SHST
Horticulture and Forestry Society of Transylvania

Gautam P and Pitchaimuthu S (2023)
Notulae Scientia Biologicae
Volume 15, Issue 1, Article number 11368
DOI:10.15835/nsb15111368
Research Article

NSB
Notulae Scientia Biologicae

Environmental variations on physicochemical properties in vasicine content of *Adhatoda vasica* - An ayurvedic medicinal plant

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Abstract

Adhatoda vasica (Acanthaceae) is a medicinal plant used in traditional formulations for the treatment of various disease conditions. The physicochemical properties fluctuate with the season and in response to stress. To obtain the highest medicinal value it is necessary to collect the plant raw material in the correct season. The present study was aim to evaluate the physiochemical fluctuations in the leaves of *A. vasica* and the vasicine, an antidiabetic constituent in these leaves. Leaves were collected in every month of a year at different time and places and authentication and standardization was done. The leaves were evaluated for the macroscopic and microscopic evaluation, quantitative microscopy, proximate phytochemical analysis, extractive values in petroleum ether, chloroform, ethyl acetate, ethanol, water, and determination of the concentration of antidiabetic component vasicine. The leaves showed morphological and microscopical variations in different seasons. The phytochemical composition was the same in all seasons however the levels of extractive values fluctuated in response to seasonal variations. Maximum levels of ethanol and water extract were obtained in the winter season also the vasicine concentration was higher in this season.

Keywords: alkaloids; antidiabetic; herbal; metabolites; variations

Introduction

Adhatoda vasica an Indian medicinal plant has been used in traditional treatments for numerous human diseases for thousands of years and they continue to be an important therapeutic aid for alleviating the ailments of human kind. In India, it is estimated that 75% of population depends on plants to therapy themselves, of those about 65% populace use medicinal plants habitually to battle certain ailments and almost 45% human use such plants in pharmaceutical industries (Dash, 2017). Its leaves are extensively used for treating cold, cough, whooping cough and chronic bronchitis and asthma as sedative expectorant, antispasmodic and anti-inflammatory drug. There is considerable demand for this plant within the country. The demand is being met from natural sources (Shabir *et al*., 2013). The Leaves have been found to be a rich source of alkaloids of which vasicine and vasicinone are bioactive. A non-nitrogenus neutral principle, vasakin, vasicinone, two new quinazoline alkaloids, one of which was named as adhvasinone and two new pyrroloquinazoline alkaloids, desmethoxyaniflorine and 7-methoxyvasicinone were identified from the ethanolic extract of the leaves. Anonymous (2004). Ethanolic extract of the leaves have hypoglycemic activity (Dhar *et al*., 1968).

Received: 20 Oct 2022. Received in revised form: 08 Mar 2023. Accepted: 15 Mar 2023. Published online: 22 Mar 2023.
From Volume 13, Issue 1, 2021, Notulae Scientia Biologicae journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

The World Health Organization (WHO) has outlined herbal medicine as culminated labelled medicinal products that incorporate lively ingredients as aerial or underground accessories of plants. Of the 3,50,000 higher plant species on earth, more than 75000 species are reported to have at least some medicinal value (Shi-Lin *et al*., 2016).

Since ages, humans have relied on nature for their basic needs for the production of foodstuff, shelters, clothing, means of transportation, fertilizers, flavors, and fragrances, and medicines.

Plants have formed the basis of sophisticated traditional systems of medicine that have been in existence for thousands of years and continue to provide humankind with new remedies (Haidan *et al*., 2016). The history of herbal medication is equally old as human history. Most of these plant-derived drugs were originally identified through the subject of traditional remedies and folk knowledge of indigenous people and some of these could not be substituted despite the tremendous progress in synthetic chemistry. Therefore, plants can be depicted as a major source of medicines, not merely as isolated active principles in standardized dosage form but also as crude drugs for the population. Modern medicines and herbal medicines are complimentary being used in areas for health care program in various developing countries including India (Sen, 2017). In the present scenario, the demand for herbal products is growing exponentially throughout the globe and major pharmaceutical companies are currently carrying on extensive research on plant materials for their potential medicinal value (Ekor, 2013). The need of new therapies for glycemic control is the fact that existing treatments have limitations because of their side effects (Chaudhary, 2017).

