

Variations In Vasicine Content Of Vasaka- An Indian Medicinal Plant

Palshikar Gautam ^{1*}, Dr. P. Shanmuga Pandiyan ²

1. Ph. D. Research Scholar, PRIST Deemed to be University, Thanjavur, Tamil Nadu, India - 613403.
2. Ph. D. Research Supervisor, PRIST Deemed to be University, Thanjavur, Tamilnadu, India- 613403.

* Corresponding author

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Abstract

Vasaka- *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 *Adhatoda 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 Secondary metabolites; Herbal medicines; Alkaloids; Antidiabetic; Seasonal variations.

Introduction

Vasaka an Indian medicinal plants have 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 80% of population depends on plants to therapy themselves, of those about 60% populace use medicinal plants habitually to battle certain ailments and almost 40% human use such plants in pharmaceutical industries (Sushree Dash, 2017). 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 2,50,000 higher plant species on earth, more than 80000 species are reported to have at least some medicinal value (Shi Lin Chen 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 Yuan 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

(Saikat 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 (Martins Ekor 2013). The need of new therapies for glycemic control is the fact that existing treatments have limitations because of their sideeffects (Arun 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 (Wesam Kooti, 2016.). Diabetes mellitus is a growing problem worldwide entailing enormous financial burden and medical care policy issues (Feneli Karachaliou, 2020.). According to International Diabetes Federation (IDF), the number of individuals with diabetes in 2011 crossed 366 million, with an estimated 4.6 million deaths each year, (N. H. Cho et al., 2018.). According to the World Health Organization (WHO), up to 90% of the population in developing countries uses plants and its products as traditional medicine for primary health care (Oyinlola Oyebode et al., 2016). The WHO has listed 21,000 plants, which are used for medicinal purposes around the world. Among these, 2500 species are in India (Uttpal 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 (Ngan 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 (Asha Thomas et al., 2020). The present study was aim to evaluate the physiochemical fluctuations in the leaves of *Adhatoda vasica* and the vasicine, an antidiabetic constituent in these leaves.

Material And Methods

The plant material was collected in every month of the year 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. The plant was authenticated and 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. 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 phyosterols using reported methods (Table 1).

A.	Month											
	1	2	3	4	5	6	7	8	9	10	11	12
L.	5.30 ±0.57	6.00 ±1.00	6.05 ±1.00	6.50 ±1.00	6.70 ±1.00	6.05 ±1.00	6.33 ±1.52	6.37 ±1.52	6.32 ±1.15	6.50 ±1.00	5.27 ±1.15	5.78 ±1.15
M.	5.34 ±0.57	6.07 ±1.00	6.04 ±1.00	6.02 ±1.00	6.01 ±1.00	6.06 ±1.00	6.03 ±1.52	6.33 ±1.52	6.36 ±1.15	6.04 ±1.00	5.72 ±1.15	5.76 ±1.15
H.	5.76 ±0.57	6.34 ±0.57	6.33 ±0.57	6.36 ±0.57	6.39 ±0.57	6.34 ±0.57	6.07 ±1.15	6.75 ±1.15	6.73 ±0.57	6.39 ±0.57	6.05 ±1.00	6.02 ±1.00

Table 1: HPTLC Analysis- High Performance Thin Layer Chromatography

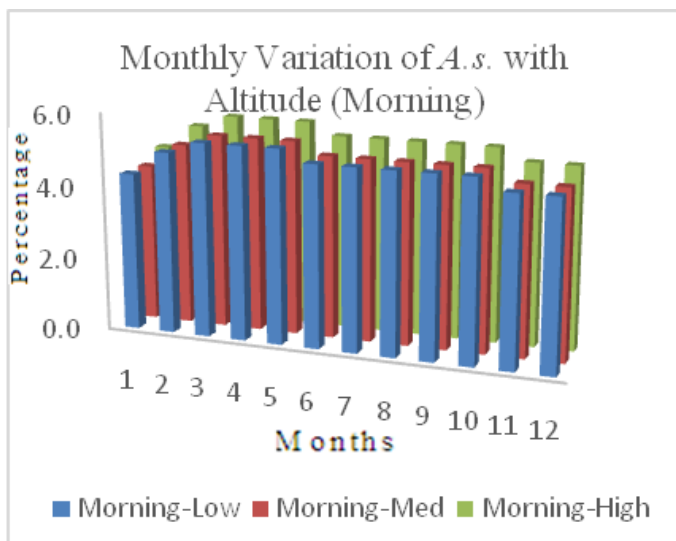
Plant Name	Phyto Constituent	Std. Area (Under Curve)	Mobile Phase	Wavelength (nm)
Vasaka	Vasicin	5053.5 AU	Ethyl acetate: Methanol: Ammonia (8:2:0.2)	254

