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***Research Article** (Open Access)*

IMPACT OF SEASONAL VARIATIONS ON ANTIDIABETIC PHYTOCHEMICALS IN *ALSTONIA SCHOLARIS*

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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. *Alstonia scholaris L.,* 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 erythrodiol 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 erythradiol 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*

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

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 (Hiren *et al.* 2013). 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 (IUCN, 2011; Marinelli, 2005). 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 (Gurib-Fakim).

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 (Vyas, 2010). 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 (Adithan, 1996; Tandon *et al.*, 2004).

The need of new therapies for glycemic control is the fact that existing treatments have limitations because of their side effects (Stumvoll *et al.*, 2005). 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 (American Diabetes Association, 2007). Diabetes mellitus is a growing problem worldwide entailing enormous financial burden and medical care policy issues (Keter and Mutiso, 2012). 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 (Dong *et al.*, 2012). 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 (WHO, 2002). The WHO has listed 21,000 plants, which are used for medicinal purposes around the world. Among these, 2500 species are in India (Modak *et al.*, 2007). 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 (Patil *et al.*, 2011).

A chromatographic fingerprint of an 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 (Patil and Shettigar, 2011). 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 (Anderson *et al.*, 2008).

MATERIALS AND METHODS

Collection and Identification of Plant material-

The plant material i.e. of *Alstonia scholaris 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./2019/06) 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 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.

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:

Table 1: Mobile phase used for HPTLC analysis

Plant Name	Phyto Consti.	Std. Area	Mob. Phase	λ max (nm)
A. scholaris	Erythrodiol	3670.5 AU	Methanol: Toluene (8:2)	254

RESULTS

3.1. Assessment of quality of plant material- A. scholaris L.

3.1.1. Macroscopic evaluation

Fig. 1: *A. scholaris L.* **leaf Summer** **Rainy season** **Winter season**

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

3.1.2 Microscopic evaluation

Fig. 2: 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

TABLE 2: Quantitative microscopy of *A. Scholaris* L. leaf

S. No	Parameter	Summer	Rainy	Winter
1	St. number	13	20	14
2	St. Index	6	12	5.8
3	Palisade ratio	7	8	5
4	Vein islet no	9	12	11
5	Vein termi no.	8	10	8.3

3.1.4 Proximate Analysis

TABLE 3: Proximate Analysis- of *A. scholaris* L. leaf

S. No	Parameter (%)	Summer	Rainy	Winter
1	F.O.M.	1.2	1.4	1.5
2	L.O.D.	5. 50	5. 20	6.25
3	Total ash	13.80	10.80	13.5
4	water soluble ash	3.70	1.40	3.50
5	Acid Insolu. Ash	2.30	1.80	1.0
6	Sulphated Ash	1.65	2.65	2.30
7	Water S. Ext. V.	15	12	32.8
8	Alcohol S. Ext. V	8.2	7.6	7.5
9	Ether S. Ext. V	3.5	4.5	3.0

3.1.5 Phytochemical studies-

TABLE 4: Preliminary phytoprofle of *A. Scholaris* leaf extract summer season

Parameter	Solvent			
	Pet. ether	Chloroform	Ethyl acetate	Ethanol
Color	Green	Green	Brown	Chocolate Brown
Consistency	Viscous	Viscous and Sticky	Viscous and Sticky	Viscous and Sticky
%Yield w/w	3.36	3.87	3.10	8.2

TABLE 5: Preliminary phytoprofle of *A. Scholaris* leaf extract rainy season

Parameter	Solvent			
	Pet. ether	Chloroform	Ethyl acetate	Ethanol
Color	Green	Green	Brown	Chocolate Brown
Consistency	Viscous	Viscous and Sticky	Viscous and Sticky	Viscous and Sticky
%Yield w/w	3.45	3.65	3.40	7.6

TABLE 6: Preliminary phytoprofle of *A. Scholaris* leaf extract winter season

Parameter	Solvent			
	Pet. ether	Chloroform	Ethyl acetate	Ethanol
Color	Green	Green	Brown	Chocolate Brown
Consistency	Viscous	Viscous and Sticky	Viscous and Sticky	Viscous and Sticky
%Yield w/w	3.60	3.70	3.40	7.5

3.1.6 Qualitative chemical tests-

TABLE 7: Qualitative chemical tests *A. scholaris* leaf extract (+ : Present, - : Absent)

