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Phytochemical analysis of *Adhatoda vasica* and identification of an isolated alkaloid Vasicine using HPTLC

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Abstract

Adhatoda vasica commonly known as adosa is a small, evergreen shrub found in India and the world and known for its effectiveness in treating respiratory ailments. The present study was carried out to qualitatively screen phytochemical constituents present in *Adhatoda vasica* and to isolate and identify an alkaloid vasicine from the plant. Aqueous and methanolic extract of the leaves were prepared and subjected for qualitative phytochemical analysis. The phytochemical analysis revealed the presence of saponins, tannins, phenols, alkaloids, terpenoids, glycosides and cardiac glycosides in aqueous extract and alkaloids, terpenoids, flavanoids, phlobatanins, glycosides and cardiac glycosides in the methanolic extract. A quinazoline alkaloid vasicine was isolated from the leaves of the plant and identified by High Performance Thin Layer Chromatography. HPTLC revealed the presence of the alkaloid vasicine in the plant sample at a concentration of 1.5 percent. Thus the method was found to be more reliable, simple and easy for identification of vasicine in the plant.

Keywords: *Adhatoda vasica*, phytochemical screening, alkaloid, vasicine, HPTLC

Introduction

Adhatoda vasica, commonly known as adosa belongs to the family Acanthaceae. It is a small, evergreen shrub found in many regions of India and throughout the world, with a multitude of uses in Ayurveda and much well-known for treating respiratory ailments. The leaf extract has numerous phytochemical constituents, especially enriched with a potential source of quinazoline alkaloids - vasicolin, adhatodine, vasicolinone and anisotine (Jain, 1982) [6]. It exhibits vast pharmacological activities like anti-asthmatic, bronchodilator, wound healing, cholagogue, anti-allergic, antitubercular, abortifacient, uterotonic, insecticidal, anti-bacterial, anticestodal, anti-inflammatory, anti-oxidant, antitussive, hepatoprotective, anti-viral, thrombolytic, antifungal, antidiabetic and immunomodulatory activity (Singh *et al.*, 2017) [12]. Hence, this study was designed to find out the phytochemical constituents in the plant and to isolate the alkaloid vasicine using established procedure and identify vasicine using High Performance Thin Layer Chromatography.

Materials and Methods

The plant material mainly the leaves of *Adhatoda vasica* were collected from the medicinal garden of Department of Veterinary Pharmacology and Toxicology during the period of January 2020. The taxonomical identification of the plant was confirmed by the Gandhigram Rural Institute, Department of Biology, Dindigul district, Tamil Nadu with the specimen accession number 863. The collected leaves were dried under shade, blended to powder with a mechanical grinder and was passed through sieve No.40 and stored in air tight container until extraction of vasicine.

Qualitative phytochemical screening

Phytochemical screening of the herbal extracts was carried out using standard chemical methods for identifying the presence of phytochemical constituents (Trease and Evans, 2003) [14].

Extraction and Identification of vasicine

Extraction of vasicine was carried out by a novel and conventional method as described by Keesara and Jat (2017) [8]. A representative quantity of 300 gms of *Adhatoda vasica* leaf powder was taken for the isolation of the alkaloid. Petroleum ether was added to deaft the material and refluxed at 60°C with 12 cycles to obtain 56.20 g of methanolic extract. This was further subjected to extraction with water and dichloromethane, wherein a mass of 20 g was obtained. On further extraction, 2.82g of yellowish - green powder was isolated and subjected to purification as per the method of Sajeeb *et al.*, (2015) [10]. The final eluted compound was diluted to a final concentration of 1mg/mL of methanol and was subjected to High Performance Thin Layer chromatography (HPTLC,; ANCHROM - CAMAG; WINCATS Version 5.0) for identification of vasicine (Das *et al.*, 2005) [5]. The recovery rate was determined with six different concentrations for the reference standard (M/s. SIGMA, ALDRICH) and sample.

Results and Discussion

The phytochemical analysis revealed the presence of saponins, tannins, phenols, alkaloids, terpenoids, glycosides

and cardiac glycosides in aqueous extract and alkaloids, terpenoids, flavanoids, phlobatanin, glycosides and cardiac glycosides in the methanolic extract (Table 1)

Kumar *et al.*, (2013) [9] screened the aqueous leaf extract of *Adhatoda vasica* for secondary metabolites and found out the presence of alkaloids, tannins, saponins, phenolics and flavonoids. The author reported highest content of tannins and alkaloids and lowest content of phenols in the extract. Arora, 2013 [11] revealed the presence of alkaloids, cardiac glycosides, saponins, reducing sugars, flavanoids and terpenoids in the methanolic extract. The methanolic extract was reported to have strong reducing power owing to which they were used as antioxidant supplement against diseases caused by the generation of reactive oxygen species (Cook and Samman, 1996) [4].

