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# Isolation & purification of vasicine from leaves of *Adhatoda vasica* by modified acid-base extraction method

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**Abstract**
*Adhatoda vasica* Nees or vasaka is a plant that is widely used in indigenous system of medicine for its expectorant activity. The plant is also useful for the treatment of cough, asthma, leprosy and other skin diseases. Vasicine (peganine) is a quinazoline alkaloid that is the major phytochemical component of the plant. In addition to vasicine, vasaka plant also contains numerous alkaloids like vasicinone, deoxy vasicine, vasicinol etc. The work aims to provide an easy and cheaper method for the extraction of vasicine from the leaves of *Adhatoda vasica*. In this work, vasicine was isolated from the leaves of *Adhatoda vasica* by the modification of traditional acid base extraction method. The alkaloid mixture isolated from the leaves was further purified by column chromatography and preparative Thin Layer Chromatography. Purified vasicine was confirmed using standard vasicine obtained from Natural Remedy, Bangalore, by observing under UV light at 254 nm. Rf value of purified vasicine was obtained as 0.561 ± 0.039, while the reference standard showed Rf value of 0.55.

**Keywords:** *Adhatoda vasica*, vasicine, quinazoline alkaloid, acid-base extraction, column chromatography, thin layer chromatography

## 1. Introduction
*Adhatoda vasica* (L) Nees [Fig 1] is a shrub belonging to the family Acanthaceae, which grows to a height of 1.5-2.5 m, with simple, opposite leaves and white, pink or purple flowers. The plant is common throughout Indian peninsula and can be seen up to an altitude of 1300 m. The plant is also known by its synonyms *Adhatoda zeylanica* Medic. and *Justicia adhatoda* L. It is also known by its common names Malabar nut tree and Sanskrit name Vasaka (Atal, 1980) [1].

The plant is well known in Ayurveda and Unani medicines and is used for the treatments of various diseases, especially respiratory tract ailments (Claeson *et al*., 2000) [2]. The leaves of the plant are used for the treatment of malarial fever, chronic fever, intrinsic hemorrhage, cough, asthma, leprosy, skin diseases and piles (Singh and Sharma, 2013) [10]. The leaves of the plant contain quinazoline alkaloids like vasicine, vasicinone and deoxy vasicine (Shinawie, 2002) [9].

Presence of vasicinolone, vasicol and peganine have been reported from roots, whereas the flowers contain quercetin and kaempferol (Rawat *et al*., 1994) [6]. More than 20 formulations containing vasaka juice is used in Ayurveda including vasarishta, triphala ghrita, vasavaleha etc. Vasicine and vasicinone present in the leaves of the plant have respiratory stimulant activity. Investigations have shown that bronchodilatory activity of vasicine is comparable to that of theophylline, both *in vitro* and *in vivo*. Deoxyvasicinone has antimicrobial, anti-inflammatory and anti-depressant activity.

**Fig 1:** *Adhatoda vasica*

The quinazoline alkaloid vasicine is the major secondary metabolite present in the leaves of *Adhatoda vasica*. It is an effective bronchodilator, which is used for the management of respiratory problems, along with vasicinone, another major alkaloid found in the plant (Suthar *et al*., 2009) [11]. In spite of its long known presence in the plant, purification of vasicine is particularly challenging due to its coexistence with many other structurally similar moieties (Rachana *et al*., 2001). In the method followed here, vasicine was purified out from the chloroform extract of *Justicia adhatoda* leaves by traditional acid-base extraction method followed by column chromatography and preparative Thin Layer Chromatography. This method offers a cheap and easier alternative to the methods currently followed.

## 2. Materials and Methods

### 2.1 Plant material

Leaves of *A. vasica* were collected from Medicinal garden of PLAFFS, TANUVAS, Chennai. The plant specimen was authenticated by Dr. Sunil Kumar, Siddha Central Research Institute, Chennai and a voucher specimen was deposited in the herbarium.

### 2.2 Chemicals and Standard

Analytical grade chemicals and reagents were procured from Merck. TLC plate silica Gel 60 F254 was obtained from Merck. The reference standard of vasicine was purchased from Natural Remedy, Bangalore.

### 2.3 Extraction of alkaloid vasicine

The leaves were air dried for two weeks at room temperature and were made into fine powder, which was kept in air tight containers. Hundred grams of leaf powder was soaked in chloroform in 1:10 ratio for a period of 48 hours and was subjected to vigorous shaking intermittently. The filtered extract was concentrated using a rotary evaporator to obtain semi solid crude extract. To the concentrated extract, 100 ml of 0.01 N HCl was added and stirred for 4 hours. The acid treated extract was filtered to obtain a clear solution which was extracted with 100 mL chloroform thrice. The aqueous layer was collected in a beaker and the organic layer containing lipids and other impurities was discarded. To the aqueous layer collected 5% ammonia solution was added until the pH reached 9.5. The basified solution was extracted with 100 mL chloroform thrice. The bottom layer was collected and was concentrated to obtain yellowish-brown colored amorphous residue.

### 2.4 Isolation and Purification of vasicine

Column chromatography was carried out to separate vasicine from the unwanted components. Silica of mesh size 120-200 was used and elution was carried out with hexane, hexane: chloroform (8:2, 6:4, 4:6, and 2:8), chloroform, chloroform: ethanol (9:1, 8:2) (Fig 2). Preparative TLC was carried out and alkaloid spots were detected under 254 nm UV light (Fig 3). Orange spots could be detected on spraying Dragendroff's reagent.

