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SEASONAL VARIATIONS ON ANTIDIABETIC PHYTOCHEMICALS IN JUSTICIA ADHATODA

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Abstract-

Background and Aim-

Plants live on a planet with days and seasons, and that affects their phytoconstituents. Challenge is, availability of active principles in medicinal plants change by seasonal fluctuations, so their dose pattern for therapeutic efficacy also gets influenced. The best duration for the harvesting of specific secondary metabolites for better yield is not fixed. Seasonal impact show changes in important constituents like polyphenol, flavonoids, glycosides, alkaloids, essential oil etc. Late summer is the best collection time for essential oil component. Winter and rainy are best season for other secondary metabolites.

Experimental procedure

The selected plant i.e. *Justicia adhatoda,* belongs to alkaloidal category with having antidiabetic activity. Plants were evaluated for pharmacognostic study which includes macroscopic and microscopic evaluation, determination of physicochemical parameters in a systematic way. HPTLC fingerprinting for vasicine was done. Study was performed for plant material with three different seasons and best results were analysed.

Results and Conclusion

All the plants showed correct taxonomy with specific morphological, microscopical and physico-chemical parameters which is helpful for the standardization of drugs. Extracts showed presence of alkaloids, terpenes, flavonoids, steroids, phenolics, saponin and carbohydrate. HPTLC fingerprinting confirmed the presence of vasicine in the plant extracts. Seasonal variations occour in plant constituent shows best collection period. Current research aims to focus on best possible season for the harvesting of some pharmaceutically important plant materials.

Keywords Secondary metabolites, Herbal medicines, alkaloids, antidiabetic, seasonal variations

抽象的-

背景和目标-

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植物生活在一个有昼夜交替的星球上，这会影响它们的植物成分。挑战是，药用植物中活性成分的可用性随季节波动而变化，因此它们的治疗功效剂量模式也会受到影响。收获特定次生代谢物以提高产量的最佳持续时间不是固定的。多酚、黄酮类、苷类、生物碱、精油等重要成分随季节变化而变化。夏末是精油成分的最佳采集时间。冬季和多雨是其他次生代谢物的最佳季节。

实验程序

所选择的植物，即Justicia adhatoda，属于具有抗糖尿病活性的生物碱类。对植物进行生药学研究评估，包括宏观和微观评估，以系统的方式确定理化参数。进行了瓦西辛的 HPTLC 指纹图谱分析。对三个不同季节的植物材料进行了研究，并分析了最佳结果。

结果和结论

所有植物均表现出正确的分类学，具有特定的形态、显微和理化参数，有助于药物的标准化。提取物显示存在生物碱、萜烯、类黄酮、类固醇、酚类、皂苷和碳水化合物。 HPTLC 指纹图谱证实植物提取物中存在 vasicine。植物成分的季节性变化显示最佳采集期。目前的研究旨在集中于收获一些药学上重要的植物材料的最佳季节。

关键词 次生代谢产物，草药，生物碱，降糖药，季节变化

1. Introduction-

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 [1]. 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. [2, 3] 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. [4] 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 [5]. 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 [6, 7]. The need of new therapies for glycemic control is the fact that existing treatments have limitations because of their side effects. [8] 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. [9] Diabetes mellitus is a growing problem worldwide entailing enormous financial burden and medical care policy issues [10]. 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 [11]. 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 [12]. The WHO has listed 21,000 plants, which are used for medicinal purposes around the world. Among these, 2500 species are in India [13]. 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 [14]. A chromatographic fingerprint of a Herbal Medicine is a chromatographic pattern of the extract of certain common chemical components of pharmacologically active and or chemical constituents. This chromatographic contour should be highlighted by the essential attributions of reliability and fuzziness or similarity and differences so as to chemically represent the herbal medicine explored [15]. Phytochemical changes due to various seasons were studied by performing HPTLC densitometric quantification. Microscopic variation observed in the quantity of cell inclusions, number of fibers and wall thickness of lignified cells. Physicochemical parameters also showed variation. [16]

Need-

- Lack of common standards and appropriate methods for evaluating traditional medicine to ensure the safety, efficacy and quality control.
- Importance and necessity to develop a standard operational procedure for the standardization of herbal drugs and formulations.
- Benchmarking the evaluation protocols including both quality control and quality assurance of herbal drugs would play a major role in providing highly reliable and effective herbals drugs and to attract international trade, thus generating revenue.
- A uniform research policy in herbal medicines is need of the hour.
- Development of standardized herbal formulations is necessary.
- Inadequate regulation and an increasing demand for better documentation of efficacy and safety of herbal remedies have countered this popularity.

