

# SCREENING THE POTENCY OF ERYTHRODIOL CONTENT IN SAPTAPARNI LEAVES

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## Abstract

Saptaparni leaf shows importance in traditional formulations for the cure of various diseases. The physicochemical characteristics changes with the season, place and time. To obtain the highest medicinal value, it is necessary to collect the plant raw material in the correct season. This research study aim to evaluate the physicochemical changes in the leaves of *Alstonia scholaris* and alkaloid erythrodiol, an antidiabetic constituent in plant leaves. Leaves were collected in every month of a year at different time and places. Subjected to authentication and standardization. The leaves were evaluated for macroscopic and microscopic evaluation, quantitative microscopy, proximate phytochemical analysis, extractive values in different non polar to polar solvents along with determination of concentration of antidiabetic component erythrodiol. 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 varied in response to seasonal changes. Maximum levels of ethanol and water extract with erythrodiol content were obtained higher in winter season.

**Keywords:** Constituents; Herbs; Alkaloids; Hypoglycemic; External variations.

## INTRODUCTION

Since ancient years medicinal plants are useful by local societies in treatment of various diseases and disorders. Near about more than 82 % of the universal population uses herbs as resources for primary healthcare and more than 70% of some allopathic medicines involve extracts deriving from plants. (Bauer A., 2014).

*Alstonia scholaris* an Indian agricultural 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 humankind. In India, it is estimated that 80% of the population depends on plants for therapy themselves, of those about 60% populace use medicinal plants habitually to battle certain ailments, and almost 40% of humans use such plants in pharmaceutical industries (Dash S., et. al., 2017). The World Health Organization (WHO) has outlined herbal medicine as culminated labeled 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 90000 species are reported to have at least some medicinal value (Chen S. L., et. al., 2016). For ages, humans have relied on nature for their basic needs for the production of foodstuff, shelters, clothing, and means of transportation, fertilizers, flavors, 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 (Yuan

H., 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 complementary and are used in areas for health care programs in various developing countries including India (Sen S. et. al. 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 M., 2013). The need for new therapies for glycemic control is the fact that existing treatments have limitations because of their side effects (Chaudhury A., et. al. 2017).

The herbal extracts which are effective in lowering blood glucose levels with minimal or no side effects are known to be used as antidiabetic remedies (Kooti W., et. al., 2016). Diabetes mellitus is a growing problem worldwide entailing enormous financial burden and medical care policy issues (Karachaliou F., et. al., 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, (Cho N. H., et. al., 2018). According to the World Health Organization (WHO), up to 90% of the population in developing countries uses plants and their products as traditional medicine for primary health care (Oyebode O., 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 (Anand U., et. al., 2019). There are about 800 plants that 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 N., et. al., 2020). HPTLC study helps to gain an idea about the chromatographic pattern of the components. The current study involves the use of HPTLC parameters to study changes in phytoconstituents. The macroscopic and microscopic study was performed (Thomas A., et. al., 2020).

High performance thin layer chromatography (HPTLC) is an important quality assessment tool for the evaluation of herbal drugs and herbal formulations. It allows for the analysis of a broad number of compounds both efficiently and cost effectively. (Shivarkar R., et. al., 2018). The present study was aim to evaluate the physiochemical fluctuations in the leaves of *Alstonia scholaris* and the erythrodiol, an antidiabetic constituent in these leaves.

## METHODOLOGY-

### *Identification of plant-*

The plant material was collected in entire months of a year from places of different altitude i.e. Low (680 meters), medium (970 meters) and high (1,600 meters), twice in a day. Plant sample was authenticated from Botanical Survey of India, Pune.

Macroscopy, microscopic and phytochemical analysis were performed. Ethanolic extract was used for further analysis due to its high percentage yield. The present work involves the compilation of data for fluctuations of active components in the alkaloidal and anti-diabetic plants due to season, time, and places.

### *Assessment of quality of plant materials*

It involves determination of Stomatal number, Stomatal index, Palisade ratio, Vein islet number, and Vein termination number.

### *Proximate analysis*

Foreign organic matter, Loss on drying, Total ash, Water soluble ash, Acid insoluble ash, Sulphated Ash, Water soluble extractives, Alcohol soluble extractives, and Ether soluble Extractive value.