The herbal extracts which are effective in lowering blood glucose level with minimal or no side effects are known to be used as antidiabetic remedies (Kooti, 2016.). Diabetes mellitus is a growing problem worldwide entailing enormous financial burden and medical care policy issues (Karachaliou, 2020.). According to International Diabetes Federation (IDF), the number of individuals with diabetes in 2010 crossed 375 million, with an estimated 4.5 million deaths each year, (Cho *et al*., 2018.). According to the World Health Organization (WHO), up to 80% of the population in developing countries uses plants and its products as traditional medicine for primary health care (Oyebode *et al.,* 2016). The WHO has listed 26,000 plants, which are used for medicinal purposes around the world. Among these, 3500 species are in India (Anand *et al.,* 2019). There are about 800 plants which have been reported to show antidiabetic potential. A wide collection of plant-derived active principles representing numerous bioactive compounds have established their role for possible use in the treatment of diabetes (Tran *et al*., 2020). HPTLC study helps to gain idea about chromatographic pattern of the components. Current study involves use of HPTLC parameters to study changes in phytoconstituents. Macroscopic and microscopic study was performed (Thomas *et al*., 2020). The present study was aim to evaluate the physiochemical fluctuations in the leaves of *A. vasica* and the vasicine, an antidiabetic constituent in these leaves. Different analytical methods are available and reported, such as HPTLC for estimation of vasicine extracted from leaves in different seasons. (Narayana *et al.,* 1995, Soni *et al.,* 2008, Das *et al.,* 2005).

Materials and Methods

The plant material was collected in every month of the year i.e. in the Rainy season (June, July, August, September), Winter (October, November, December, January) and Summer (February, March, April, May), from places of different altitude i.e. Low (560 meters), medium (920 meters) and high (1,312 meters), twice i.e. morning and evening. A voucher specimen (No. BSI/WRC/100-1/Tech./2019/02) was deposited in the Herbarium of Botanical Survey of India, Pune.

This study involves collection of data for changes of active components in alkaloidal and anti-diabetic plant due to season, time and places. The plant was authenticated by Botanical survey of India, Pune. Morphological and microscopic study and phytochemical screening was performed. Plant material collected in different seasons, time and places was extracted successively and as the percent yield of ethanol meth found to be more as compare to other solvent extracts so ethanolic extract content was used for further analysis.

Assessment of quality of plant materials

The plant materials were assessed as per WHO guideline. Fresh plant parts were subjected to color, odor and taste, determination of shape, size, surface characteristics and appearance.

For microscopically examinations, free hand sections of the fresh leaf were cut, cleared with chloral hydrate solution and water, and stained with a drop of hydrochloric acid and phloroglucinol. Photomicrographic images were taken by using Trino CXR camera.

Quantitative microscopy

Leaves were subjected to quantitative microscopy for the following values using reported method-stomatal number, stomatal index, palisade ratio, vein islet number, vein termination number.

Proximate analysis

Proximate analysis of powdered plant material was carried out using reported methods.

Following determinations were done; foreign organic matter, loss on drying, total ash, water soluble ash, acid insoluble ash, sulphated Ash, water soluble extractives, alcohol soluble extractives, ether soluble extractive value.

Phytochemical screening

The air-dried powder (1 kg) of plant was extracted in Soxhlet apparatus with solvents of increasing polarity as follows: Petroleum ether; Chloroform; Ethyl acetate; Ethanol. Each time before extracting with the next solvent, the material was dried. All the extracts were concentrated by distilling the solvent and the extracts were dried on water bath. Then consistency, color, appearance of the extracts and their percentage yield were noted.