Objective-

To evaluate best season for collection of herbal raw material so as to gain more percentage of active constituents and leads to potent formulation.

2. Material Methods-

2.1 Collection and Identification of Plant material-

The plant material i.e. of *Justicia adhatoda L.* was collected in the three different seasons i.e Summer (May), Rainy (September) and Winter (January) from the Botanical garden of JSPM's Jayawantrao Sawant College of Pharmacy and Research, Pune, Maharashtra. Authentication was done by Taxonomist of the Botanical Survey of India, Pune. A voucher specimen (No. BSI/WRC/100-1/Tech./2019/02) was deposited in the Herbarium of Botanical Survey of India, Pune.

2.2 Assessment of quality of plant materials-

The plant materials were assessed as per WHO guideline.

2.2.1 Macroscopic evaluation-

Fresh plant parts were subjected to color, odor and taste, determination of shape, size, surface characteristics and appearance.

2.2.2 Microscopic evaluation-

For microscopical 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.

2.2.3 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

2.2.4 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

2.3 Phytochemical screening:

The 100 gm air dried powder extracted in soxhlet apparatus with 300 ml solvents of increasing polarity as- 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.

Fig- 1 Hot continuous extraction- Soxhlet extraction

2.3.1 Establishment of qualitative phytoprofile of successive solvent extracts. (chemical tests):

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.

2.4 HPTLC Analysis:

Plant Name	Phyto Consti.	Std. Area	Mob. Phase	λ max (nm)
J. adhatoda	Vasicine	5053.5 AU	Ethyl acetate: Methanol: Ammonia (8:2:0.2)	254

Table- 1 Mobile phase used for HPTLC analysis

3. Result-

3.1. Assessment of quality of plant material- *J. adhatoda L.*

3.1.1. Macroscopic evaluation-

Fig. 2 *J. adhatoda L.* leaf Summer Rainy season Winter season

Colour is green, Unpleasant odour, bitter taste, shape: is oblong simple, petiolate, exstipulate crenate or entire margin, a tapering base and acuminate apex, leathery touch, smooth and shining texture.

3.1.2 Microscopic evaluation

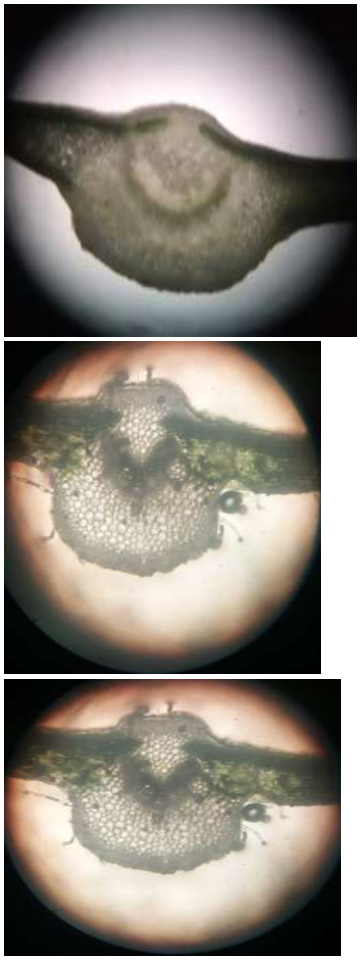


Fig. 3 T. S. Summer Rainy season Winter season

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 125µ) trichomes, Prismatic form of calcium oxalate crystals and starch grains are present.

