ISSN: 0975-3583,0976-2833 VOL 12, ISSUE 01, 2021

SEASONAL VARIATIONS ON ANTIDIABETIC PHYTOCHEMICALS IN *SYZYGIUM CUMINI L.*

Gautam Palshikar[1], P. Shanmuga Pandiyan[2]
1- PhD Research Scholar, PRIST Deemed to be University, Tamil Nadu, India.
2- Research Supervisor, PRIST Deemed to be University and Professor and Dean at School of Pharmacy, Sathyabama Institute of Science &Technology, Chennai, India.

Abstract-

Introduction

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.

Objective

Plants live on a planet with ages 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.

Methods

The selected plant i.e. *Syzygiumcumini L.* belongs to alkaloidal category with having antidiabetic activity. Plant was evaluated for pharmacognosticstudy which includes macroscopicand microscopic evaluation, determination of physicochemical parameters in a systematic way. HPTLC fingerprinting for stigmasterol was done. Study was performed for plant material with three different seasons and best results were analysed.

Results and Conclusion

Selected plantshowed correct taxonomy with specific morphological, microscopical and physico-chemical parameters which is helpful for the standardization of phytoconstituents. Extractsshowed presence of alkaloids, terpenes, flavonoids, steroids, phenolics, saponin and carbohydrate. HPTLC fingerprinting confirmed the presence of stigmasterolin 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.

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 includingIndia [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

ISSN: 0975-3583,0976-2833 VOL 12, ISSUE 01, 2021

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 reliabilityand 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-

Both the plant material i.e. of *Syzygiumcumini L.*was collected in the three different seasons i.e Summer (May), Rainy (September) and Winter (January) from the Botanical garden of JSPM's Jayawantraosawant 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./2019/04) was deposited in the Herbarium of Botanical Survey of India, Pune.

2.2 Assessment of quality of plantmaterials-

The plant materials were assessed as per WHO guideline.

2.2.1 Macroscopicevaluation-

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

2.2.2 Microscopicevaluation-

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 Quantitativemicroscopy-

Leaves were subjected to quantitative microscopy for the following values using reportedmethod.

- Stomatal number
- Stomatal index
- Palisaderatio
- Vein isletnumber
- Vein terminationnumber

ISSN: 0975-3583,0976-2833 VOL 12, ISSUE 01, 2021

2.2.4 Proximateanalysis-

Proximate analysis of powdered plant material was carried out using reportedmethods.
Following determinations were done

- Foreign organic matter
- Loss ondrying
- Total ash
- Water soluble ash
- Acid insoluble ash
- Sulphated Ash
- Water soluble extractives
- Alcohol soluble extractives
- Ether soluble Extractivevalue

2.3 Phytochemicalscreening:

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.

Figure 1Hot 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 reportedmethods.

ISSN: 0975-3583,0976-2833 VOL 12, ISSUE 01, 2021

2.4 HPTLC Analysis:

Table 1Chromatographic Conditions used for HPTLC Analysis

Instrument used	CAMAG make HPTLC
Software	winCATS
Sample Applicator	Linomat 5 and Hamilton 100 µL syringe
Stationary Phase	HPTLC plates silica gel G 60F254
Application Volume	10 µl
Development Mode	TLC Twin Trough Chamber
Derivatization mode	Dip tank for about 1 minute
Spray reagent	Anisaldehydesulphuric acid reagent

a) Samplepreparation:

Accurately weighed 20 mg of each extracts individually into volumetric flask and 10 mL methanol was added to it. Dissolved it and filtered it with whatman filter paper no. 1 and used for HPTLC profiling.

b) Standardpreparation:

Accurately weighed 10 mg of each standard individually into volumetric flask and 10 mL methanol was added to it. Dissolved it and filtered it with whatman filter paper no. 1 and used for HPTLC profiling.

Procedure-

HPTLC was performed on ethanolic extract of crude drug. All the extracts were subjected to qualitative evaluation of various phytoconstituents using precoated TLC silica gel G 60F254 (Merck) plates as stationary phase and most suitable mobile phase to give better chemoprofile of the constituents. Linomat 5 applicator and Hamilton 100 µLsyringe was used for application. Silica gel G 60 F254 precoated HPTLC plates (E. Merck Germany) were employed for fingerprinting, in order to achieve better separation of all the components in the extract. The plates were pre-washed in methanol for removing any impurities picked up by plate during storage in the laboratory environment.

