European Journal of Academic Essays 2(2): 65-69, 2015
ISSN (online): 2183-1904
ISSN (print): 2183-3818
www.euroessays.org

# Chromatographic Fingerprint Profile of Alkaloids of *Abrus precatorius* Linn. by HPTLC

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**Abstract:** Fingerprint analysis approach using HPTLC profile has become the most potent tools for quality control of herbal medicines because of its simplicity and reliability. It can serve as a tool for identification, authentication and quality control of herbal drugs. The aim of the present study is to determine the chemical profile and alkaloid composition of the medicinally important plant *Abrus precatorius* Linn. Leaves of plant under study, exhibit antifertility activity and is used in preparation of herbal medicine1. Hence to prove its authenticity and diversity in Alkaloid composition, the development of chemical fingerprint is need of the hour. The study was planned to develop a fingerprint profile of leaf extract of *Abrus precatorius* Linn. Alkaloidal fraction from the leaves of this plant was developed in the mobile phase - Toluene : Ethyl acetate : Diethyl amine ( 7:2:1) and scanned under visible light at 540 nm and UV at 254 nm & 366 nm. Chromatogram was then derivatized with Dragendroff's reagent followed by heating at 105 0C. The plant showed specific fingerprints at 254 nm and 366 nm. HPTLC fingerprints of alkaloidal fraction were obtained and Rf values were recorded. The alkaloidal bands can be used to discover bioactive products that may serves leads for the development of the new pharmaceuticals that address hither to unmet therapeutic needs.

**Keywords:** *Abrus precatorius* Linn., HPTLC fingerprints, Alkaloids

## 1. Introduction

The leaves, root, stem and seeds of *Abrus precatorius* Linn. is used for its medicinal properties from ancient times. The leaves are pinnate and glabrous, with many leaflets (12 or more) arranged in pairs. The leaflets are oblong, measuring 2.5 cm long and 1.5 cm wide[1]. Hot water extract of dried leaves and roots are applied to the eye for eye diseases[2]. Water extract of leaves and stem is taken orally by males as an aphrodisiac and by females to facilitate child-birth[3]. Decoction of dried leaves and root boiled in milk is used as a tonic[4]. Fresh leaf juice is taken orally for coughs. Fresh leaves are taken orally for coughs[5]. The leaves and roots are sweetish and traditionally used to cure fever, stomatitis, asthma, bronchititis and wound healing property [6-7].

The medicinal properties of a plant is attributed to wide array of secondary metabolites found in plants. The present work is concentrated on one such secondary metabolite - alkaloids in leaves of *Abrus precatorius* Linn. Development of chemical fingerprints using HPTLC is an effective tool for linking the chemical constituents' profile of the plant with botanical identity of plants[8]. The development of chromatogram of Alkaloid extract as a whole serves as template for identification and authentication of plant material thus negating the use of expensive marker compounds.

## 2. Material and Methods

*Abrus precatorius* Linn. was collected from Western Ghats region of Sindhudurg District of Maharashtra and was identified at Department of Botany, Elphinstone College. The leaves were air dried for 12 days, pulverized and stored in an air tight container for preliminary pharmacognostic and HPTLC finger print studies. The alkaloidal extract was prepared and was used for HPTLC studies.

### 2.1 Test for alkaloids

About 5 gms. of the dry crushed plant material was treated with liquor ammonia for 40 to 60 minutes and then extracted in ethanol for 48 hours at 30°C. The ethanol extract was distilled under vacuum, transferred to a china clay dish, and evaporated to dryness. The dried extract was treated with dilute H2SO4 . The acid extract was centrifuged and tested for alkaloids with Dragendroff's reagents.[9-10]

### 2.2 Preparation of Alkaloidal fraction:

The powdered material was wet with a half diluted NH4OH and lixiviated with ethyl acetate for 24 h at RT. The organic phase is separated from the acidified filtrate and basified with NH4OH (pH 11- 12).It was extracted with chloroform (3X), condensed by evaporation and used for chromatography. The alkaloid spots were separated using the

solvent mixture Toluene : Ethyl acetate : Diethyl amine ( 7:2:1) . The colour and Rf values of the separated alkaloids were recorded both under ultraviolet (254nm and 366nm) and visible light (540nm) before and after derivatising with Dragendorff 's reagent.[9-10]