Karthikeyan *et al.*, (2009) [7] subjected the crude ethanolic extract of the leaves of *Adhatoda vasica* for phytochemical screening and found the presence of phenols, tannins, alkaloids, anthraquinones, saponins, flavanoids, aminoacids and reducing sugars in the leaves of the plant.

The present study also revealed the presence of numerous phytochemical constituents in line with the previous reports and thereby justifying its use for various ailments.

Table 1: Phytochemical compounds in the aqueous and methanolic extract of *Adhatoda Vasica*

S. No.	Phytochemical	Aqueous Extract	Methanolic Extract
1	Saponins	+	-
2	Tannins	+	-
3	Phenolics	+	-
4	Alkaloids	+	+
5	Terpenoids	+	+
6	Flavanoids	-	+
7	Amino acids and protein	-	-
8	Carbohydrates	-	-
9	Phlobatannin	-	+
10	Volatile oils	-	-
11	Hydrolysable tannin	-	-
12	Glycosides	+	+
13	Cardiac Glycosides	+	+
14	Vitamin C	-	-

+ Present, - Absent

HPTLC Determination of Vasicine: Vasicine was characterized by the acid - base extraction procedure and employing Methanol: Toluene: Dioxane: Ammonia (2:2:5:1) as the mobile phase. The alkaloid vasicine was successfully isolated from the plant *Adhatoda vasica* [Figure 1] which showed single spot at retention factor (Rf) value of 0.479 ± 0.048 corresponding to the reference standard vasicine. Both

the isolated compound and the reference standard showed chromatogram peak corresponding to this Rf value at the wavelength of 254nm [Figure 2 and 3] and 450nm [Figure 4 and 5]. The validation parameters for determination of vasicine are presented in figure 6. Thus the compound identified was confirmed to be vasicine and the percentage of extraction was found to be at the concentration of 1.5 percent.

