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Estimation of vasicine in the leaves of *Adhatoda vasica* (L). Nees with varying seasons and methods

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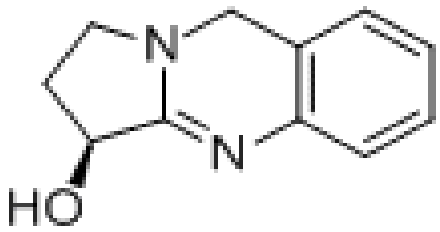
Abstract
Adhatoda vasica (*Vasa*) leaf is a commonly used drug in Ayurveda as a bronchodialator. A number of quinazoline alkaloids (Vasicine, vasicinone, vasinol, vasicol, adhatonine) essential oils (betane), vitamins (c, b-carotene), steroids (vasakin) and many fatty acids contribute to the medicinal activity of the plant. Vasa leaf juice (*swarasa*) is used in many Ayurvedic formulations. In this work the classical method of extracting the juice (*swarasa*) from the leaf is compared with commercial and chemical methods of extraction. The work is an effort to compare the methods extract maximum quantity of juice in terms of its major alkaloid vasicine. The work also aims to identify the best season for sample collection. For this juice samples were evaluated using HPTLC technique. The present study revealed that the method pudapakam gives maximum vasicine content when compared to the other methods and best season for sample collection is September.

Keywords: *Vasa* juice, HPTLC, vasicine, pudapakam

Introduction
The plant Adhatoda vasica, a member of Acanthaceae family is a well known drug in Ayurveda and Unani Medicine (the wealth of India 1948). It is being used in India for years for the treatment of respiratory ailments. It is well established now that, vasicine is the major, as well as, the most important active principle of this medicinal plant. It is reported to be responsible for most of its activities including: antioxidant, anti-inflammatory and bronchodilatory activity. It is an optically active molecule in its natural condition but, gets racemized when extracted. Although, its importance has been recognized since years and it is being used extensively in many herbal formulations for respiratory diseases and disease related to female reproductive system, limited data is available for its molecular mechanism of action [1]. A number of quinazoline alkaloids (Vasicine, vasicinone, vasinol, vasicol, adhatonine) essential oils (betane), vitamins(c, b-carotene), steroids (vasakin) and many fatty acids contribute to the medicinal activity of the plant [2]. It is an important drug prescribed for chronic fever, intrinsic haemorrahage, cough and asthma, leprosy, skin diseases and piles (Sharma PV 1996). The plant has been included in the WHO manual The Use of Traditional Medicine in Primary Health Care which aims to profit health workers to keep them informed of the therapeutic utility of surrounding flora (WHO 1990). It is reported to be an expectorant, abortifacient, antimicrobial, antitussive and anticancerous (Singh et al 2011. Among the many alkaloids vasicine was reported to have bronchodilatory, respiratory stimulant and uterine stimulant effect. It was first isolated from by Sen and Ghose in 1924 [3]. In Ayurvedic formulations it is used in Vasarishta, Mahatiktaka ghritha, vasavaleha, Panchatiktaka ghrita, Vasakasava etc. [4, 5]. The drug is employed in different forms such as fresh juice, decoction, powder, alcoholic extract and liquid extract or syrup.
In the present work the samples collected in various seasons[6] were compared and various methods of preparation of the extract were also compared including traditional method for vasicine content [7]. For the study HPTLC technique has been adopted. The extracts were prepared in different methods.