**2.3 Development of HPTLC Fingerprint profile**:

The HPTLC analyses were performed on aluminum plates pre-coated with silica gel 60F254 (Merk, Germany). 5µl & 10 µl of extract were applied on the plate of 10 X 10 cm by HPTLC as bands of 10 mm width of each with the help of CAMAG linomat IV sample applicator. The plates were developed in a CAMAG twin- trough chamber previously equilibrated with a mobile phase for 20 minutes. The solvent system of Toluene : Ethyl acetate : Diethyl amine ( 7:2:1) was used to develop HPTLC fingerprint profile for alkaloids 4. The plate was developed up to 8 cm, air dried and scanned at wavelength of 254 & 366 nm using CAMAG TLC Scanner 3. The chromatograms were recorded. Then the plate was derivatized with Dragendorff 's reagents and heated at 105 0C on hot plate till the development of colour of bands and observed under UV and white light. The colour of recorded bands and Rf values were recorded[11].

## 3. Result

**3.1 Test for alkaloids:**

The positive test with Dragendroff's reagent indicates presence of Alkaloids.

**3.2 HPTLC Fingerprints of Alkaloids:**

HPTLC chromatogram was best observed before derivatization at 366 nm. 10µl aliquot showed better result and separation. The solvent system of Toluene : Ethyl acetate : Diethyl amine ( 7:2:1) showed better band separation.(Figure 1-3)

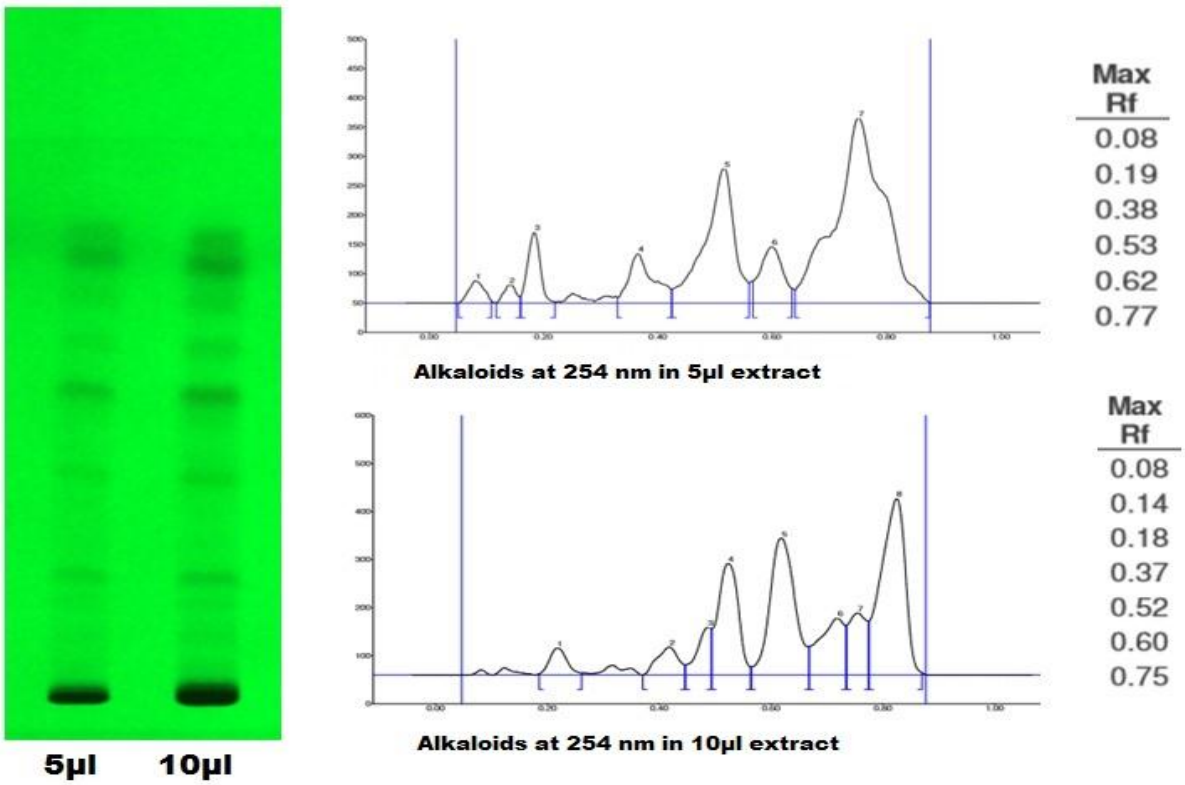


Figure 1: Chromatogram and densitogram with corresponding Rf values of alkaloids of *Abrus precatorius* Linn. at 254nm after derivatisation