Result

Plant leaf color is green, Characteristic odor and taste, shape is oblong simple, petiolate, exstipulated. 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. Enclosed by a parenchymatous bundle sheath. Vessels with pitted thickening, anomocytic or anisocytic Stomata, glandular, multicellular uniseriate (60 to 125 μ) trichomes, Prismatic calcium oxalate crystals and starch grains are present. Constituents in leaf shows presence of alkaloids and its percentage varies at different season, time and places (Table 2; Figure 1).

Table 2: Monthly Variation with high Altitude at Morning (% of alkaloids) n= 3 P< 0.05.

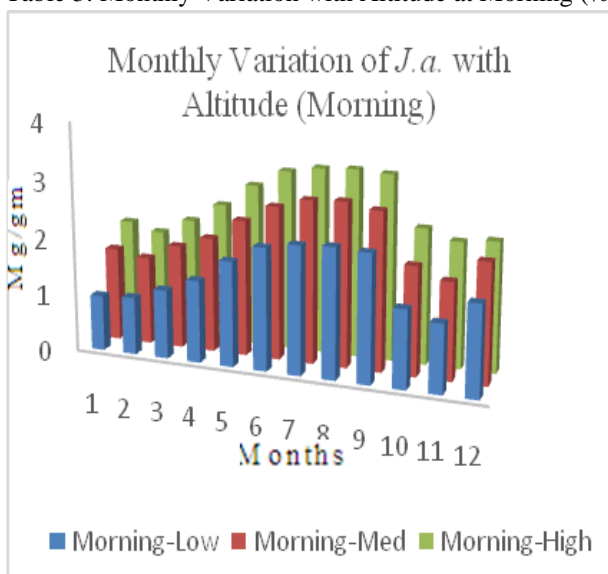
A= Altitude, L= Low, M= Middle, H= High



Med.= Medium (Altitude)

Fig. 1 Monthly Variation with Altitude (Morning)