### Phytochemical study

The air-dried powder (1 Kg) of the plant was extracted in soxhlet apparatus with solvents of different polarity by using successive solvent extraction method. All the extracts were concentrated by distilling the solvent and the extracts were dried in a water bath. Then consistency, color, the appearance of the extracts, and their percentage yield were noted.

### Qualitative analysis of 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, tannins, proteins, amino acids, saponins, and phyosterols using reported methods (Tab. 1).

Table 1: HPTLC Analysis- High Performance Thin Layer Chromatography

Plant Name	Phyto Constituents	Standard Area (Under Curve)	Mobile Phase	Wavelength (nm)
<i>A. scholaris</i>	Erythrodiol	3680.6 AU	Benzene: Ethanol (8:2)	254

## RESULT AND DISCUSSION

It is Large tree type plant grows up to 20-25 metres in height; Leaves are green in Color odor and taste is characteristic, simple, whorled, estipulate; petiole 6-10 mm long, stout, glabrous; lamina 5-15 x 5-8 cm, obovate, oblanceolate; base cuneate; apex obtuse; margin entire, glabrous, subcoriaceous; lateral nerves many, slender, prominent, glabrous, parallel, looped near the margin forming intramarginal nerves; intercostae reticulate, obscure.

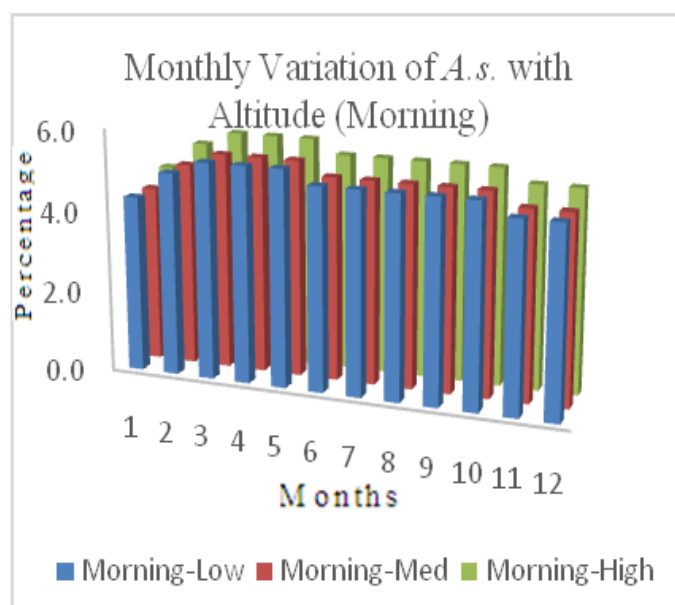
Outermost layer of cell wall made of unilayered epidermis with closely packed barrel nature parenchyma cells. Vascular Bundle is curved shaped, conjoint, collateral and closed. Inside presence of parenchymatous bundle sheath. Thick Vessels, anomocytic Stomata, glandular (50 to 120 $\mu$ ) trichomes, rosette type of crystals of calcium oxalate and starch grains are present. Alkaloids are present in leaf and its percentage varies at different season, time and places (Table 2; Figure 1).

Table 2: Monthly fluctuations of alkaloids with Altitude at time. n= 3 P< 0.05.

A.	Month											
	1	2	3	4	5	6	7	8	9	10	11	12
L.	5.33 $\pm 0.57$	6.00 $\pm 1.00$	6.33 $\pm 1.52$	5.33 $\pm 1.52$	5.33 $\pm 1.15$	6.00 $\pm 1.00$	6.00 $\pm 1.00$	6.00 $\pm 1.00$	6.00 $\pm 1.00$	6.00 $\pm 1.00$	5.67 $\pm 1.15$	5.67 $\pm 1.15$
M.	5.33 $\pm 0.57$	6.00 $\pm 1.00$	6.33 $\pm 1.52$	6.33 $\pm 1.52$	6.33 $\pm 1.15$	6.00 $\pm 1.00$	6.00 $\pm 1.00$	6.00 $\pm 1.00$	6.00 $\pm 1.00$	6.00 $\pm 1.00$	5.67 $\pm 1.15$	5.67 $\pm 1.15$
H.	5.67 $\pm 0.57$	6.33 $\pm 0.57$	6.67 $\pm 1.15$	6.67 $\pm 1.15$	6.67 $\pm 0.57$	6.33 $\pm 0.57$	6.33 $\pm 0.57$	6.33 $\pm 0.57$	6.33 $\pm 0.54$	6.33 $\pm 0.57$	6.00 $\pm 1.00$	6.00 $\pm 1.00$

