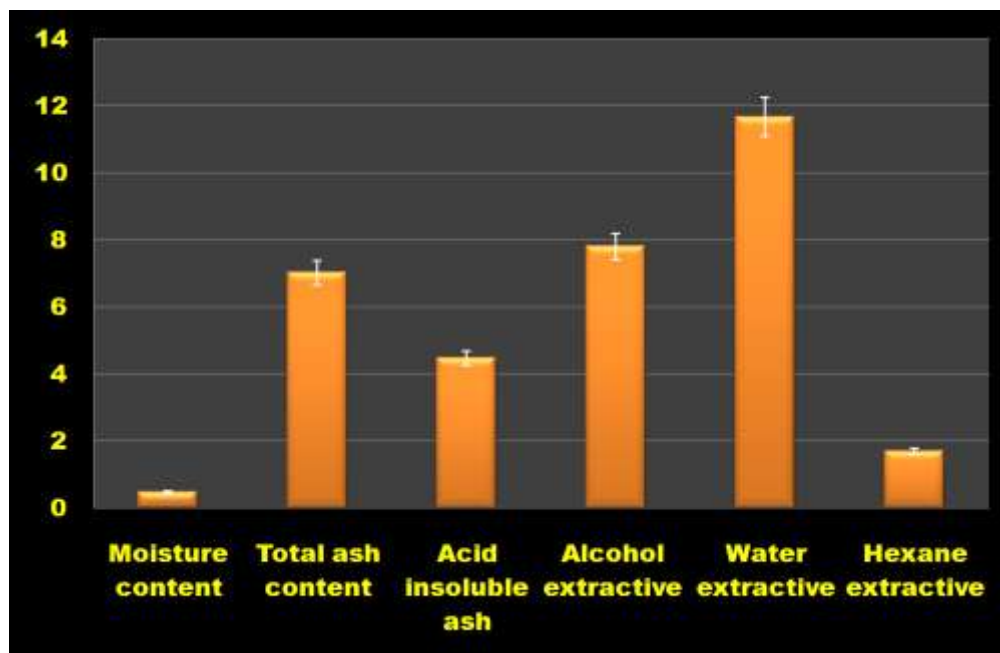
## Results and Discussion:-

The root of *Abrus precatorius* L. contains important source of bioactive constituents for the growth of new chemotherapeutic agents. The standardization of crude drug helps to determine the quality and purity of the drug in the powdered form. The root of *Abrus precatorius* L. subjected to physicochemical parameters such as foreign matter, moisture content, ash values, extractive values and their observations were depicted in **Table 1 & Graph 1**. The foreign matter studies used to determine the contaminations such as stones, sand, harmful and poisonous foreign matter and presence of chemical residues. This parameter used to eliminate the toxin materials present in the plant materials. The moisture content analysis of the drug should be minimized in order to prevent from decomposition due to chemical change or contamination of crude drug. The ash value studies are used to determine the purity and quality of crude drugs. The low ash values specify the purity of drug and the high ash values indicate the adulteration, substitution and contamination of crude drug.

Extractive values analysis used to evaluate the chemical constituents present in the herbal drug and also helps to identify the solubility nature of specific components in each solvents. These values mainly used to estimate the exhausted and adulterated drugs. Water-soluble extractives are used to determine the water soluble active component of herbal drugs such as sugar, glycosides, tannins, acids, mucilage etc. Alcohol-soluble extractive values are an important method to estimate the various components like resins, tannins, saponins, alkaloids etc. Hexane-soluble extractives are indicative of volatile and non volatile substances.