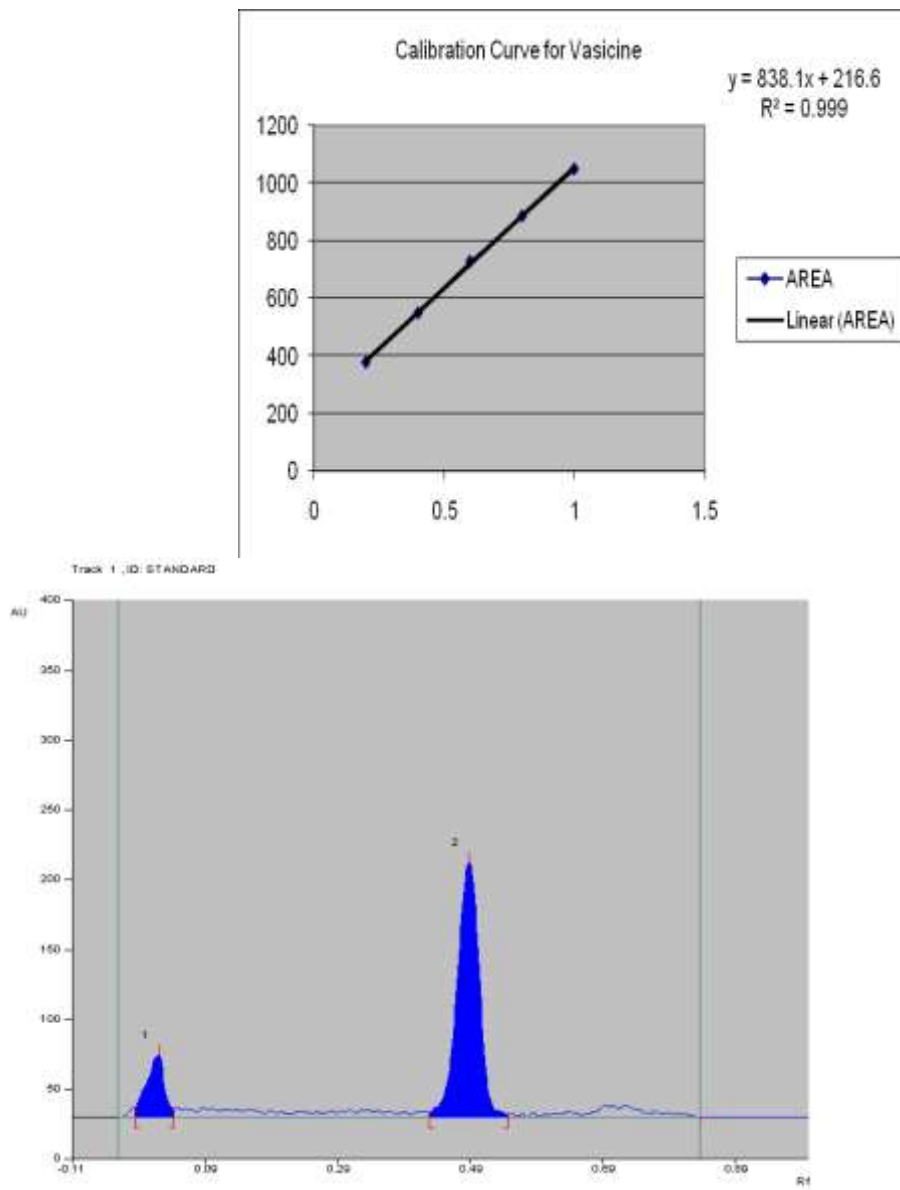
Table 3: Monthly Variation with Altitude at Morning (% Yield mg/g) n= 3 P< 0.05.



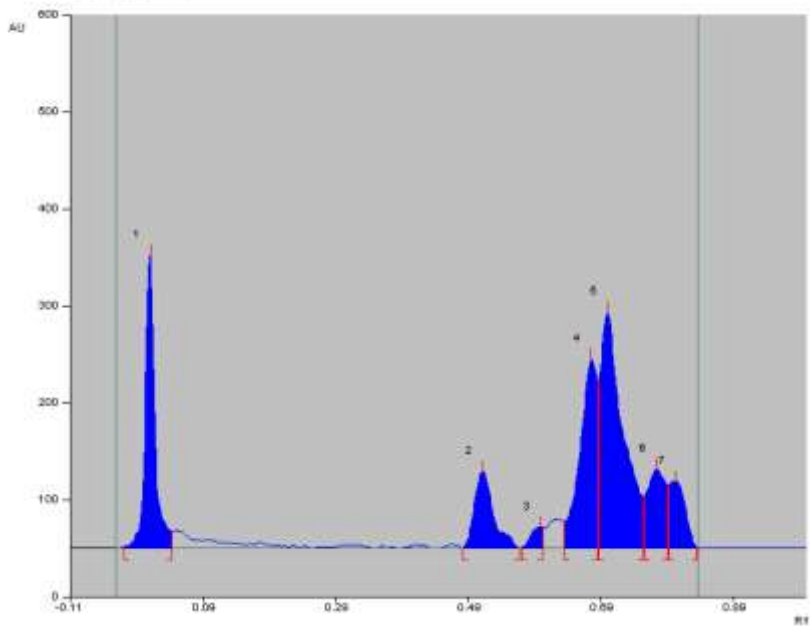
Mg/gm= Milligram/ gram, Med= Medium (altitude)

Figure 2: Monthly Variation of with Altitude (Morning)