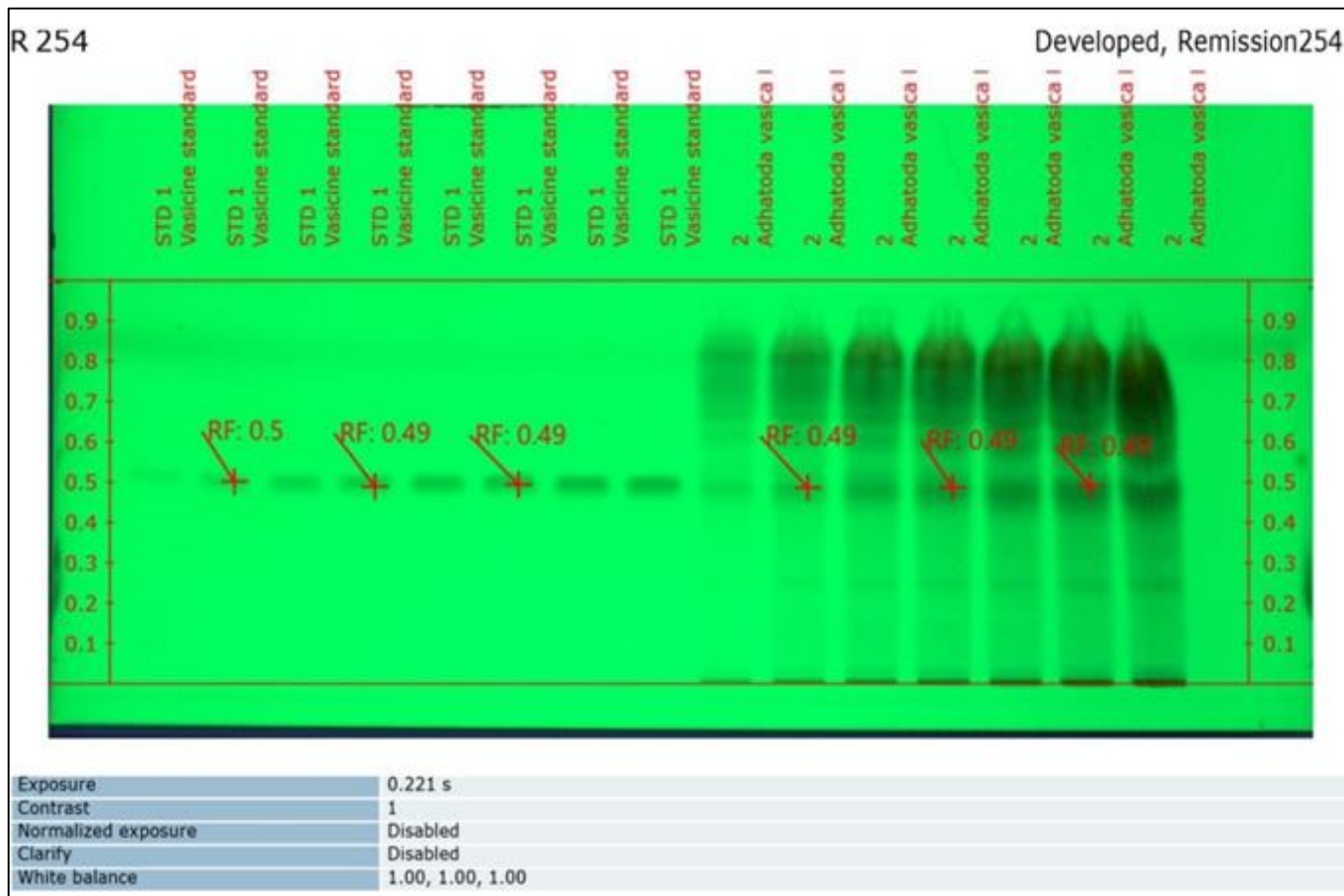


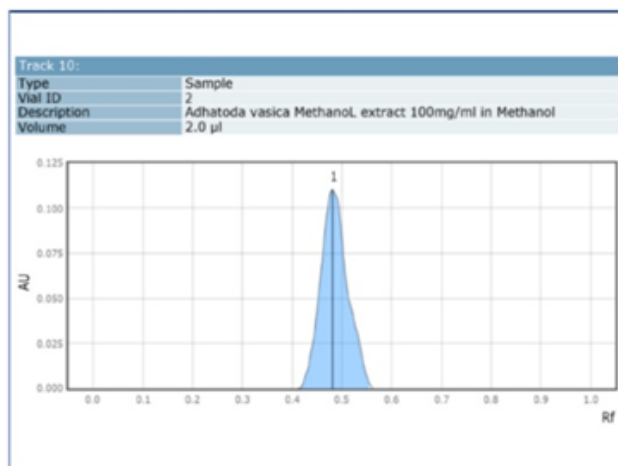
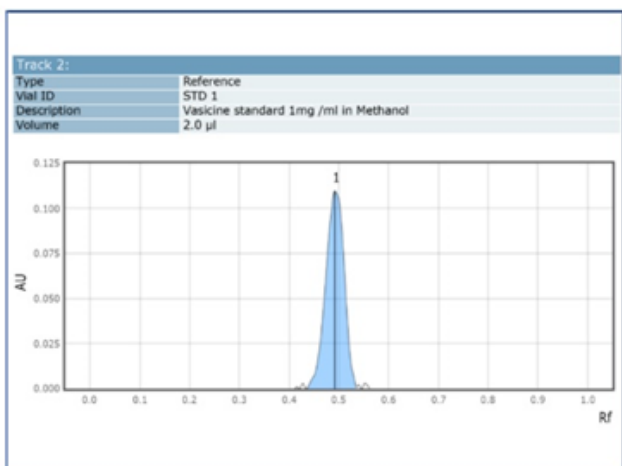
Fig 1: Identification of Vasicine at Wavelength 254nm

Brain (1983) [2] eluted vasicine and vasicinone with methanol-dichloromethane- perchloric acid (50:50:0.01) at 300 nm using High Performance Liquid chromatography. The author noticed a minimum detectable level of 20 ng for vasicine and 10 ng for vasicinone. The author achieved satisfactory separation of the two alkaloids and suggested HPLC as an effective method for the identification of vasicine and vasicinone from *Adhatoda vasica*.

Sharma *et al.*, (1992) [11] employed reverse phase HPLC method for estimating vasicine in 2 polyherbal drug formulations - Shereeshadi Kashaya and Yastyadivati and the vasicine content was found to be 18.1 mg/100 g in Shereeshadi Kashaya and 0.7 mg/100g in Yastyadivati. The author reported a recovery rate of 99% and the minimum

detectable level was 50 ng.

Srivastava *et al.*, (2001) [13] reported a high performance liquid chromatographic method for the determination of the quinazoline alkaloids vasicine and vasicinone. Peak purity and similarity of the two alkaloids have been studied using photodiode array detector and the separation was performed with acetonitrile – phosphate buffer (15:85) using a Hibar Merck make C18 column. The author studied the effects of different solvents and reported a maximum concentration of the alkaloid in the methanolic extract. The method was reported to be simple, sensitive, rapid and reproducible for the quantification of pharmacologically important alkaloids vasicine and vasicinone.