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

3.1.7 HPTLC analysis-

CONC µg/ml	AREA (AU)
1	929.3
2	1425.3
3	1862.3
4	2272.8
5	2761.4

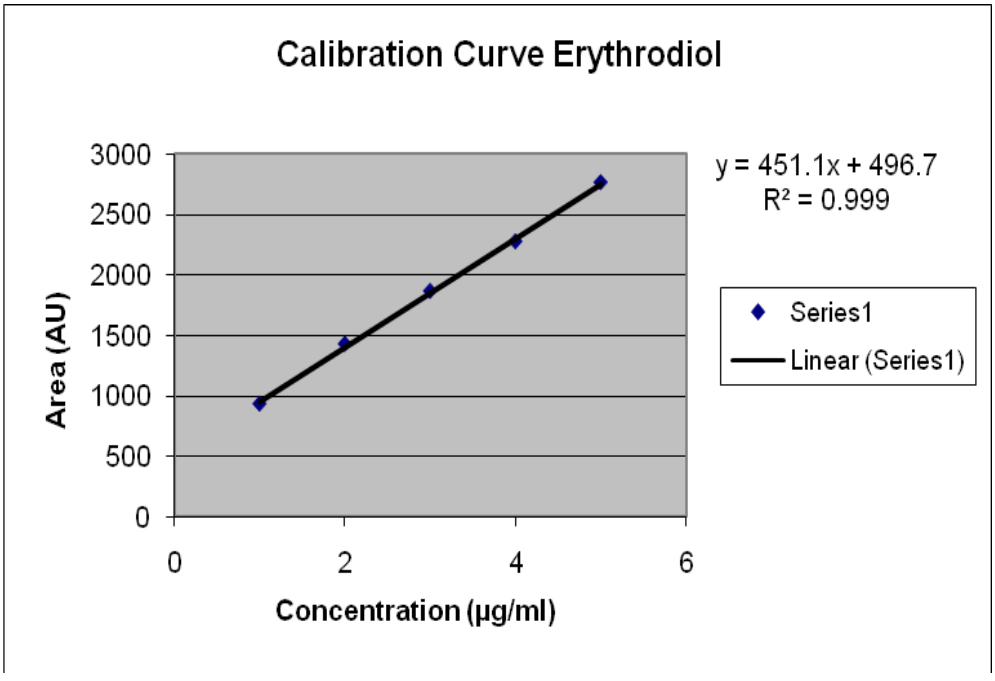
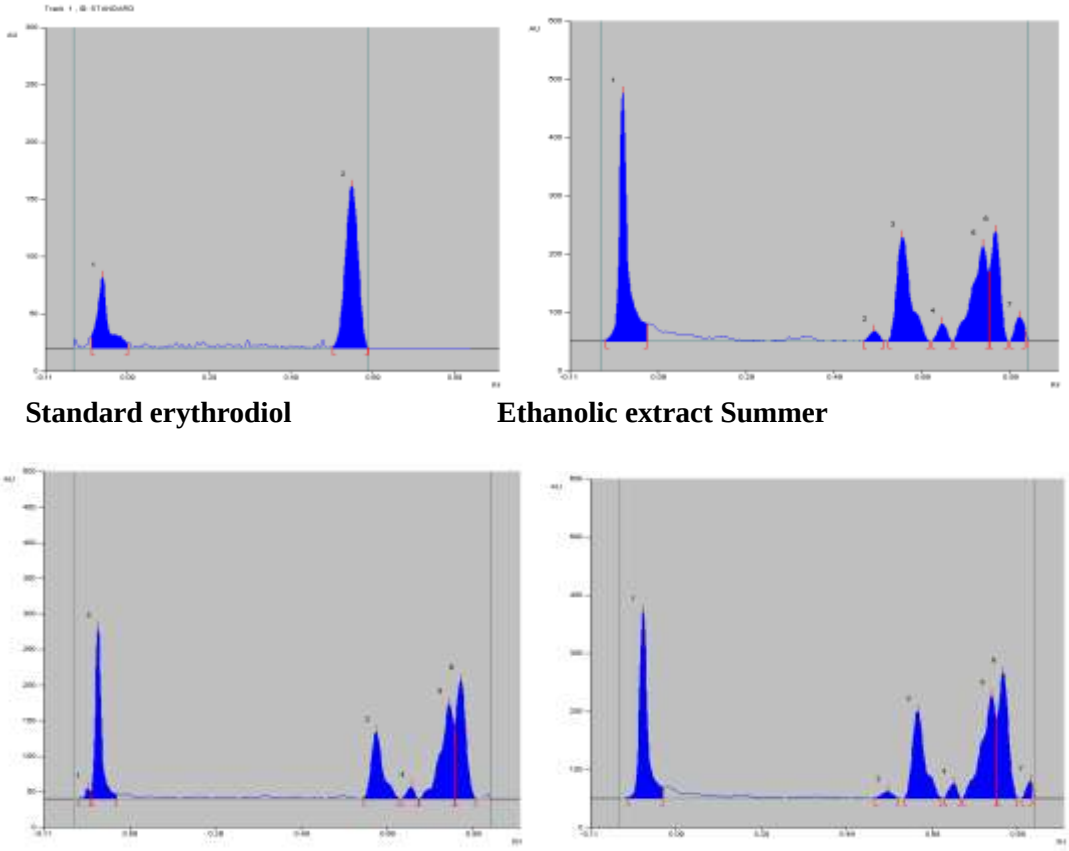


Fig. 3: Calibration curve for Erythrodiol



Standard erythrodiol **Ethanolic extract Summer**

Ethanolic extract Rainy **Ethanolic extract Winter**

Fig. 4: Densitogram of erythrodiol from *A. scholaris*

TABLE 8: Result of HPTLC analysis of *A. Scholaris leaf extract*

Rf Value	Season	Area (AU)	Yield (mg/g)
0.64	**Summer**	**4657.6**	**3.1**
	Rainy	2072.6	1.37
	Winter	3595.2	2.39

The ethanolic extract of *A. scholaris* in three different seasons contains 3.1, 1.37 and 2.39 mg/g Erythrodiol respectively, It shows that in summer season Erythrodiol content is more in *A. scholaris* leaves.

DISCUSSION

The study of morphological, microscopical and physico-chemical parameters of *A. Scholaris* 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. *A. Scholaris*. 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 antidiabetic constituents i.e. in summer season erythradiol 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.

A. scholaris have shown varying degrees of HPTLC Chromatogram for erythradiol 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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CONFLICT OF INTEREST

The authors declare that the research was conducted in absence of any conflict of interest. This research did not receive any specific grant from funding agencies in the public, commercial or not for profit sectors.

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