Vasicine



Molecular formula $C_{11}H_{12}N_2O$
Formula weight 188.226

II. Objectives

The objectives of the present work is to compare the quantity of vasicine obtained from Adhatoda vasica

- collected at different seasons
- by different methods of preparation of extracts

III. Materials and methods

1. **Plant material collection and identification**
   Adhatoda vasica leaves were collected from the Botanical Garden in the Pharmacognosy Unit of Government Ayurveda College, Trivandrum (Kerala State 8.4916ºN, 76.9787ºE). Identification of the Plant was authenticated by the Departmental Botanist and subjected to detailed phytochemical studies.
2. **Instrumentation**
   Camag HPTLC instrument with win CATs software, automatic sample spotter (Camag, Muttenz, Switzerland), syringe: 100 µL (Hamilton), Automatic Development Chamber, Camag TLC scanner 3 linked to "winCATS" software (Camag), HPTLC plates 20 x 10 cm, precoated with silica gel 60 F_{254} TLC plate (0.2 mm uniform thickness)
3. **Standard Marker**
4. Vasicine obtained from Fluka (accuracy≥/95%)
5. **Procedures**

a. Method of Extraction

Extract from Adhatoda vasica leaves was prepared by the following different methods.

Procedure 1 Steaming

First method employed was steaming. In this method 50 g of the fresh leaves were subjected to steam without adding any water to the leaves for 20 minutes. The leaves were then taken in 4 layers of muslin cloth and squeezed to get the extract and coded as S1.

Procedure 2 Grinding

In the second method 50g of the fresh leaves was crushed ground and made into paste using mortar. It was then squeezed through 4 layers of muslin cloth and juice was collected, coded as S2.

Procedure 3 Pudapaka vidhi

In the third method called **"Pudapakam"** 50 g of fresh leaves were crushed, ground and it was again covered with Adhatoda leaves. This was covered with clay of 1.5 inches thickness and heated in coal until the clay part became red hot. After self-cooling it was squeezed through 4 layers of muslin cloth to collect the juice. This is coded as S3.

Procedure 4 Modified Pudapaka vidhi (oven heating)

In the fourth method 50g of the fresh leaves was ground and made to a fine paste and covered with Adhatoda leaves. Then it was again covered with clay. It was heated at 180^0C in oven for 2 hours. After cooling in air, it was squeezed to collect the juice as before and coded as S4.

Procedure 5 Alcohol extract

50 g of fresh leaves were dried under shade and alcohol extract was prepared by refluxing with alcohol for 1 hour and made upto 100 ml in a standard flask and this sample was coded as S5.

Procedure 6 Decoction

Decoction was the last method tried. In this 50 g of fresh leaves were shade dried and decoction was prepared using 800 ml of water reduced to 100 ml and this is coded as S6.
In all the above procedures the volume of juice collected was recorded.

4.b. Sample preparation

All the juices prepared (procedures 1-4) and also the decoction from method 6 was extracted using ethyl acetate. The solvent evaporated, the residue redissolved in methanol and made upto 100 ml.

4.c. TLC Analysis

TLC analyses of the extracts were performed on silica gel G plates[11]. Aliquots of the extracts were applied on the plates as spots, plates were developed in TLC chamber previously saturated with the mobile phase. Different mobile phases were tried and Chloroform: methanol: ammonia 13:1:0.2 V/V/V was identified as the best method. The chromatogram was developed. The chromatogram were visualized under UV 254 and 365 nm and then sprayed with Dragondroff's reagent.

4.d. HPTLC analysis

Quantitative estimation of vasicine was carried out using Camag HPTLC system with marker compound vasicine. Stock solution of vasicine was prepared in ethanol (0.1 to 1.5 µg/mL). The standard solutions were injected on a TLC plate using Linomat V. 12 µL each of the suitably diluted sample solutions were applied on the plate. The plate was then developed in the mobile phase using the Automatic Development Chamber and was scanned at 289 nm. The peak areas were recorded. The vasicine content was estimated from the calibration curve of the standard and expressed as percentage of vasicine[8].

Results and Discussion

Discussion

The leaf juice of Adathoda vasica is used in several polyherbal formulations of Ayurveda (AFI 2003), in which the fresh plant material is crushed and squeezed to yield the juice. But A. vasica leaves do not produce the juice easily simply by crushing and squeezing. So a distinct method *Pudapaka vidhi* is prescribed in Sarngadhara samhitha, an Ayurvedic text book of pharmaceutics, however this method is very elaborate, tedious, time consuming and difficult to handle when the quantity is large. So a modified method using oven is tried instead of coal. Commercial methods adopt steaming and alcohol extraction. So a comparison has been done among these six methods in terms of measuring quantity of leaf juice obtained from same amount of sample (50 g) and the amount of vasicine in each preparations.