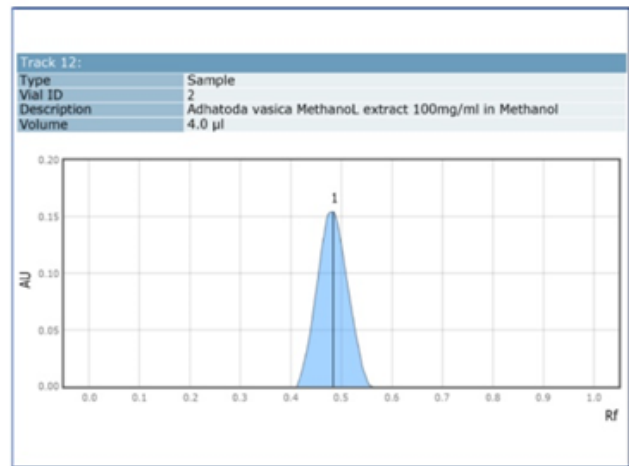
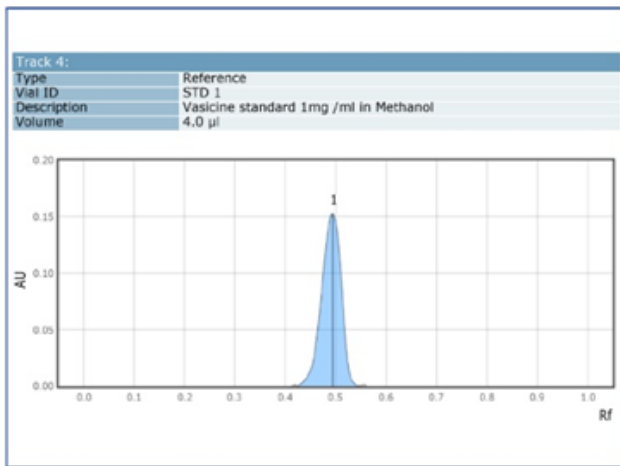
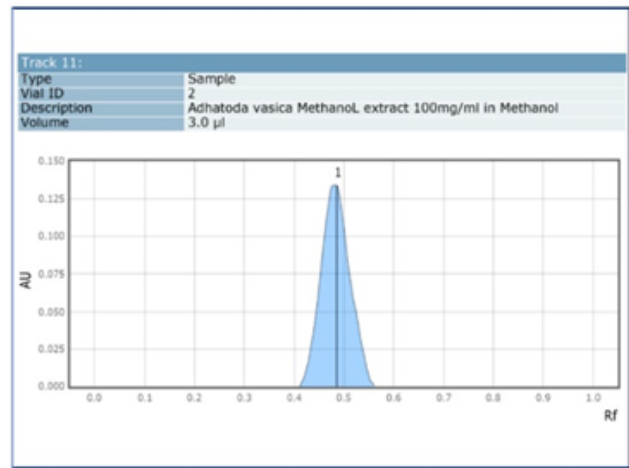
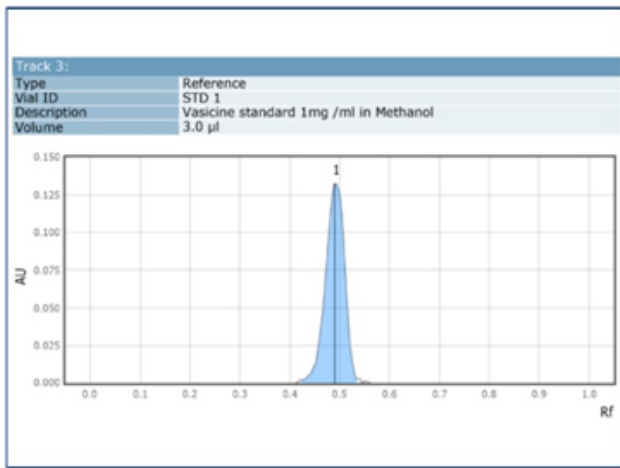
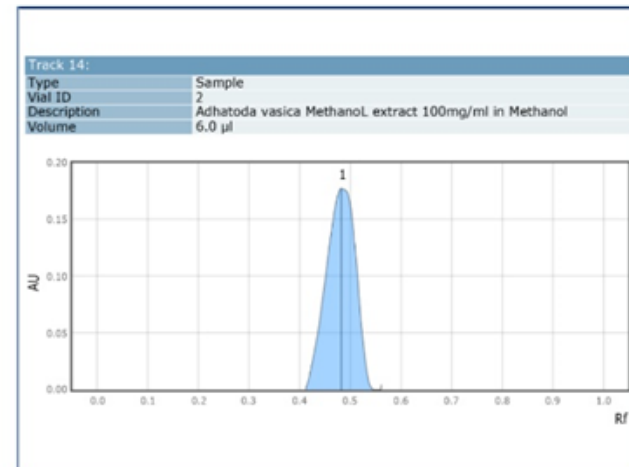
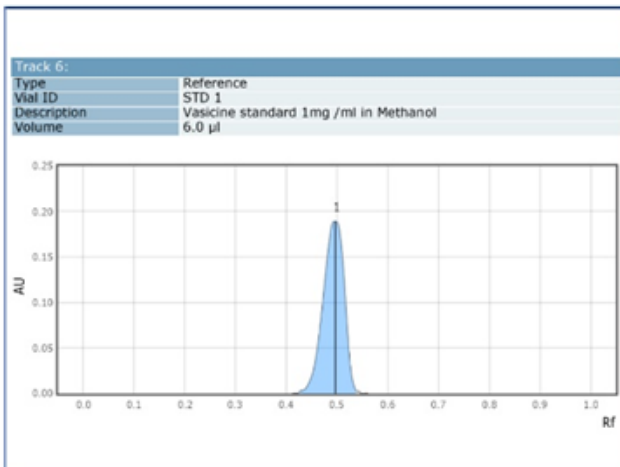
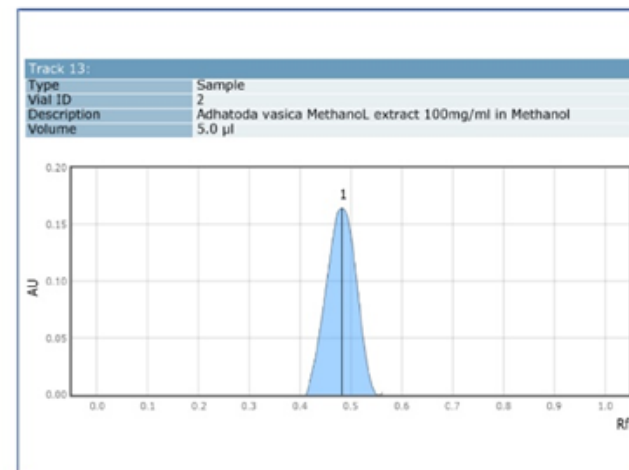
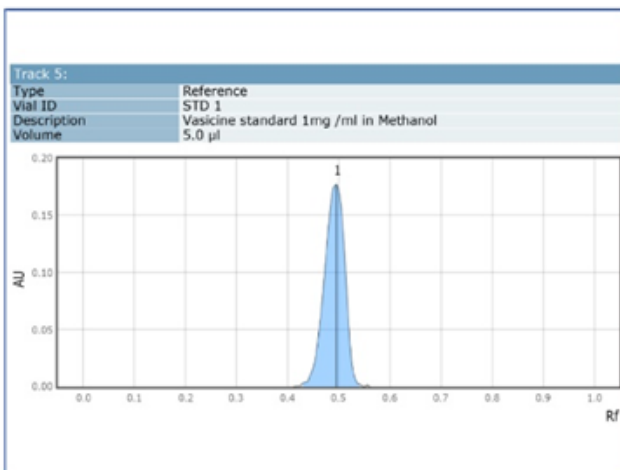