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

Figure 1: Monthly Variation with Altitude and time



Med. = Medium (Altitude)

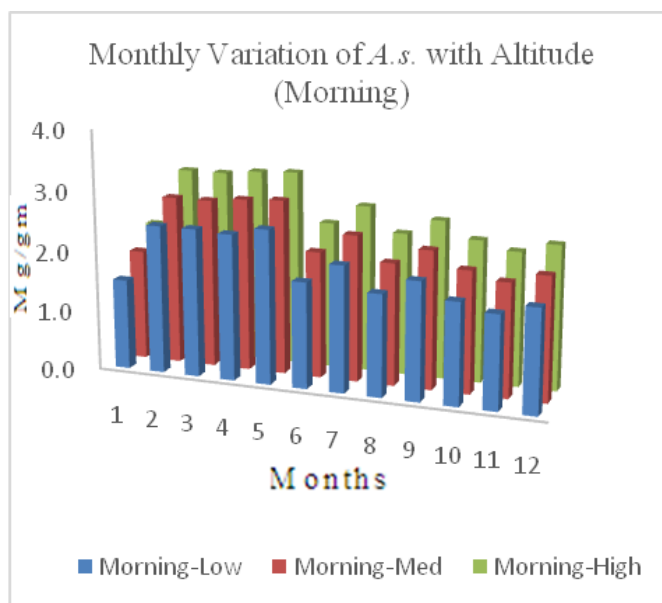
HPTLC densitogram of erythrodiol % Yield mg/g in leaf extract show variations at different season, time and places. In summer season i.e. in February to May, at morning time and at high altitude it shows more yield and according to ANOVA-  $P < 0.05$  followed by Tuckey's test it is significantly variable to other season of rainy and winter, time and places of low and middle altitude (Table 3; Figure 2).

Calibration curve of erythrodiol (Figure 3) show variations of concentration of erythrodiol with Area under curve.

Table 3: Monthly Variation in % Yield mg/g with Altitude and time  $n = 3$   $P < 0.05$ .

A.	Month											
	1	2	3	4	5	6	7	8	9	10	11	12
L.	2.50 ±0.60	3.50 ±0.55	3.50 0.64	3.40 ±0.75	3.60 ±0.46	2.80 ±0.47	3.10 ±0.70	2.70 ±0.78	3.00 ±0.81	2.70 ±0.51	2.60 ±0.64	2.70 ±0.49
M.	2.80 ±0.55	3.80 ±0.51	3.80 ±0.52	3.90 ±0.66	3.90 ±0.52	3.10 ±0.62	3.40 ±0.56	3.00 ±0.66	3.30 ±0.79	3.00 ±0.55	2.90 ±0.69	3.10 ±0.58
H.	3.20 ±0.05	4.10 ±0.05	4.10 ±0.11	4.20 ±0.10	4.20 ±0.11	3.40 ±0.15	3.80 ±0.15	3.40 ±0.20	3.60 ±0.25	3.40 ±0.05	3.20 ±0.11	3.40 ±0.10