The quantity of juice obtained for the same amount of sample (50 g) varied from method to method. The values are compared in the following table.

Table 1: Comparison of quantity of juice by different methods

Method	Quantity of juice obtained (ml)
Steaming and squeezing	18
Grinding and squeezing	25
Pudapakam and squeezing	19
Heating at 180^0C and squeezing	24
Alcohol extract	--
Decoction	--

2. Charecterisation of secondary metabolites

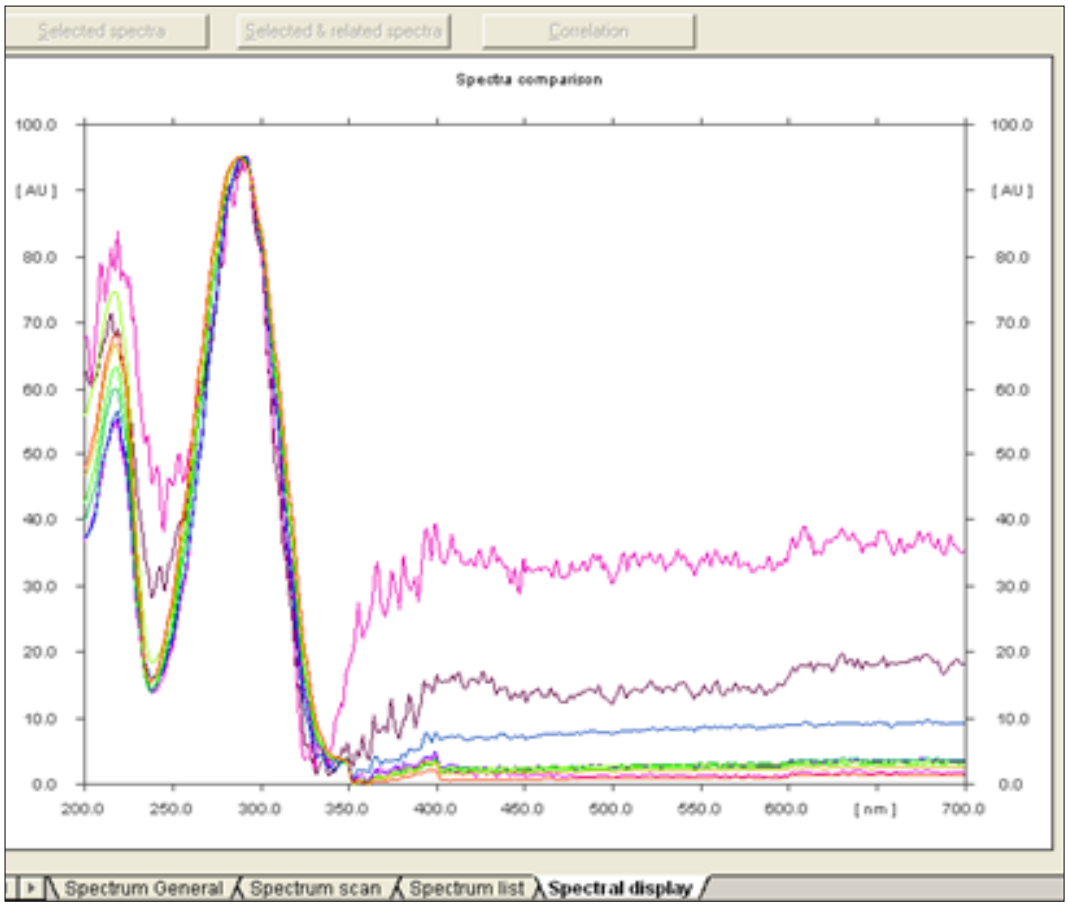
The secondary metabolite present in A. vasica was first confirmed as alkaloid by qualitative analysis of the extract. In this study the detection of alkaloid in the samples was again confirmed by TLC studies. TLC study was done under UV light of 245 and 365 nm and identified on the basis of their colour and R_f values. Alkaloid developed orange colour with Dragendorff's reagent[9]. Rf value obtained is 0.4, which corresponds to that of vasicine.[10]