Fig 2: HPTLC chromatogram of reference and sample at wavelength - 254nm – concentration 2µL, 3µL, 4µL



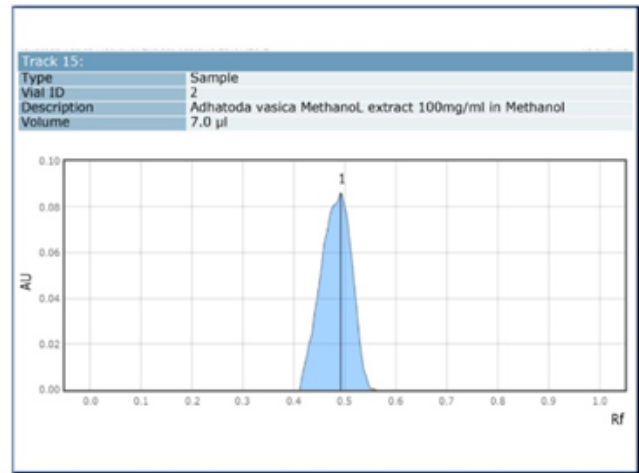
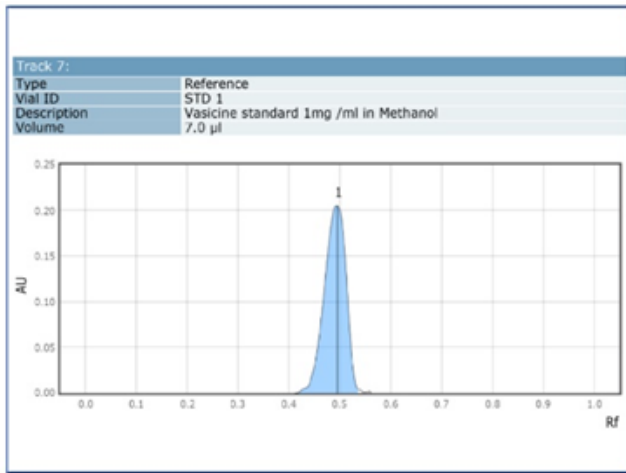