3.1.3 Quantitative microscopy-

S. No	Parameter	Summer	Rainy	*Winter*
1	St. number	6.4	7.2	6.7
2	St. Index	11.4	11.7	12.5
3	Palisade ratio	1.3	1.3	1.4
4	Vein islet no	23	25	23
5	Vein termi no.	35	36	36

TABLE 2 Quantitative microscopy of *J. adhatoda L.* leaf

3.1.4 Proximate Analysis-

S. No	Parameter (%)	Summer	Rainy	*Winter*
1	F.O.M.	1	1.4	1
2	L.O.D.	4.55	4.20	10.2
3	Total ash	6.75	9.5	8.5
4	water soluble ash	1.70	2.50	3.50
5	Acid Insolu Ash	1.25	1.70	1.0
6	Sulphated Ash	1.70	2.50	2.30
7	Water S. Ext. V.	10.10	10	32.8
8	Alcohol S. Ext. V	8.6	8.7	8.7
9	Ether S. Ext. V	4	4	3

TABLE 3 Proximate Analysis of *J. adhatoda L.* leaf

Phytochemical studies-

Parameter	Solvent			
	Pet. ether	Chloroform	Ethyl acetate	Ethanol
Color	Green	Green	Brown	Brown
Consistency	Viscous	Viscous and	Viscous an	Viscous an

		Stick y	d Sti ck y	d Sti ck y
%Yie ld w/w	2.3 6	2.02	1.7 8	8.6

TABLE 4 Preliminary phytoprofle of *J. Adhatoda* leaf extract summer season

Para meter	**Solvent**			
	Pet. ether	**Chlor ofor m**	**Ethyl aceta te**	**Etha nol**
Color	Gr een	Green	Brow n	Br ow n
Consi stenc y	Vis co us	Visco us and Stick y	Vis co us an d Sti ck y	Vis co us an d Sti ck y
%Yie ld w/w	2.3 0	2.10	1.8 0	8.7

TABLE. 5 Preliminary phytoprofle of *J. Adhatoda* leaf extract rainy season

Para meter	**Solvent**			
	Pet. ether	**Chlor ofor m**	**Ethyl aceta te**	**Etha nol**
Color	Gr een	Green	Brow n	Br ow n
Consi stenc y	Vis co us	Visco us	Vis co us	Vis co us
%Yie ld w/w	2.3 2	2.00	1.7 8	8.5

TABLE 6 Preliminary phytoprofle of *J. Adhatoda* leaf extract winter season

3.1.5 Qualitative chemical tests-

S. No.	**Type of phytoconstituent**	**Season**		
		Summer	**Rainy**	**Winter**
1	Alkaloids	+	+	+
2	Amino acids	-	-	-
3	Carbohydrates	+	+	+
4	Flavonoids	+	+	+
5	Glycoside	+	+	+
6	Phenolic compounds	-	-	-
7	Proteins	-	-	-
8	Steroids	+	+	+
9	Saponins	+	+	+

TABLE 7 Qualitative chemical tests of leaf extract (+: Present, -: Absent)

3.1.6 HPTLC analysis -

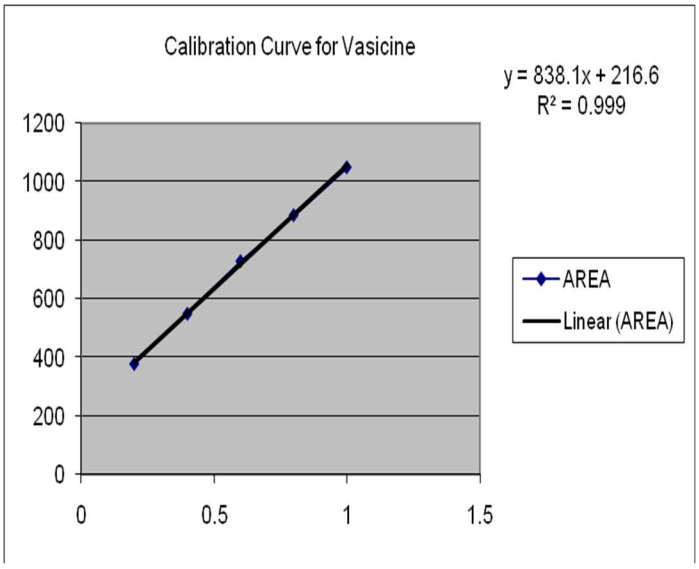
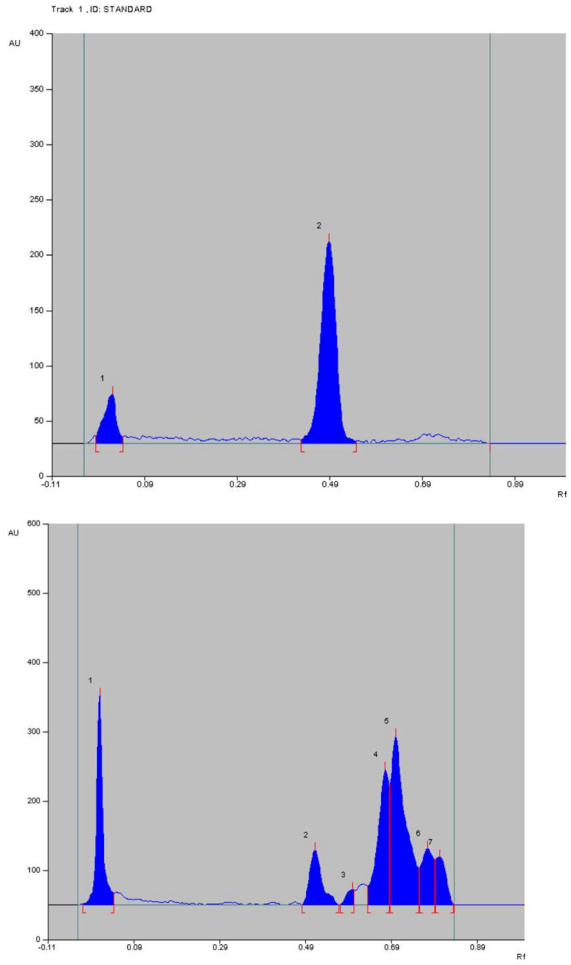