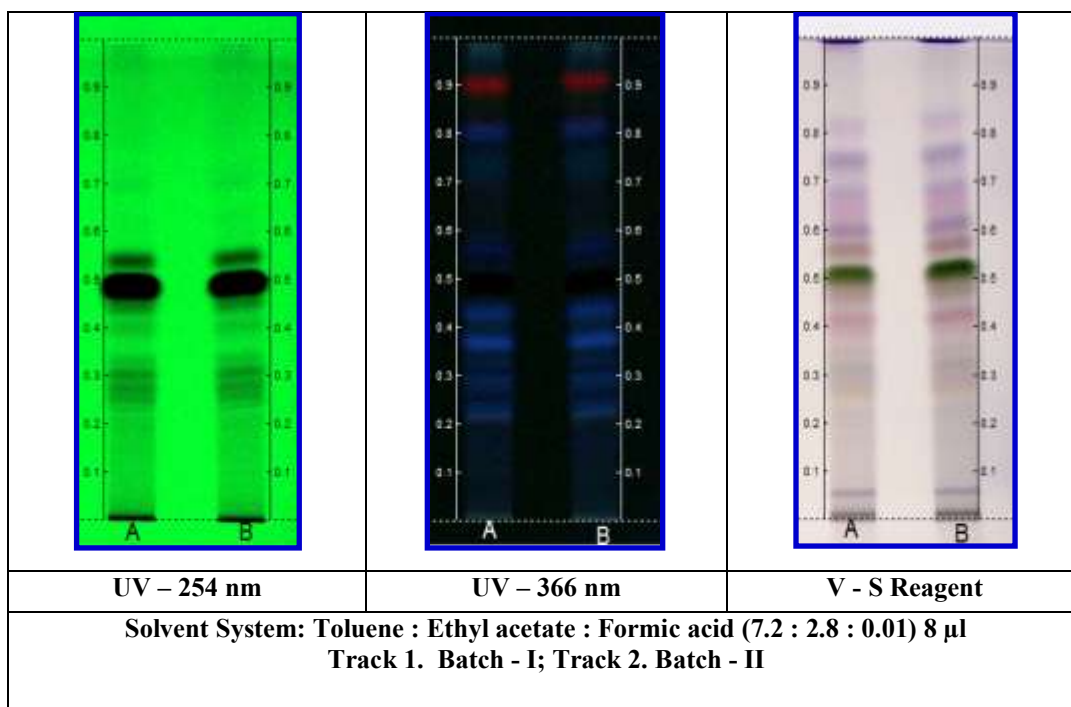
**Table 1:-** Physicochemical parameters of *Abrus precatorius* L. root.

Parameters analyzed	Results
Foreign Matter (% w/w)	0.02 %
Moisture content (% w/w)	0.47 %
Total ash content (% w/w)	7.01 %
Acid-insoluble ash content (% w/w)	4.46 %
Alcohol-soluble extractive matter (% w/v)	7.80 %
Water-soluble extractive (% w/v)	11.65 %
Hexane soluble extractive (% w/v)	1.68 %
pH values (5% Aqueous solution)	5.5