Plant extractswere applied on HPTLC plate and developed in given solvent system to a distance of 8 cm. The plates were dried at room temperature in air, scanned after spraying with detection reagent (Anisaldehyde sulphuric acid) and heated at 110^0 C for 5 minutes). The *Rf*values and yield of the resolved bands were noted.

Table 2Mobile phase used for HPTLC analysis

Plant Name	PhytoConsti.	Std. Area	Mob. Phase	λ max(nm)
S. cumini	Stigmasterol	2301.0 AU	Methanol: Toluene (7:3)	254

3. RESULT-

3.1 Assessment of quality of plant material- *S. Cumini*L.

3.1.1 Macroscopic evaluation-

Colour is green, astringent odour, sour taste, Size is 8 x 14 cm,Shape is oblong-ovate to elliptic, broad and less acuminate apex. Base Cuneate- obtuse,margin entire, leatherytouch, smooth and shining Texture.

ISSN: 0975-3583,0976-2833 VOL 12, ISSUE 01, 2021

Figure 2: Morphology of S. cumini L. leaf

3.1.2 Microscopic evaluation-

Cell wall is single layered compactly arranged barrel shaped epidermal cells with thin cuticle and lower epidermis was found filled with brown content, Vascular bundle is radially arranged Arc shaped, Outer phloem fibers and sieve elements, inner xylem vessel, xylem parenchyma and its fiber, cyclo-staurocytic and randomly distributed stomata, unicellular trichomes, presence of starch grains and cluster crystal of Calcium oxalate.

Figure 3: Microscopy of Syzygiumcumini L. leaf

3.1.3 Quantitative microscopy-

Table 3Quantitative microscopy of S. cumini L. leaf

S. No	Parameter	Summer	Rainy	Winter
1	St. number	8	14	8
2	St. Index	14	18	13
3	Palisade ratio	7.5	7	6.5
4	Vein islet no	8	9	9
5	Vein termi no.	9	11	9

3.1.4 Proximate Analysis-

Table 4Proximate Analysis- of S. cumini L. leaf

S. No	Parameter (%)	Summer	Rainy	Winter
1	F.O.M.	1.5	1.1 %	1.3
2	L.O.D.	5.70	4.30	4.50
3	Total ash	5.20	6.20	3.20
4	Water soluble ash	2.60	2.20	2.30
5	Acid Insolu Ash	2.80	1.70	1.70
6	Sulphated Ash	1.00	1.60	0.60
7	Water S. Ext. V.	13	13	18
8	Alcohol S. Ext. V	8.1	8.6	7.9
9	Ether S. Ext. V	3.5	4.5	4

ISSN: 0975-3583,0976-2833 VOL 12, ISSUE 01, 2021

3.1.5 Phytochemicalstudies-

Table 5Preliminary phytoprofle of S. cumini leaf extract summer season

Parameter	Solvent			
	Pet. ether	Chloroform	Ethyl acetate	Ethanol
Color	Green	Green	Brown	Brown
Consistency	Viscous	Viscous and Sticky	Viscous and Sticky	Viscous and Sticky
%Yield w/w	2.63	3.42	2.76	8.1

Table 6Preliminary phytoprofle of S. cumini leaf extract rainy season

Parameter	Solvent			
	Pet. ether	Chloroform	Ethyl acetate	Ethanol
Color	Green	Green	Brown	Brown
Consistency	Viscous	Viscous and Sticky	Viscous and Sticky	Viscous and Sticky
%Yield w/w	2.63	3.42	2.76	8.6

Table 7Preliminary phytoprofle of S. cumini leaf extract winter season

Parameter	Solvent			
	Pet. ether	Chloroform	Ethyl acetate	Ethanol
Color	Green	Green	Brown	Brown
Consistency	Viscous	Viscous and Sticky	Viscous and Sticky	Viscous and Sticky
%Yield w/w	2.63	3.42	2.76	7.9

3.1.6 Qualitative chemical tests-

Table 8Qualitative chemical tests S. cumini 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	+	-	+