Fig 3: HPTLC chromatogram of reference and sample at wavelength - 254nm – concentration 5µL, 6µL, 7µL

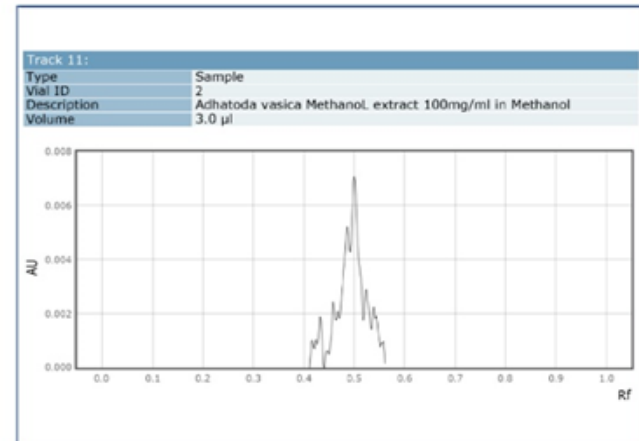
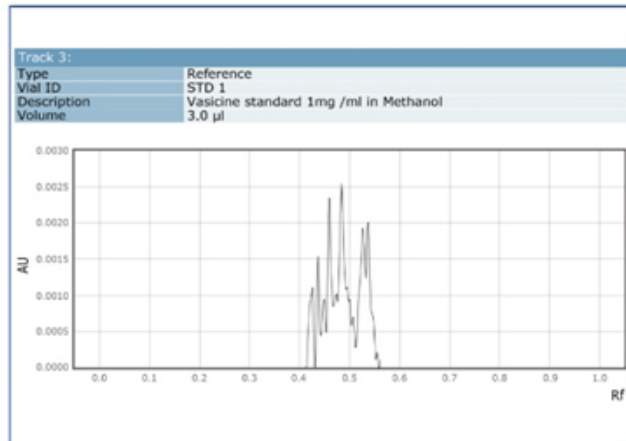
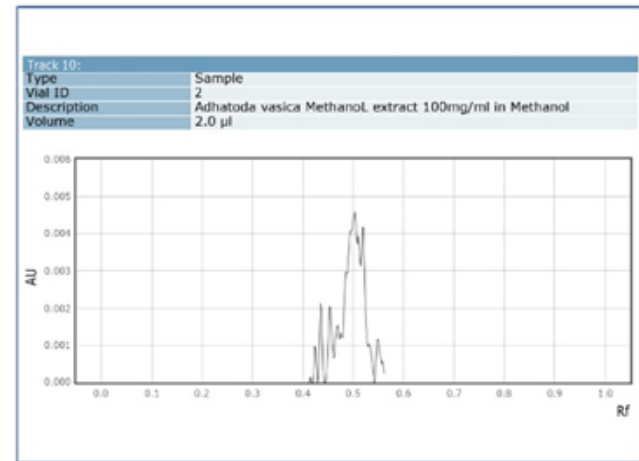
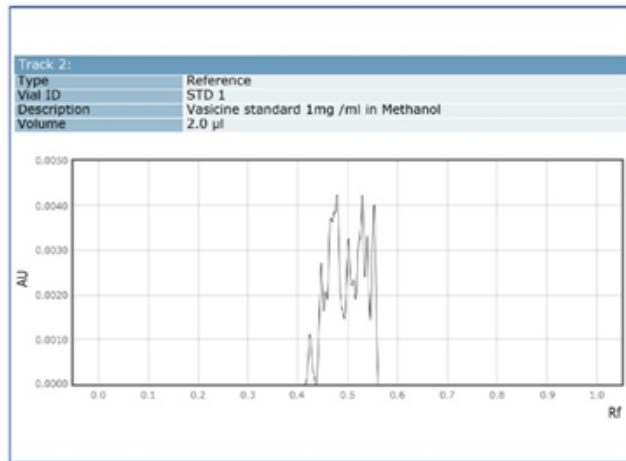
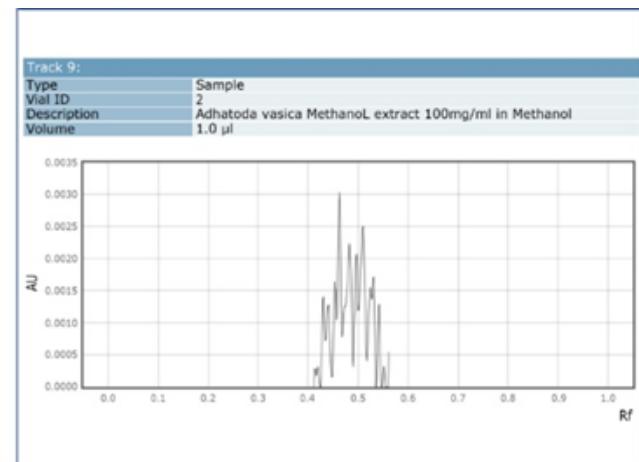