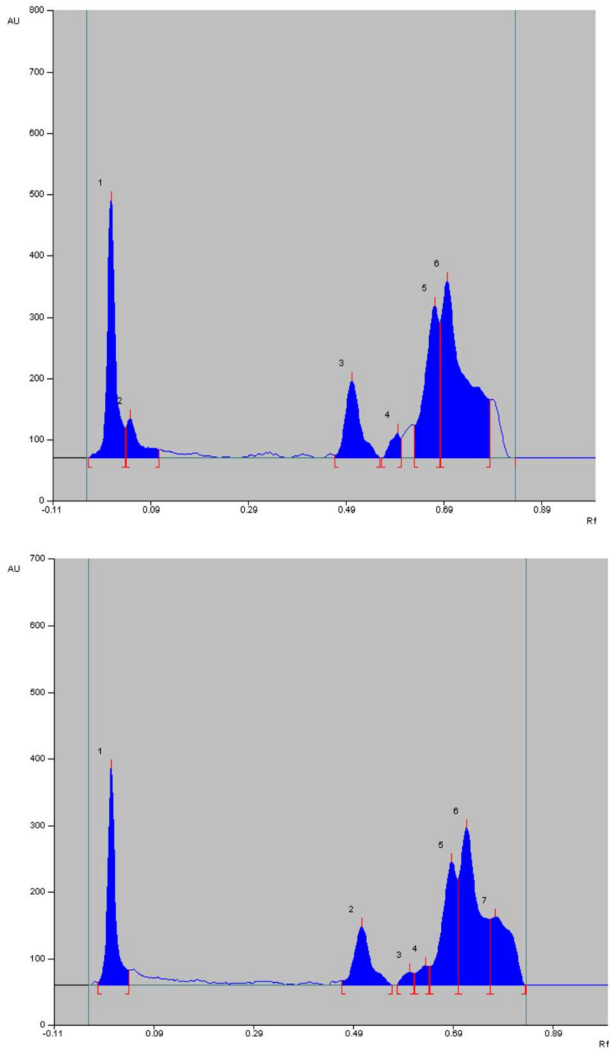
Fig. 4 Calibration curve for vasicine

CONC µg/ml	**AREA (AU)**
0.2	379.9

0.4	550.8
0.6	730
0.8	887
1	1049.9



Standard vasicine Ethanolic extract (Summer)



Ethanolic extract (Rainy)
Ethanolic extract (Winter)

Fig. 5 Densitogram of standard and extracts

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

TABLE 8 HPTLC analysis of *J. Adhatoda*

Leaf extract

The ethanolic extract of *J. adhatoda* in three different seasons contains 1.89, 3.20 and 2.36 mg/g vasicine respectively, It shows that in

Rainy season Vasicine content is more in *J. Adhatoda* leaves.

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 belong 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.

CONFLICT OF INTEREST-

The authors certify that, they have no involvement in any organization or entity with any financial or non-financial interest in the subject matter or materials discussed in this paper.

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