Establishment of qualitative phytoprofile of successive solvent extracts-

The extracts obtained from successive solvent extraction were then subjected to various qualitative chemical tests to determine the presence of various phytoconstituents like alkaloids, glycosides, carbohydrates, phenolics and tannins, proteins and amino acids, saponins and phytosterols using reported methods (Table 1).

Table 1. HPTLC Analysis- High Performance Thin Layer Chromatography

Plant Name	Phyto Constituent	Std. Area (Under Curve)	Mobile phase	Wavelength (nm)
A. vasica	Vasicin	5053.5 AU	Ethyl acetate: Methanol: Ammonia (8:2:0.2)	254

Results and Discussion

Plant leaf color is green, characteristic odor and taste, shape is oblong simple, petiolate, exstipulate entire margin, a tapering base and acuminate apex, leathery touch, smooth and shining texture. Cell wall is single layered epidermis made up of compactly arranged barrel shaped parenchymatous cells. Vascular bundle is arc shaped, conjoint, collateral and closed.

Table 2. Monthly variation with high altitude at morning

Altitude	Month											
	1	2	3	4	5	6	7	8	9	10	11	12
Low	4.30 ±0.57	5.00 ±1.00	5.05 ±1.00	5.50 ±1.00	5.70 ±1.00	5.05 ±1.00	5.33 ±1.52	5.37 ±1.52	5.32 ±1.15	5.50 ±1.00	4.27 ±1.15	4.78 ±1.15
Medium	4.34 ±0.57	5.07 ±1.00	5.04 ±1.00	5.02 ±1.00	5.01 ±1.00	5.06 ±1.00	5.03 ±1.52	5.33 ±1.52	5.36 ±1.15	5.04 ±1.00	4.72 ±1.15	4.76 ±1.15
High	4.76 ±0.57	5.34 ±0.57	5.33 ±0.57	5.36 ±0.57	5.39 ±0.57	5.34 ±0.57	5.07 ±1.15	5.75 ±1.15	5.73 ±0.57	5.39 ±0.57	5.05 ±1.00	5.02 ±1.00

(% of alkaloids) n= 3 P< 0.05.

Table 3. Monthly variation with altitude at morning

Altitude	Month											
	1	2	3	4	5	6	7	8	9	10	11	12
Low	1.00 ±0.20	1.10 ±0.26	1.25 ±0.55	1.43 ±0.11	1.82 ±0.05	2.10 ±0.05	2.27 ±0.05	2.34 ±0.05	2.23 ±0.11	1.42 ±0.05	1.25 ±0.10	1.62 ±0.60
Medium	1.624 ±0.20	1.56 ±0.32	1.84 ±0.65	2.03 ±0.10	2.46 ±0.11	2.78 ±0.05	2.84 ±0.05	2.93 ±0.05	2.82 ±0.15	1.97 ±0.10	1.72 ±0.10	2.15 ±0.60
High	2.03 ±0.58	1.84 ±0.05	2.17 ±0.60	2.44 ±0.11	2.83 ±0.05	3.17 ±0.05	3.22 ±0.05	3.36 ±0.05	3.24 ±0.11	2.49 ±0.05	2.23 ±0.10	2.32 ±0.05

(% Yield mg/g) n= 3 P< 0.05.

Enclosed by a parenchymatous bundle sheath. Vessels with pitted thickening, anomocytic or anisocytic stomata, glandular, multicellular uniseriate (50 to 120µ) trichomes, prismatic calcium oxalate crystals and starch grains are present.

Chemical constituents in leaf shows presence of alkaloids and its percentage varies at different season, time and places (Table 2; Figure 1).