Fig 4: HPTLC chromatogram of reference and sample at wavelength - 450nm – concentration 1µL, 2µL, 3µL

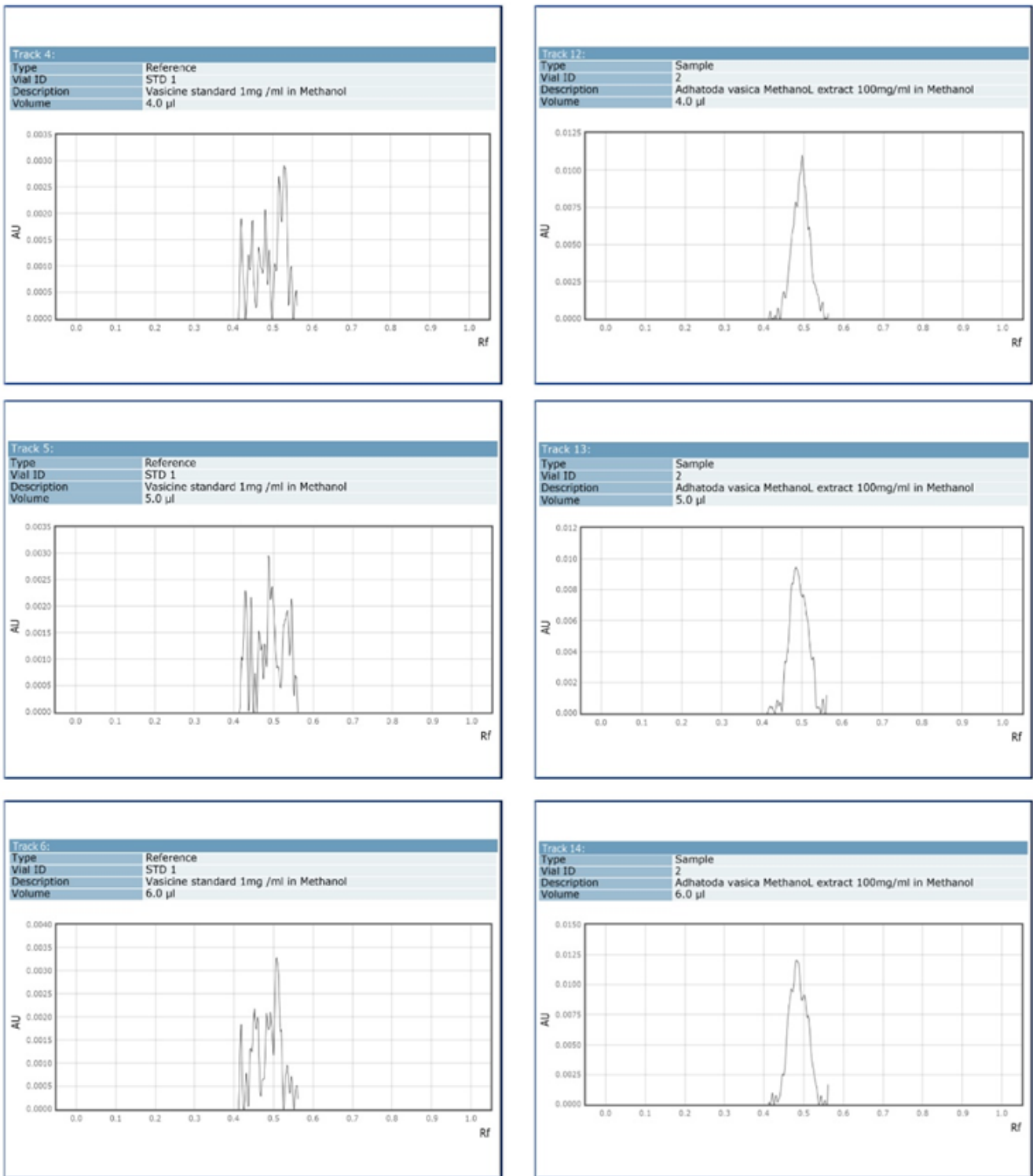


Fig 5: HPTLC chromatogram of reference and sample at wavelength - 450nm – concentration 4µL, 5µL, 6µL