3. HPTLC analysis

Quantitative estimation of vasicine was carried out using Camag system using marker vasicine. For the estimation of the vasicine in test samples, same volume of the samples and standards were plotted on a TLC plate and developed in the solvent system Chloroform: methanol: ammonia 13:1:0.2 V/V/V and was scanned at 289 nm[12]. By comparing the peak heights and also the peak area of the samples with the standard the concentration of samples determined and percentage calculated.

4. Comparison of methods:

The samples were subjected to different methods of preparation such as steam, grinding, pudapaka vidhi, oven heating, alcohol extract and decoction. The quantity of vasicine obtained in these methods were compared using vasicine standard as marker adopting HPTLC technique.



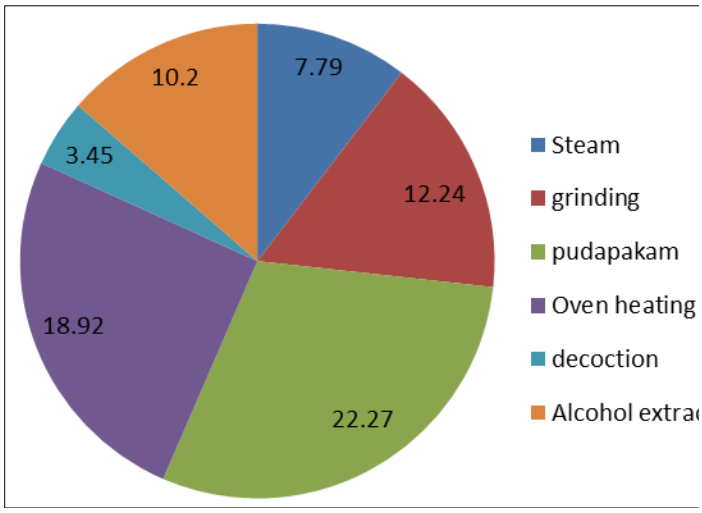
Picture 1: Spectral display of vasicine from different methods

These values are depicted in a pie chart for more easy comparison.

Table 2: Quantity of vasicine by different methods

Method	% of vasicine
Steaming S1	7.79
Grinding S2	12.24
Pudapaka vidhi S3	22.27
Oven heating (modified pudapakam) S4	18.92
Alcohol extract S5	3.45
Decoction S6	10.20

% of vasicine



Picture 2: comparison of different methods showing percentage of vasicine

It is seen from the area profile and concentration that the pudapakam method as prescribed by the Acharyas is the best method. It is seen that Pudapakam is giving the maximum concentration of the alkaloid vasicine when compared with the other methods.