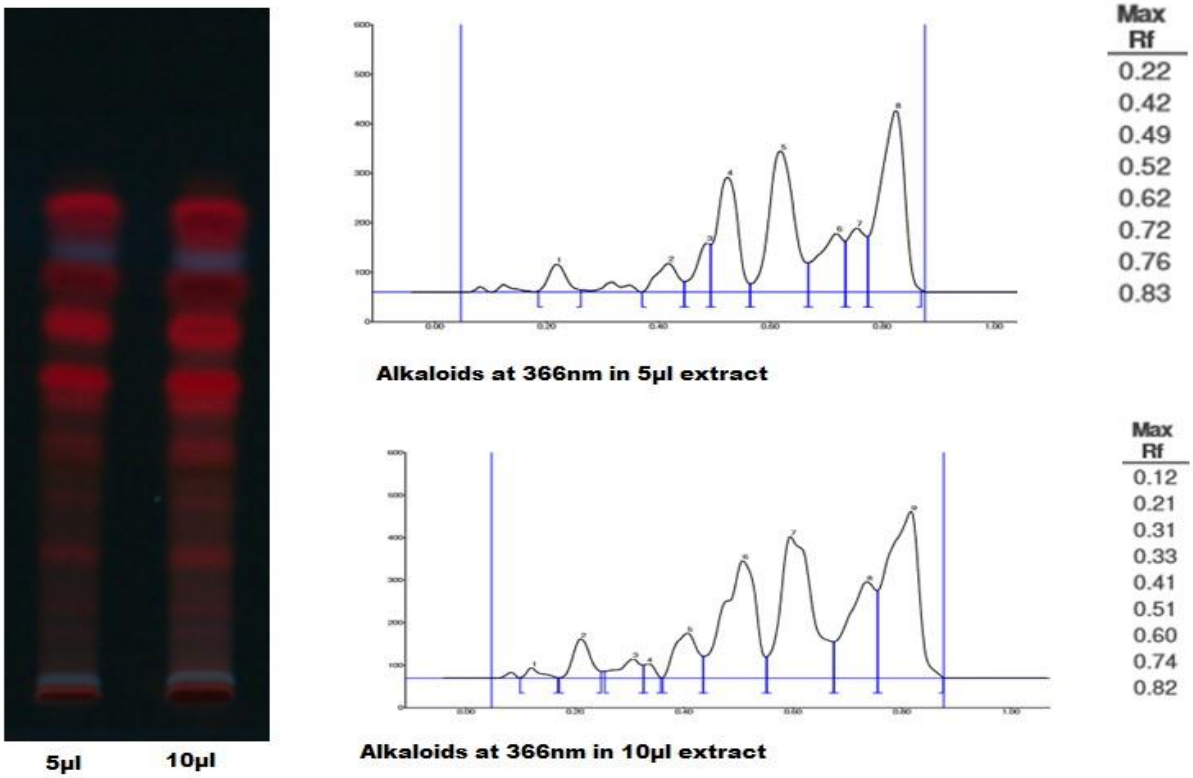


**Figure 2:** Chromatogram and densitogram with corresponding Rf values of alkaloids of *Abrus precatorius* Linn. at 366nm after derivatisation