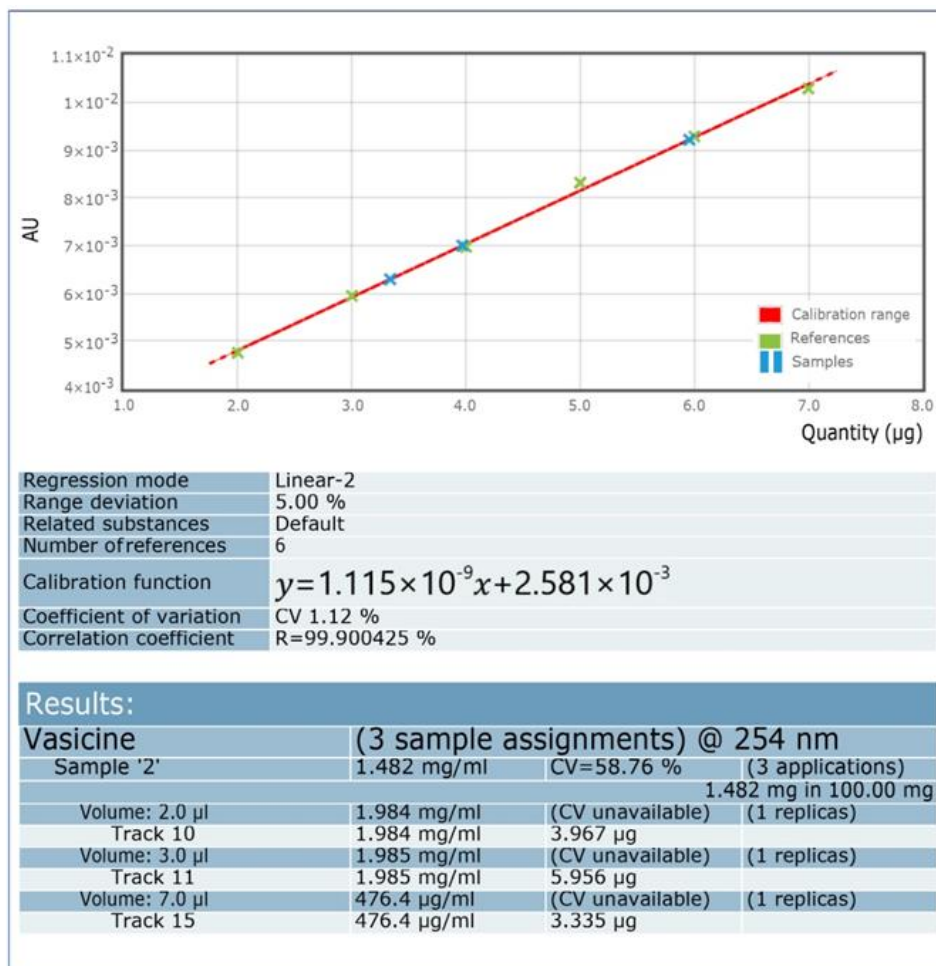


Fig 6: Validation parameters of HPTLC for the determination of vasicine

Claeson *et al.*, (2000) [3] identified vasicine from *in vitro* culture of *Adhatoda vasica* by Thin Layer Chromatography (TLC). The secondary metabolite present in the culture was detected as an alkaloid using the TLC plate samples under UV light at 245 and 365 nm which emitted an orange colour with dragendorff's reagent at an Rf value of 0.4. The plant harbours a rich source of the quinazoline alkaloids, vasicine, vasicinone, deoxyvasicinone, vasicol, adhavasicinone and some minor compounds in the same series. Vasicinone was reported to be formed by oxidation of vasicine at C-8 position (Srivastava *et al.*, 2001) [13].

In concurrence with the earlier reports, the current study successfully detected the quinazoline alkaloid vasicine present in the plant extract prepared from the local origin by HPTLC method. Thus this technique can very well be adopted for screening numerous plant samples from different geographical agroclimatic conditions as it was proved to be more reliable, with high sensitivity and accuracy.

Conclusion

From the present study, it can be concluded that the alkaloid vasicine present in the plant *Adhatoda vasica* can be separated by acid base extraction and identified by HPTLC which is a simple and reliable technique. Further the isolated compound can be used as reference standard in HPTLC analysis of vasicine.

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Conflict of Interest

The authors express no conflict of interest.

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