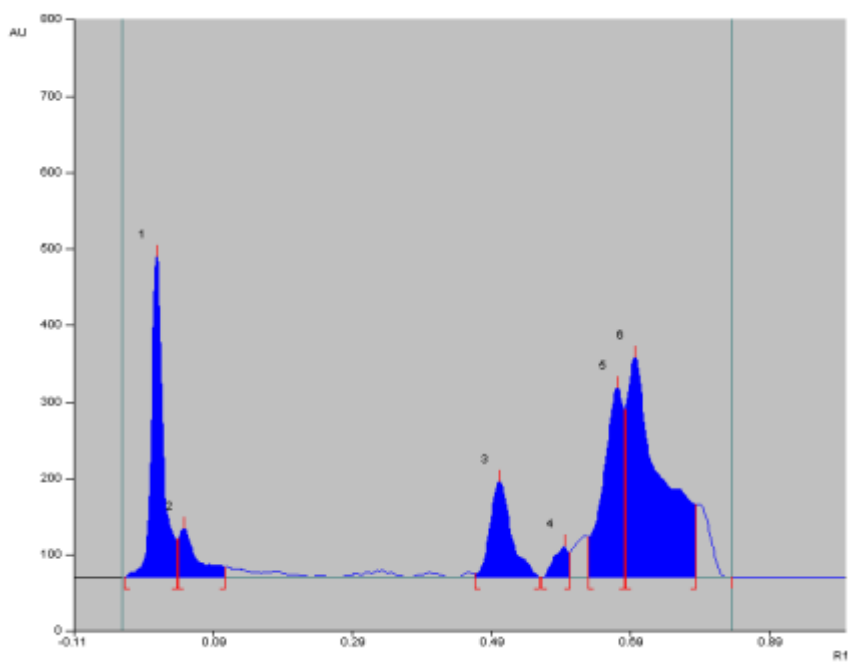
Figure 3: Calibration curve of vasicine



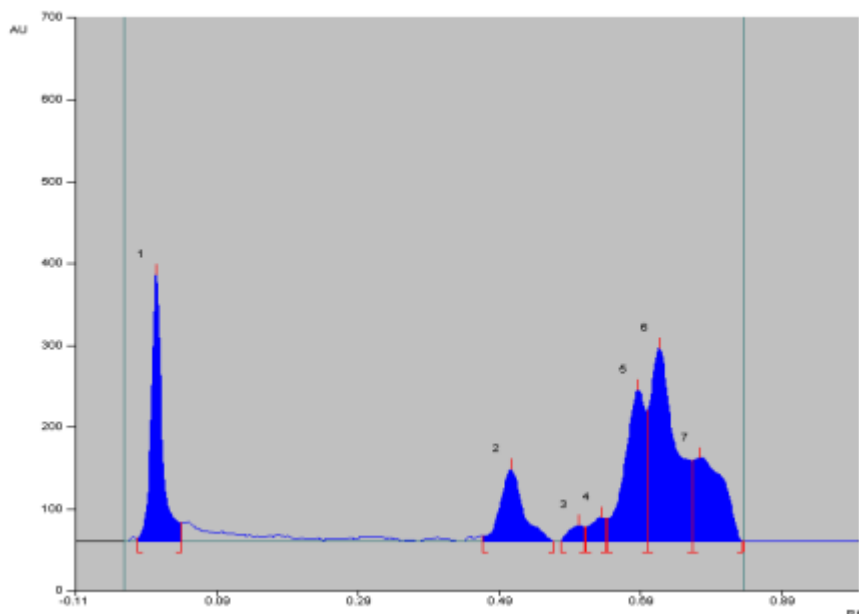
Chromatogram of Standard Vasicine



Chromatogram of Vasicine Summer season



Chromatogram of Vasicine Rainy season



Chromatogram of Vasicine Winter season

Figure 4: HPTLC Densitogram of vasicine

Table 4: HPTLC analysis-

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

The values are expressed as mean SEM; $P < 0.05$, (Two way ANOVA followed by Tukey's multiple comparison test). The Vasicine content in ethanolic extract of *J. adhatoda* is more and values are significantly variable in June-Sept. (rainy season) at morning time with high altitude place.

Discussion

The study of morphological, microscopical and physico-chemical parameters of *Justicia adhatoda* 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. *Justicia adhatoda* 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 seasons there is a change in HPTLC pattern of the constituents i.e. in rainy season Vasicine content is more. It helps to identify best season 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.

Conclusion

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. *Justicia adhatoda* 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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Disclosure of Conflict of Interest

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