Table 4. Concentration and Area Under Curve (AUC)

Concentration (µg/ml)	Area (AUC)
0.2	379.9
0.4	550.8
0.6	730
0.8	887
1	1049.9

Table 5. HPTLC analysis of *A. vasica* leaf extract

Rf Value	**Season**	**Area (AU)**	**Yield (mg/g)**
0.49	Summer	1868	1.89
	Rainy	**3149**	**3.20**
	Winter	2329	2.36

HPTLC chromatogram of vasicine % Yield mg/g in leaf extracts show variations at different season, time and places. In months of June-Sept. (rainy season) at morning time with high altitude place it shows more yield. The values are expressed as mean SEM; P<0.05, (Two-way ANOVA followed by Tukey's multiple comparison test) (Table 3; Figure 2). Calibration curve (Figure 3) show variations of concentration of vasicine with area under curve. (Table 4; Figure 3). The presence of vasicin in ethanolic extract was confirmed by HPTLC fingerprinting and the yield was 3.20 mg/ gm at Rf value 0.49 (Table 5; Figures 4, 4-A, 4-B and 4-C).

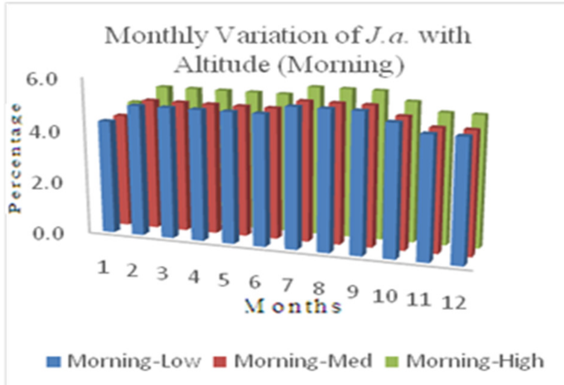


Figure 1. Monthly variation with altitude (Morning)
Med.= Medium (Altitude)

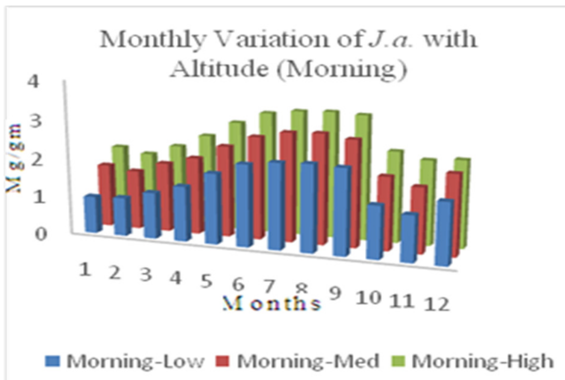


Figure 2. Monthly variation of with altitude (Morning)
Mg/gm= Milligram/ gram, Med= Medium (altitude)