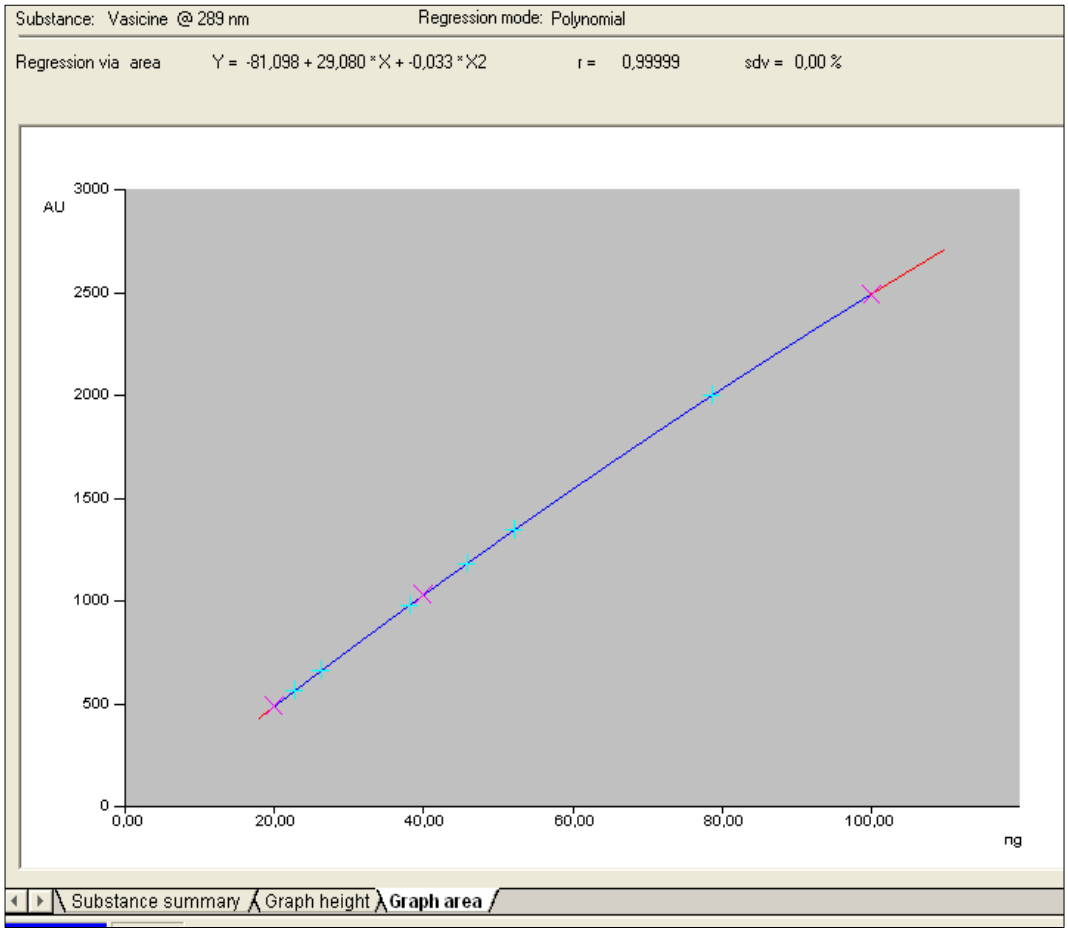
5. Comparison of seasons

The samples collected during six different seasons ie, shad ritu kalas were compared by taking the extract by steaming method. All the six samples were subjected to HPTLC analysis following the procedures explained earlier. The vasicine content in different seasons are listed in the following chart.

Table 3: Comparison of vasicine in different seasons

Season	% of vasicine
November	7.8
January	3.2
March	3.8
May	5.7
July	6.8
September	11.6

The pictorial representation by HPTLC method using vasicine standard as marker for the comparison of seasons is depicted in the following graphs.



Picture 3: Estimation of vasicine content in different seasons

Conclusion

The following conclusions are made from the study:

1. Quantity of juice prepared from Adhatoda vasica varies from method to method even if the same quantity of leaves were taken for the preparation.
2. The concentration of vasicine changes when Adhatoda extract is prepared by different methods.
3. Percentage of vasicine changes from season to season even if extracted by the same method.

From the study it is inferred that vasicine can be extracted from Adhaoda leaves in any seasons, but the quantity of vasicine varies from season to season; and the best season for maximum yield is September. Of the different methods tried

like steaming, grinding, pudapakam, oven heating, decoction and alcohol extract, the method that gave maximum quantity of vasicine is the traditional method of pudapakam. So in summary, to get maximum quantity of the alkaloid vasicine the leaves are to be collected in the month of September and to be extracted by the method pudapakam.

Acknowledgement
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