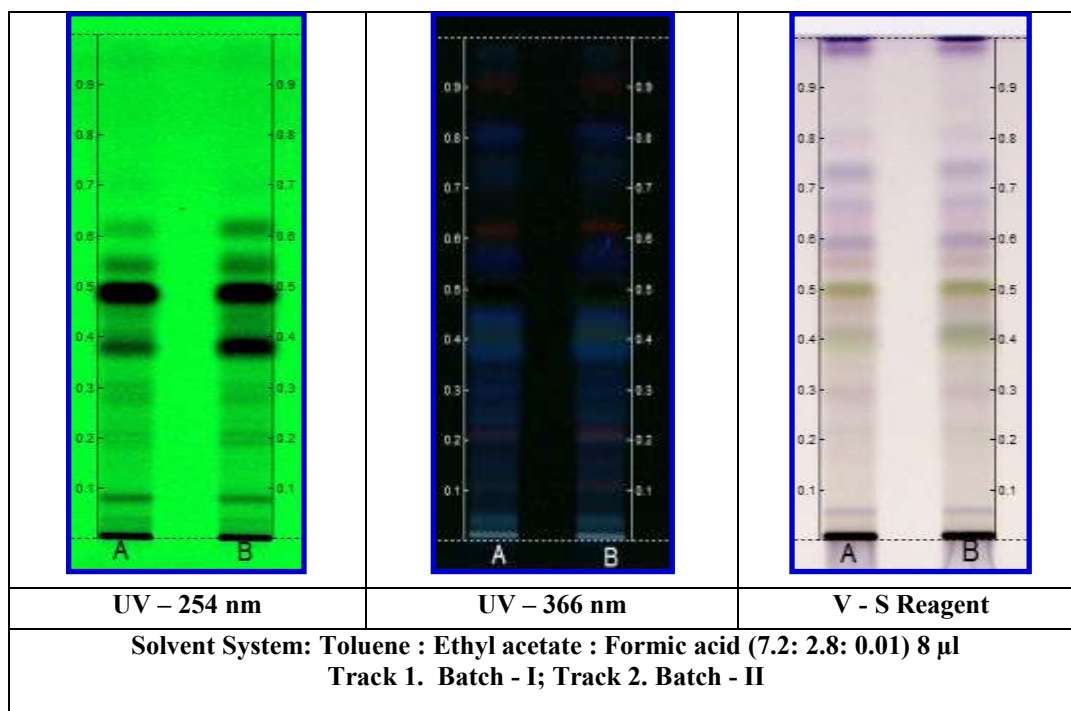
**Graph 1:-** Physicochemical parameters of *Abrus precatorius* L. root L.

HPTLC analyses plays significant role in preliminary separation and determination of chemical constituents present in the plant materials. This technique is also efficient for quick evaluation of the quality of herbal drug. The qualitative chromatographic analysis for *Abrus precatorius* L. root extracts are studied in two different solvents such as ethanol and chloroform. In both the solvent, numbers of spots were observed under UV 254 and 366 nm is shown **Figure 1 & 2**.

The HPTLC finger print profiles of *Abrus precatorius* L. root in alcohol and chloroform extract are shown in **Figure 3 to 6**. The  $R_f$  values are the evidence for the presence of specific compounds in chloroform and alcohol extracts of *Abrus precatorius* L. root. The difference in  $R_f$  values in most of the appeared peaks reflected qualitative variation in the phytochemicals. Densitometry is an instrumental technique that is more accurate than visual. In this method the resolved spots are scanned and their densities were determined with a densitometer. Densitometric chromatogram of both chloroform and alcohol extracts of *Abrus precatorius* root at two different wavelengths 254 and 366 nm are depicted (**Figure 7 & 8**). After scanning the plate was dipped in vanillin-sulphuric acid reagent followed by heating at 110°C about 5 min and observe under visible light, the plate shows major spots are shown in **Figure 1 & 2**.



**Figure 1:-** Thin Layer Chromatography of Chloroform extract of *Abrus precatorius* L. root.



**Figure 2:-** Thin Layer Chromatography of Ethanol extract of *Abrus precatorius* L. root.



**Figure 3:-** HPTLC finger print and  $R_f$  values of *Abrus precatorius* L. root in Chloroform extract at 254 nm (Absorbance mode).



**Figure 4:-** HPTLC finger print and  $R_f$  values of *Abrus precatorius* L. root in Chloroform extract at 366 nm (Absorbance mode).



