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Seasonal and Geographical Variations in Cellular Characters and Chemical Contents in *Desmodium gangeticum* (L.) DC. – An Ayurvedic Medicinal Plant

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ABSTRACT

Desmodium gangeticum (L.) DC. often referred to as *prsniparni* by ayurvedic physicians in Kerala and Tamilnadu, is a member of the well known group of plants -*dasamoola* (roots of ten plants). It has been mentioned to have high therapeutic value in Ayurveda and it is used as a major ingredient in more than 68 ayurvedic formulations. The present study deals with the major variations observed in anatomical, physico-chemical and phytochemical characters in *D. gangeticum* due to change in season and region. Phytochemical changes due to various seasons and different regions were studied by performing HPTLC densitometric quantification of lupeol in methanol extract of roots. Microscopic variation observed in the quantity of cell inclusions, number of fibers and wall thickness of lignified cells. Physicochemical parameters also showed variation. The percentage of lupeol was very low in young plants then increase with growth and maximum percentage of lupeol was observed in the flowering stage. In the case of geographical variation the quantity of lupeol was high in the roots collected from high altitude area and lowest in the ones collected from the plains. The study showed that as the 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. The region where the plants grow has also influence in the chemical constituents of the plants.

Key-words: *Desmodium gangeticum*, *prsniparni*, *dasamoola*, physico-chemical, phytochemical, lupeol.

1. Introduction

Season has impact on availability of active principles in medicinal plants. According to principles of western herbal medicine, therapeutic efficacy varies during different times or seasons of the year. The constituents and active principles vary quantitatively at different seasons of the year and the majority of plant materials are usually best collected during season when the herbs are at peak maturity and concentration ^[1]. In ancient ayurvedic texts, Charaka and Susrutha mentioned timely collection of medicinal plant parts and specific seasons are mentioned for the collection of plants according to the part of plant which is used for the medicine preparation. In *Ashtangahridaya* ^[2], the factors affecting the quality of the herbs have been stated viz, (i) period of harvesting, (ii) age, (iii) soil, (iv) altitude, (v) collecting person and (vi) post-harvest conditions. Appropriate period of collection of plant part was mentioned in Charaka samhita (Kalpam), according to which the roots should be collected only after the completion of seed shedding and in the case of fruits time should be near the ripening period i.e., full grown but unripe ^[3]. It is self-explanatory that ancient physicians were aware about relation between period of collection and distribution of active principles. In the present study, variation in histological and phytochemical characters of the medicinal plant *Desmodium gangeticum* due to the variation in season and place of collection has been carried out.

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2. Materials and Methods

2.1 Plant Materials

The plant is an erect, diffusely branched under shrub, 90-120 cm in height with a short woody stem and numerous prostrate branches provided with soft grey hairs. Leaves simple, ovate lanceolate and membranous. Flowers white or purple or lilac in elongate lax terminal or axillary racemes. Fruits moniliform 6-8 glabrescent, pods sparsely pubescent with hooked hairs, joints separating when ripe into indehiscent one seeded segments, seeds compressed and reniform (Plate 1). It is distributed in all parts of India in dry conditions. In Kerala and Tamil Nadu the roots of this plant are used as *prishniparni* and is an ingredient in more than 68 ayurvedic formulations [4-6].

2.2 Method of Study

D. gangeticum being an annual plant roots were collected in various months/period of growth. Generally the plant of *D. gangeticum* start germination with the onset of monsoon and attain maturity by the month of December-January. So young plants were collected in July, plants before flowering were collected in August, plants in flowering and fruiting time were collected in October and plants after fruit setting i.e., fully matured plants were collected in December. Plants at the time seed shedding were collected in January. Roots were also collected from various agro-climatic conditions like places of high, medium and low altitudes. Comparison of microscopic characters of the roots collected in various periods of growth was carried out by taking hand and microtome sections and staining with various histochemical stains using standard procedures [7, 8]. Histological characters like variation in the presence of lignin, variation in cell inclusions like starch, tannin, crystals etc. were observed. Phytochemical changes due to various seasons and different regions were studied by performing HPTLC densitometric quantification of lupeol in methanol extract of roots collected in various seasons and from different places. Seasonal variation in physicochemical parameters was also determined and compared as per standard methods [8].