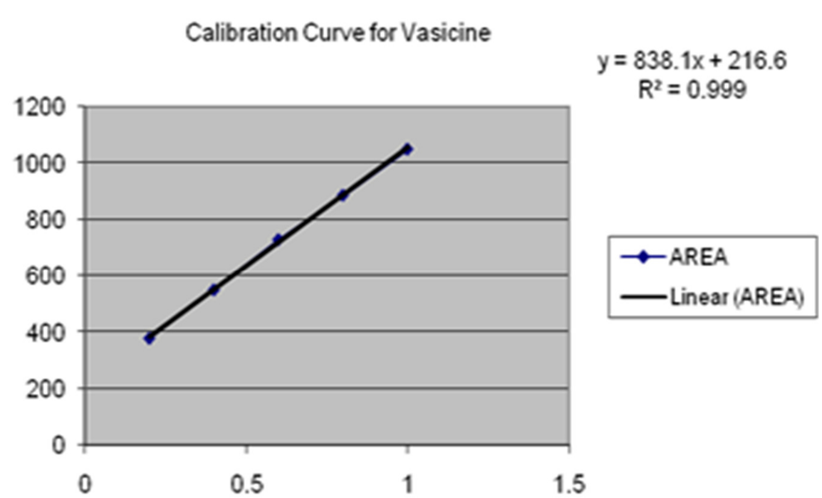
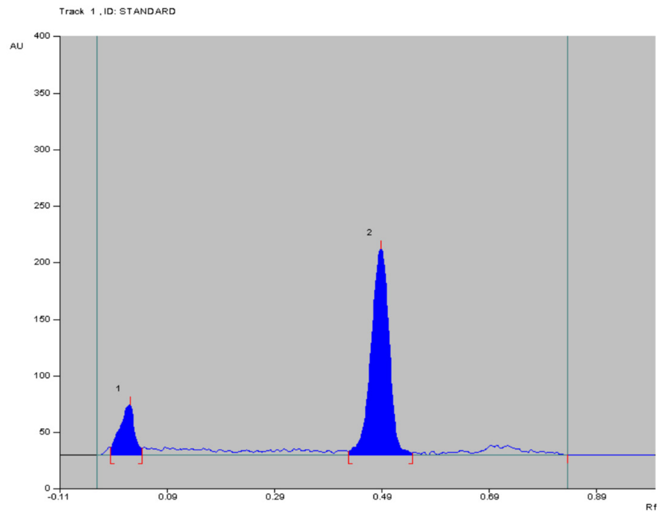
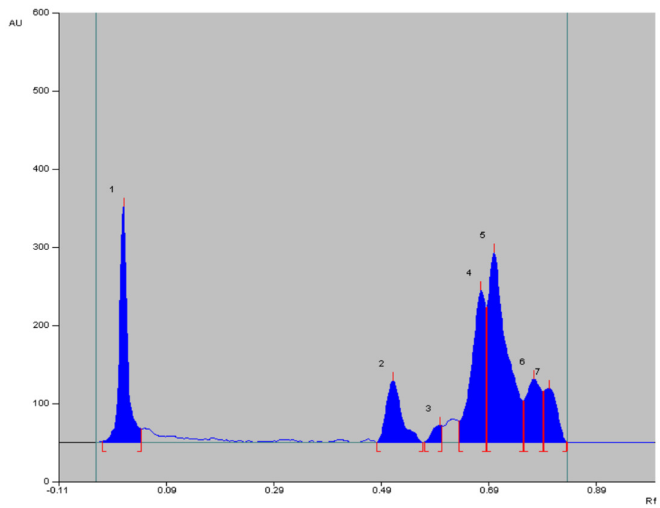


Figure 3. Calibration curve of vasicine


Track 1 , ID: STANDARD
AU
400
350
300
250
200
150
100
50
0
-0.11
0.09
0.29
0.49
0.69
0.89
Rf
1
2

(A)


AU
600
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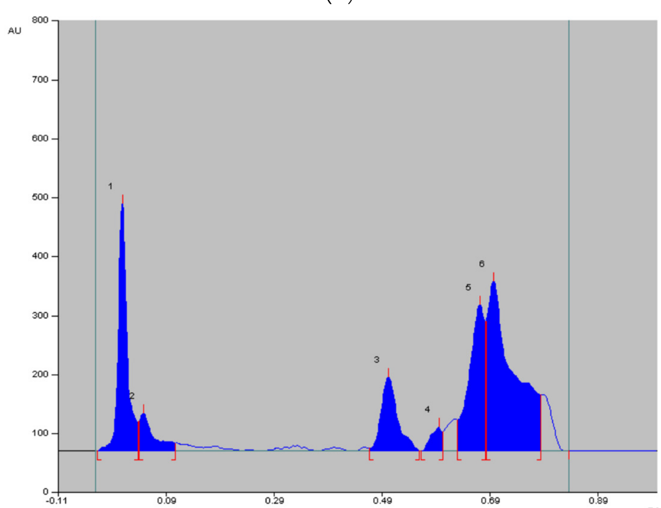
(B)