ISSN: 0975-3583,0976-2833 VOL 12, ISSUE 01, 2021

3.1.7 HPTLC analysis-

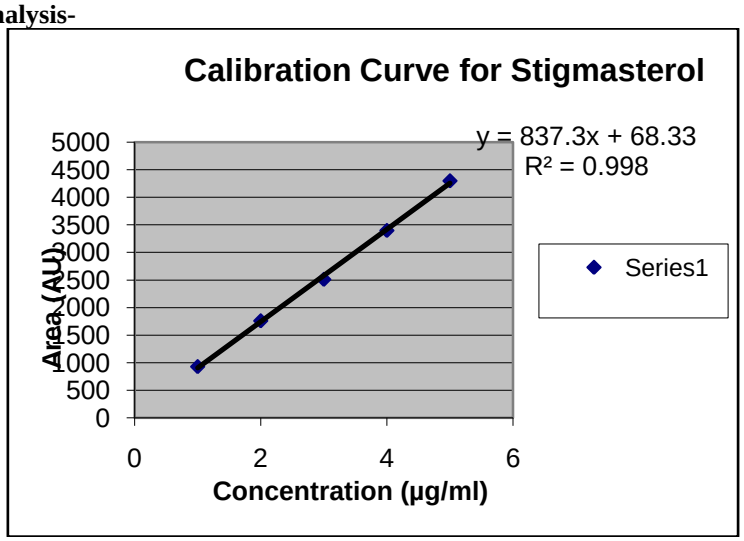
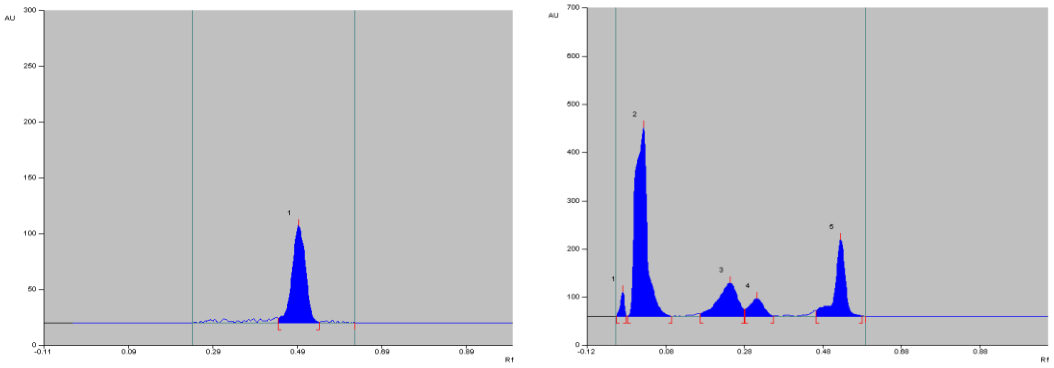
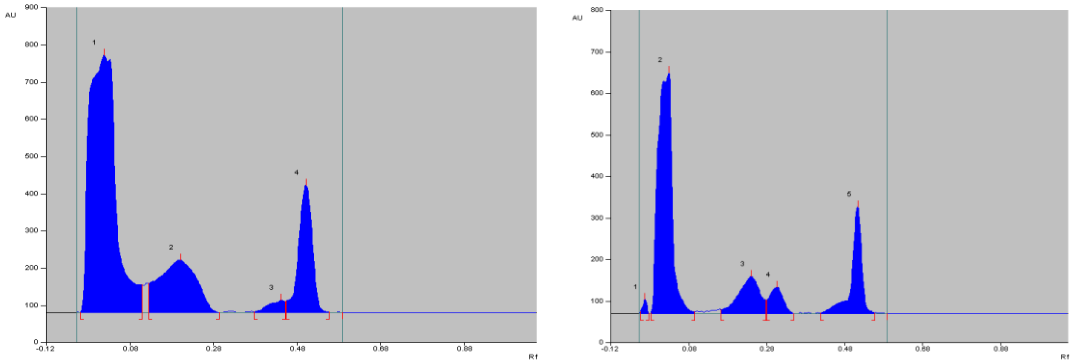


Figure 4: Calibration curve for stigmasterol

CONC µg/ml	AREA (AU)
1	931
2	1761
3	2512
4	3398.9
5	4298.7



Standard stigmasterolEthanolic extract (Summer)



Ethanolic extract (Rainy) Ethanolic extract (Winter)

Figure 5: Densitogram of stigmasterol from *S. cumini*

ISSN: 0975-3583,0976-2833 VOL 12, ISSUE 01, 2021

Table 9HPTLC analysis of S. cumini Leaf extract

Rf Value	Season	Area (AU)	Yield (mg/g)
0.51	Summer	3302.7	0.98
	Rainy	**8819.5**	**2.63**
	Winter	5292.8	1.57

The ethanolic extract of *S. cumini*in three different seasons contains 0.98, 2.63 and 1.57 mg/g Stigmasterolrespectively, It shows that in rainy season Stigmasterol content is more in *S. cumini* leaves.