3. Results & Discussion

Transverse section of the mature root is circular in outline with outer cork region, composed of 3-7 layers of thin-walled, tangentially elongated cells; a narrow cortex having 4-10 layers of thin-walled, tangentially elongated cells having a few isolated cortical fibres; phloem composed of parenchyma, sieve tubes, companion cells and fibres, traversed by phloem rays; outer layers of phloem cells collapsed in outer region; phloem fibres are lignified and circular in cross section with a narrow lumen; phloem rays uni to multiseriate; outer phloem region contain prismatic crystals of calcium oxalate; central wood region is wide consisting of usual xylem elements like vessels, tracheids, parenchyma, and fibres; and traversed by xylem rays; vessels, lignified, large, narrow, with both simple and bordered pitted thickening; xylem rays thick-walled possessing simple pits; starch grains simple, round to oval measuring 7-25 μ in dia. and prismatic crystals of calcium oxalate present in phloem and xylem.

3.1 Variation in Microscopic Characters

Throughout the growth period and in all the seasons, the basic characters of cell inclusions like starch grains and prismatic crystals of calcium oxalate and type of cells like xylem vessels, phloem fibres and xylem fibres and their pattern of arrangement are the

same. Variation shows in the quantity of cell inclusions and amount of phloem fibres and xylem fibres and in the wall thickness of vessel elements (Plate 2).

3.2 Variation in Physicochemical Parameters

The value of moisture content was high in the plants collected in the rainy season and it gradually decreases with the season. The water soluble extractive and alcohol soluble extractives were high in the month of January. Similarly the ash value and acid insoluble ash are maximum in January. The comparative values are shown in the table (Table 1).

3.3 Variation in Phytochemical Constituents

Roots were collected in various months/period of growth for HPTLC densitometric quantification of lupeol (Plate 3). The percentage of lupeol was very low in the month of June i.e., in the young plants. Then they increase with growth and maximum percentage of lupeol was observed in the month of October (Table 2&3) which was the full bloom period of the plant.

3.4 Variation Due to Geographical Conditions/ Soil Type

Roots were collected from places having different Agroclimatic conditions i.e. from high altitude, medium altitude to plains. The soil type also varies from lateritic to sandy soil when coming from high altitude to low altitude. HPTLC densitometric quantification of lupeol in methanol extract of roots collected from various geographical conditions was carried out. The result showed that the quantity of lupeol was high in the roots collected from high altitude and lowest in the ones collected from the plains (Table 2&3).

There was an earlier report by Mamman *et al.* [9] which states that the samples of *Leptadenia* collected during all the three seasons showed hardly any variation in constituents. This indicates that climate does not affect the chemical spectrum for *Leptadenia*. In this study, plant showed variation quantitatively from season to season. The variation is seen to be coinciding with the growth period of the plant i.e., lowest percentage is seen in the young stage and highest percentage in fully flowered stage. It was also seen that in the fully ripened fruiting plants the percentage of lupeol decreases in *D. gangeticum* and this result is strongly supported by the report by Raja *et al.* [10], in which it is reported that *Adhatoda* exhibited high percentage of vasicine content in March (3%) when it was in full bloom and 1.4 % in September when was in partial flowering. During the vegetative stage the plant contains very low concentration of vasicine content.

The plant materials collected from different regions revealed that in all the plants selected for the study, the highest concentration of the marker compound was observed in plants collected from the high altitude area; i.e., from the hilly areas. This result is also contradictory to the study conducted by Mammen *et al.* [9], where they reported that in *Leptadenia* the fingerprints of the three extracts of the plants collected from Gujarat, Maharashtra and Kerala were very similar to each other and the advantage of the observation is that the collection of the plant can be done at any season or from any region of the country. But in the present study the plants collected from the wild in hilly areas stand superior to other regions. In classical texts scholars have mentioned the habitats from where plants need to be collected. According to Ashtangahridaya [11], there are three types of habitats viz. *jangalam* (arid), *anoopam* (marshy) and *sadharanam* (normal) and it is insisted to collect plants from *jangalam* and *sadharanam*. Here the

hilly areas can be equated with arid areas and so this opinion justifies the result of the variation due to soil type.