AU
800
700
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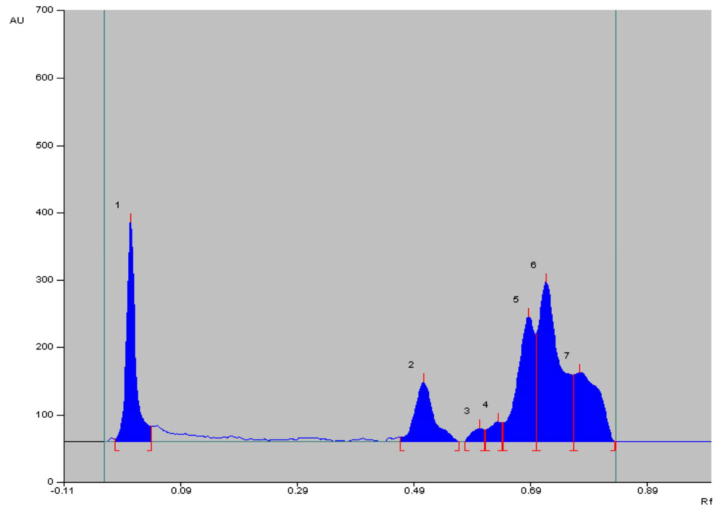
(C)



(D)

Figure 4. Densitogram of vasicine (A); Densitogram of vasicine from ethanolic extract (summer) (B); Densitogram of vasicine from ethanolic extract (rainy) (C); Densitogram of vasicine from ethanolic extract (winter) (D)

Discussion

The study of morphological, microscopical and physico-chemical parameters of *Adhatoda vasica* help to differentiate the plant from its other species. The pharmacognostic profile of plants presented here may be useful to supplement information with regard to its identification and will be helpful in establishing standardization criteria.

Present work is an attempt to compile data regarding variations of chemical constituents due to seasonal changes in selected plants i.e. *Adhatoda vasica* L. The plant belongs to alkaloid category and possessing antidiabetic activity. The plant was authenticated by botanical survey of India, Pune. Morphological and microscopic study was performed. The powdered drugs were subjected to phytochemical screening. Plant material in different seasons was extracted successively and as the percent yield of ethanolic extract found to be more as compare to other solvent extracts and according to solubility of selected phytoconstituents in ethanol, ethanolic extract was selected for further analysis. Qualitative chemical examination of extracts revealed presence of alkaloids, and other chemical components. Literature study proves that these constituents have antidiabetic activity.

The presence of vasicine in ethanolic extract of plant was confirmed by HPTLC fingerprinting and the content yield was calculated from AU. It was observed that, in different season's places and time, there is a change in HPTLC pattern of the constituents i.e. in rainy season from June to September, at high altitude and at morning 6 am vasicine content was found more. It helps to identify best season and place for collection of plant material from the source so as to gain high yield of active component and to increase the efficacy of the formulation.

Conclusions

Seasonal variation is associated with the vegetative and reproductive stages of the plant, it has direct influence with the variation in chemical constituents of the plants. As per Ayurveda, there exists a huge collection of plants with antidiabetic potential. Only few of them have been scientifically proven and a lot more have yet to be explored and proved. *A. vasica* have shown varying degrees of HPTLC Chromatogram for vasicine and hence affects hypoglycemic potency in different seasons of collection. Future studies may target isolation, purification, and characterization of bioactive compounds present in these plants and formulation of a potent antidiabetic dosage form. The outcome of such studies may provide a starting point for selection of a particular season for collection of raw material to develop potential antidiabetic drugs.

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Authors' Contributions

Both authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

Acknowledgements

This work was supported by the botanical survey of India for authentication of plant samples. B.V.D.U.'s Poona College of Pharmacy for analytical study of extracts, JSPM's Jayawantrao Sawant College of Pharmacy and Research, Hadapsar, Pune for providing Laboratory facilities to perform the research work.

List of abbreviations

A.U.C.- Area Under Curve
BSI- Botanical Survey of India
F.O.M.- Foreign Organic Matter
HPTLC- High Performance Thin Layer Chromatography
I.D.F.- International Diabetes Federation
L.O.D.- Loss on Drying OTC- Over the Counter
R & D- Research and Development
T. S.- Transverse Section
U.S- United States
WHO- World Health Organization

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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