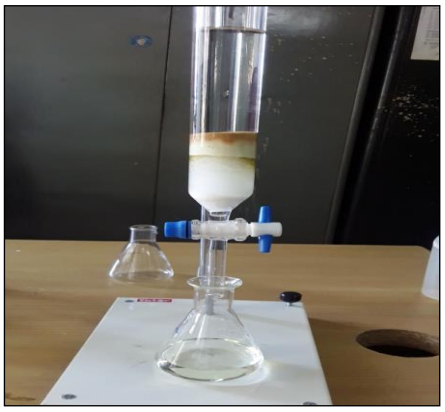

**Fig 2:** Column chromatography with different combinations of hexane, chloroform and ethanol

Stock solution of vasicine was prepared by dissolving 1 µg of accurately weighed standard in 1 ml methanol. Thin layer chromatography was carried out using ethyl acetate: methanol: ammonia in 8:2:0.2 ratio, and the presence of vasicine was checked in the collected fractions by comparing with the standard vasicine obtained from Natural Remedy, Bangalore, under UV light of 254 nm wavelength. On spraying the plate with Dragendroff's reagent, orange colored spots appeared indicating the presence of vasicine.

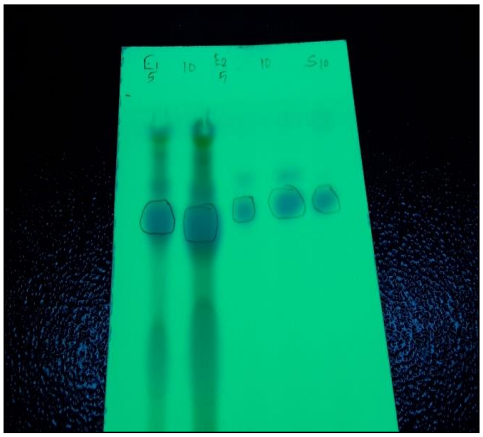

**Fig 3:** Preparative Thin Layer Chromatography plate under UV light 254 nm (Mobile phase-Chloroform: Ethanol-8:2)

## 3. Results and Discussion

On conducting column chromatography, presence of vasicine could be detected in Chloroform: Ethanol 8:2 fraction in preparative TLC. Vasicine purified out from TLC plates via preparative TLC was confirmed by running TLC along with standard vasicine from Natural Remedies, Bangalore. Standard vasicine showed and Rf value of 0.55 and the purified sample showed a similar value of 0.561±0.039 hence confirming that the compound isolated and purified from vasaka leaves is vasicine.

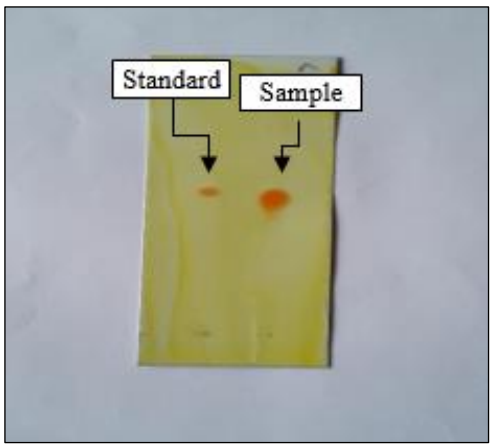


**Fig 4:** TLC plate of purified vasicine (T) against 1000 ppm Standard (S) at 5 µL each detected by Dragendorff reagent (Mobile phase- ethyl acetate: methanol: ammonia in 8:2:0.2)

In the procedure followed for isolation and purification of vasicine from the leaves of *Adhatoda vasica*, crude extraction was carried out using chloroform (Sajeeb *et al*., 2016) [7]. The percentage yield of crude extract from the given amount of leaf powder was 4.962%. On Thin Layer Chromatography the presence of vasicine was noted in chloroform: ethanol fraction of 8:2 (Duraipandiyan *et al*., 2015) [3].

On visualization in UV chamber at 254 nm (Keesara *et al*., 2015), vasicine could be visualized as a single spot against that of the standard. On spraying the plate with Dragendroff's reagent, a single orange spot was observed [Fig 4] (Sharma *et al*., 2018) [8]. The presence of numerous alkaloids in Vasaka plant makes the separation and isolation of vasicine commercially unviable. The current method, which is a modification of the traditional acid base method of extraction is a simple, viable and reproducible method for the separation, isolation and purification of vasicine. Retention Factor value (Rf value) of vasicine was calculated by comparing the distance travelled by the sample against that of the solvent (Table1).

**Table 1:** Rf value of vasicine against the standard

| TLC | Rf value |
|---|---|
| 1 | 0.587 |
| 2 | 0.55 |
| 3 | 0.574 |
| 4 | 0.495 |
| 5 | 0.61 |
| 6 | 0.551 |
| mean | 0.561 |
| Standard deviation | 0.0395 |

## 4. Conclusion

From the above study it can be concluded that vasicine, the major alkaloid *of Adhatoda vasica* can be extracted from the leave of the plant by simple modification of the traditional method of acid base extraction, in a cost effective manner.

## 5. Acknowledgments

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