DISCUSSION-

The study of morphological, microscopical and physico-chemical parameters of *Syzygiumcumini L.* 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 standardizationcriteria.

Present work is an attempt to compile data regarding variations of chemical constituents due to seasonal changes in selected plants i.e. *Syzygiumcumini L.*This plants belong to alkaloid category and possessing antidiabetic activity. The plants were authenticated by Botanical survey of India, Pune. Morphological and microscopic study of all the plants were performed. The powdered drugs were subjected to phytochemical screening. Each 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 stigmasterolin ethanolic extracts of plants 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 stigmasterolcontent 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.

4. CONCLUSION-

In the plant, the concentration of active principles is high in full bloom period, it is the best period for collection for high percentage yield of active phytoconstituent. 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.

*Syzygiumcumini*have shown varying degrees of HPTLC Chromatogram for stigmasterol,hence affectshypoglycemic 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.

FUNDING SOURCE-

There is no funding Source for this study.

ACKNOWLEDGMENT-

I most sincerely convey my deep sense of gratitude to 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 Jayawantraosawant College of Pharmacy and Research, Hadapsar, Pune for providing Laboratory facilities to perform the research work.

ISSN: 0975-3583,0976-2833 VOL 12, ISSUE 01, 2021

REFERENCES-

1. Hiren B S, Manisha D, Sheju T (2013) Compounds of selected Indian medicinal plants perspective on phytochemical and biochemical. Int. J. Chem. Res.
2. IUCN Species Survival Commission Medicinal Plant Specialist Group. http://www.iucn.org/themes/ssc/sgs/mpsg/main/Why.html. (2011) Accessed November 17.
3. Marinelli J, ed. Plant: The Ultimate Visual Reference to Plants and Flowers of the World. New York, NY: DK; (2005).
4. Gurib-Fakim A. Medicinal plants: traditions of yesterday and drugs of tomorrow. Mol Aspects Med. (2006); 27:1-93.
5. Vyas BA (2010) Phytopharmacological action of *Pergulariadaemia*with special reference to its actions and mechanism of action as diuretic and anti- inflammatory agent. Ph. D thesis.Veer Narmad South GujaratUniversity.
6. Adithan C, (1996), Pharmacological research in India, Indian J Pharmacol, 28, 125- 128.
7. Tandon V., Kapoor B., Gupta B.M., (2004), Herbal drug research in India: A trend analysis using IJP as a marker, Indian J Pharmacol, 36,96-100.
8. Stumvoll M, Goldstein BJ, van Haeften TW. Type 2 diabetes: Principles of pathogenesis and therapy. Lancet. (2005); 365: 1333–46.
9. American Diabetes Association.Standards of medical care in diabetes. Diabetes Care (2007); 30:4–41.
10. L K Keter and P C Mutiso, "Ethnobotanical studies of medicinal plants used by Traditional Health Practitioners in the management of diabetes in Lower Eastern Province, Kenya," (2012).Journal of Ethnopharmacology. vol. 139, no. 1, pp. 74–80.
11. H. Dong, N Wang, L Zhao, and F. Lu, "Berberine in the treatment of type 2 diabetes mellitus: a systemic review and meta-analysis," vol. 2012, Article ID 591654, 12 pages, (2012).Evidence-Based Complementary and Alternative Medicine.
12. World Health Organization, "Traditional medicine-growing needs and potential" (2002). WHO Policy Perspective on Medicines, vol. 2, pp. 1–6.
13. M Modak, P Dixit, J Londhe, S Ghaskadbi, and T P ADevasagayam, "Indian herbs and herbal drugs used for the treatment of diabetes," (2007).Journal of Clinical Biochemistry and Nutrition. vol. 40, no. 3, pp. 163–173.
14. R. Patil, B. Ahirwar, and D. Ahirwar, "Current status of Indian medicinal plants with antidiabetic potential: a review," (2011). Asian Pacific Journal of Tropical Biomedicine.vol. 1, no. 2, pp. S291–S298.
15. P S Patil and R Shettigar, "An advancement of analytical techniques in herbal research," (2010). Journal of Advanced Scientific Research. 1 (1), pp. 8-14.
16. Anderson D, Sweeney D, Williams T, (2008), statistics for business and economics, thomson south western.