Figure 2: Monthly Variation of with Altitude and time



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

Figure 3: Calibration curve for Erythrodiol

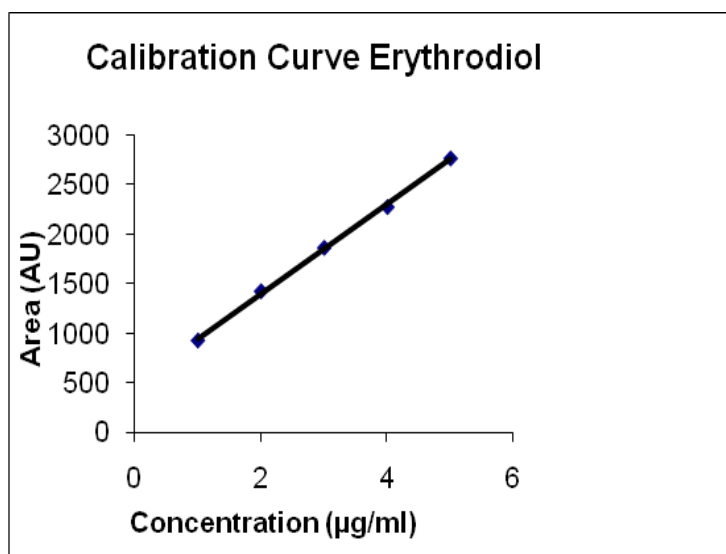
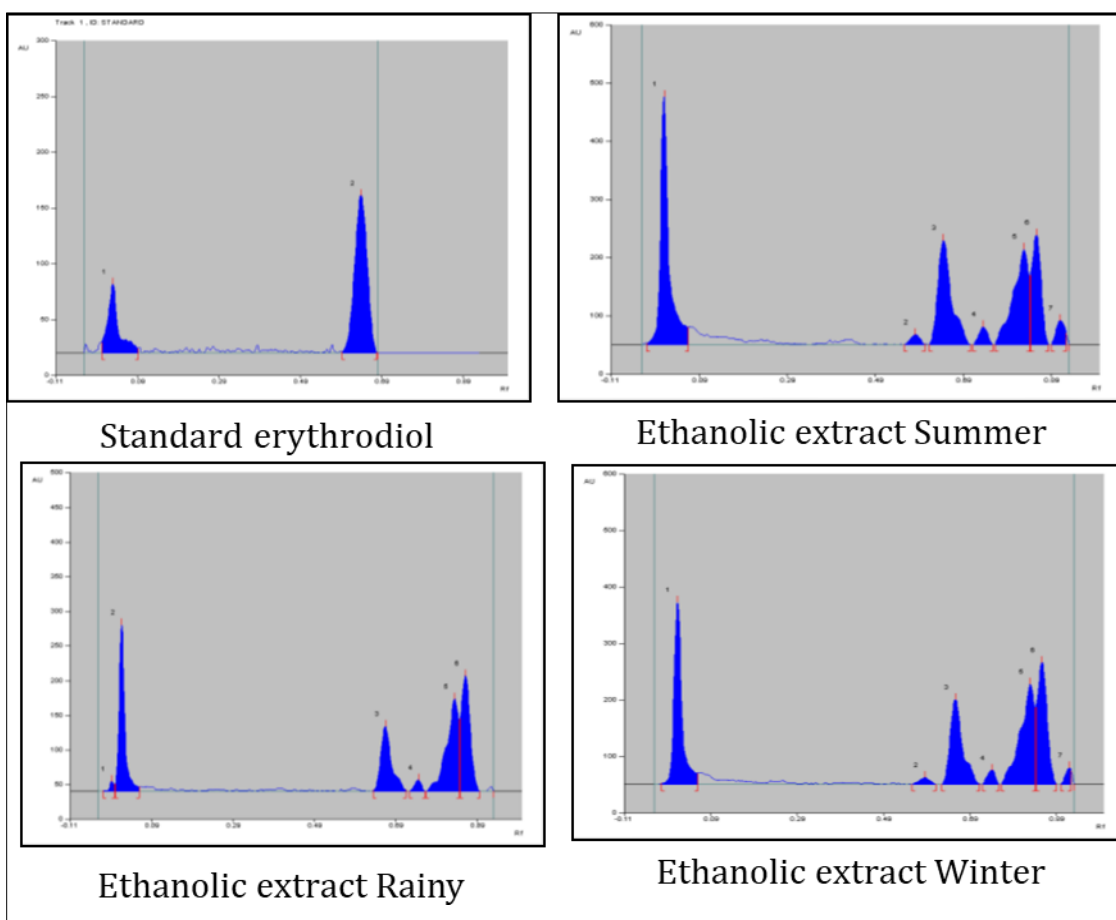


Figure 4: Chromatogram of erythrodiol



Qualitative study of extracts shows availability of alkaloidal constituents such as erythrodiol, oils, lipids, glycosides etc. It was observed that, at every month of the year, at different time and places, there is a change in HPTLC pattern (Figure 2). Erythrodiol content is significantly variable in summer season i.e. in February to May, at morning time and high altitude. This study initiates primary aspects for selecting specific season, place and time to procure crude drugs for formulating treatment for diabetes.

## CONCLUSION-

From the study it is proved that as the seasonal variation is related with the season, time and place of collection of the plant, study shows direct relation to fluctuations of active components in medicinal herbs. In the given crude leaf, the concentration of active component is more in summer season at morning time and high altitude, it is the suitable duration for collection for getting more yield.

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## STATEMENT FOR CONFLICT OF INTEREST-

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

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