**Figure 5:-** HPTLC finger print and  $R_f$  values of *Abrus precatorius* L. root in Ethanol extract at 254 nm (Absorbance mode)





Figure 6:- HPTLC finger print and  $R_f$  values of *Abrus precatorius* L. root in Ethanol extract at 366 nm (Absorbance mode)

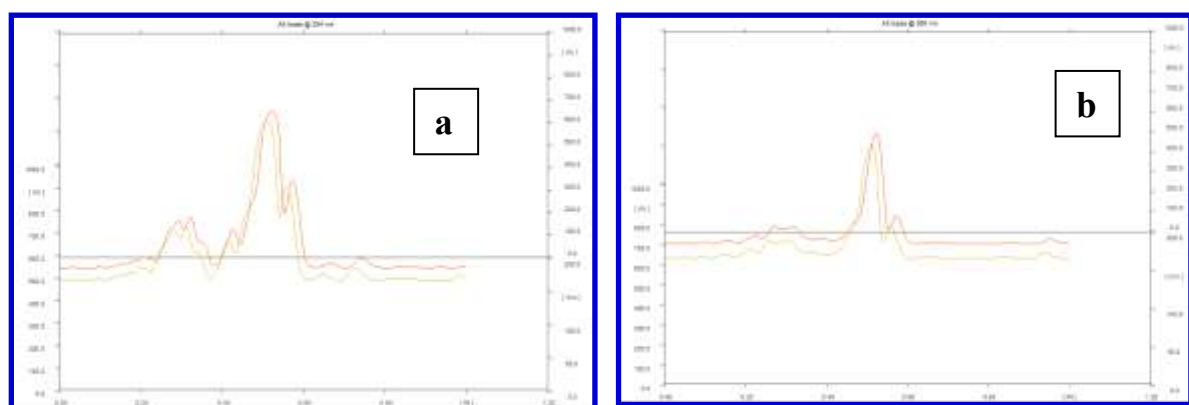


Figure 7:- Densitometric chromatogram of *Abrus precatorius* L. root in Chloroform extract at a) 254 and b) 366 nm (Absorbance mode).

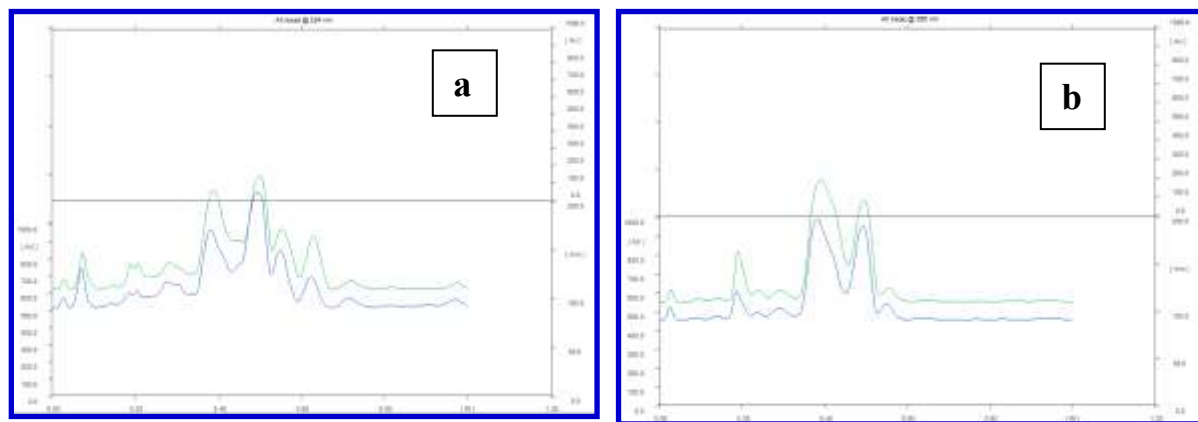


Figure 8:- Densitometric chromatogram of *Abrus precatorius* L. root in Ethanol extract at a) 254 and b) 366 nm (Absorbance mode).

### Conclusion:-

Physicochemical standards are great significance in assuring the quality, authenticity as well as purity and efficacy of the drug. The results indicated that the chloroform and alcohol extracts of *Abrus precatorius* L. root contained a number of bioactive compounds. Hence, the qualitative evaluation of HPTLC finger print profiles could be useful in the authentication and quality control of the drug and to ensure the therapeutic efficacy.

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