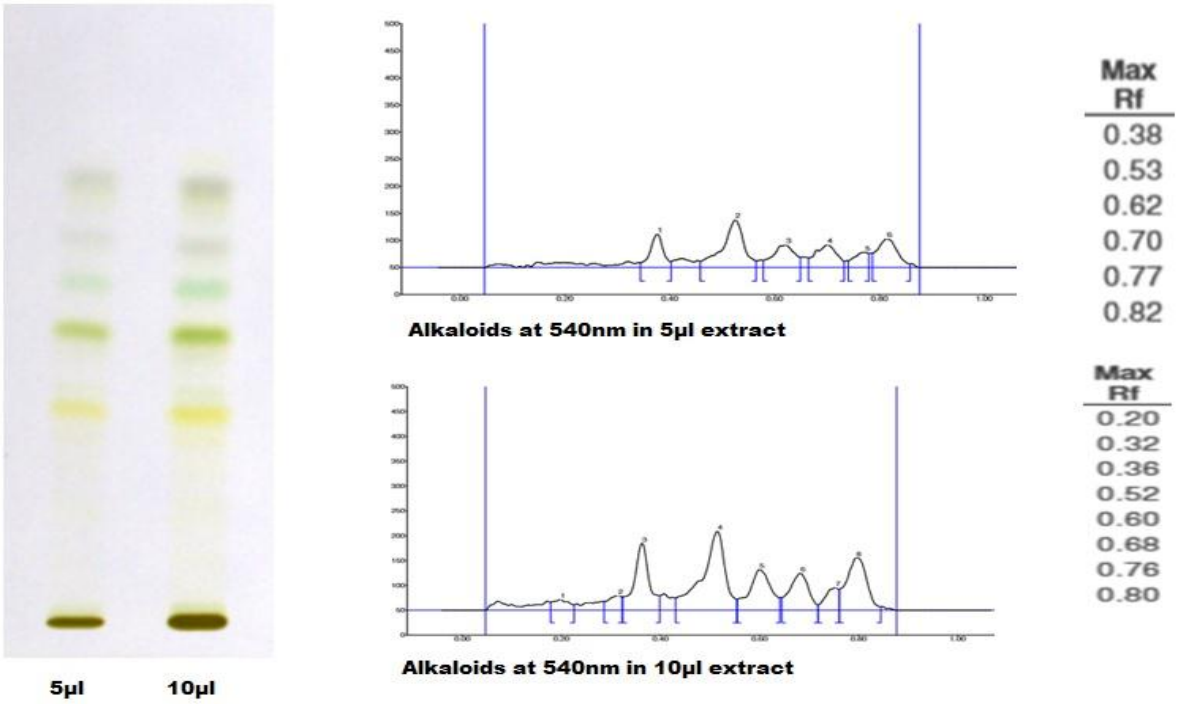


**Figure 3:** Chromatogram and densitogram with corresponding Rf values of alkaloids of *Abrus precatorius* Linn. at 540nm after derivatisation

## 4. Discussion

Alkaloids have potent physiological effects on mammalian systems as well as other organisms, and as a consequence, some constitute important therapeutic agents. Therefore determination of alkaloids is very importantly related to the quality of medicinal plants. Atropine, morphine, quinine and vincristine are representative of a host of alkaloids used to treat a range of disease conditions that range from malaria to cancer[12]. The plant shows good separation of alkaloids which can be worked upon. This fingerprint can also be used as a marker fingerprint for leaf of *Abrus precatorius* Linn. for the purpose of standardisation and authentication.

## 5. Conclusion

Standardisation of plant materials is the need of the day. An HPTLC fingerprint is suitable for rapid and simple authentication and comparison of subtle differences among samples of identical plant resource from different geographic locations[13]. The chromatographic fingerprint developed for this plant represents a comprehensive qualitative approach for the purpose of authentication, evaluation of quality and ensuring the consistency and stability of leaves of *Abrus precatorius* Linn. in herbal drugs and their related products. The plant can be used to discover bioactive products that may serves leads for the development of the new pharmaceuticals that address hither to unmet therapeutic needs. These plant derived bioactive compounds in addition of being developed directly as drugs can also served as prototype drug molecules known as “Lead Compounds” and as pharmacological probes to help better understand biochemical and physiological mechanisms[14]. Bioactivity guided fractionation can lead to the isolation of active principle of this plant and some of the chemical entities with acceptable pharmaceutical qualities can be developed as drugs in their original form directly. In addition to their medicinal use some secondary metabolites from these plants can also serve as powerful “pharmacological tool” to help explain the mechanism underlying human diseases and also serves as “phytochemical markers” for correct identification of plants[15].

## Acknowledgment

The author is thankful to University Grants Commission, New Delhi, India for funding the research project which helped me to carry out the present work.

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## Author Profile

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