3.5 Tables and Figures

Table 1: Variation in physicochemical parameters

Month	Moisture content	Alcohol soluble extractive	Water soluble extractive	Ash value	Acid insoluble ash
June	10.6	2.9	4.3	3.01	0.73
August	9.4	3.4	4.92	3.64	0.74
October	8.5	3.9	5.87	3.77	0.74
December	6.08	4.89	6.93	3.87	0.75
January	5.2	5.92	7.04	3.88	0.76

Table 2: HPTLC Densitometric quantification of lupeol in methanol extract of roots collected in various seasons/period of growth.

Month	% of lupeol
June	0.0019 %
August	0.0022 %
October	0.0037 %
December	0.0023
January	0.0026.

Table 3: HPTLC densitometric quantification of lupeol in methanol extract of roots collected from various places

Place of collection	% of Lupeol
High altitude	0.033
Medium altitude	0.015
Low altitude	0.011



Fig 1: *Desmodium gangeticum* (L.) D.C. A. Habit; B. Flowering twig; C. Fruiting twig with simple alternate leaves; D. Fresh mature root.

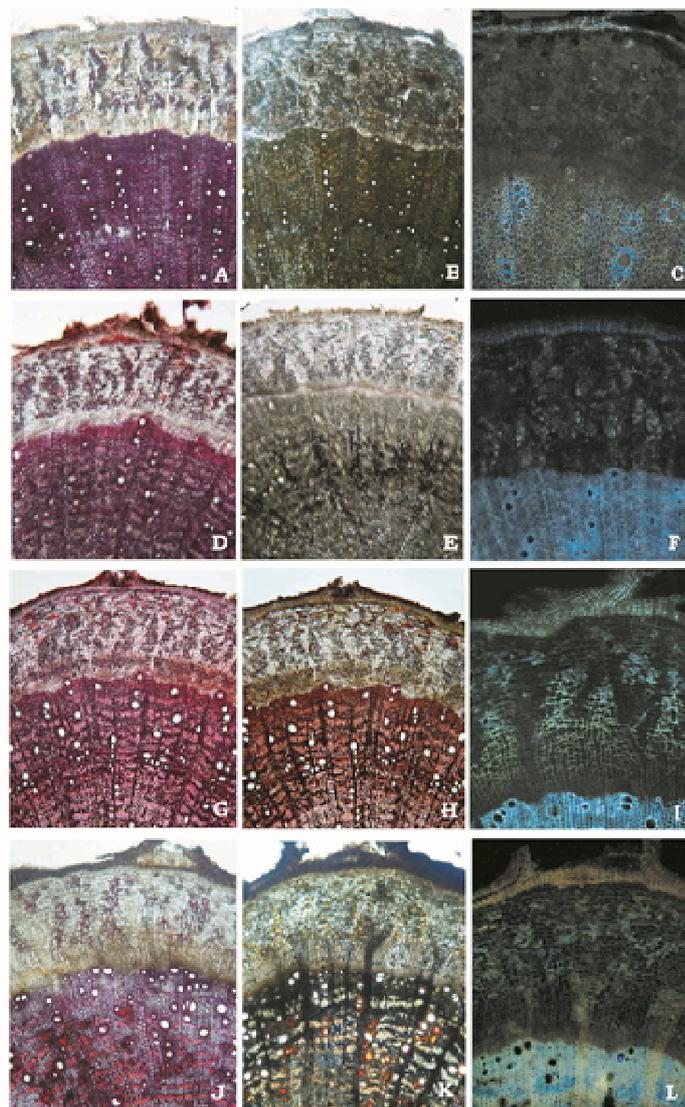


Fig 2: Seasonal/age variation in microscopic characters of *D. gangeticum* root.

A&B. TS of root of young plants collected in June x100; C. Fluorescent microscopy of young root x 200; D&E. TS of root of plants collected before flowering in August x 100; F. Fluorescent microscopy x 200; G&H. TS of root of plant with flowers and fruits collected in October x 100; I. Fluorescent microscopy of root x 200; J&K. TS of root of plant with fully matured roots collected in December x 100; L. Fluorescent microscopy x 200.

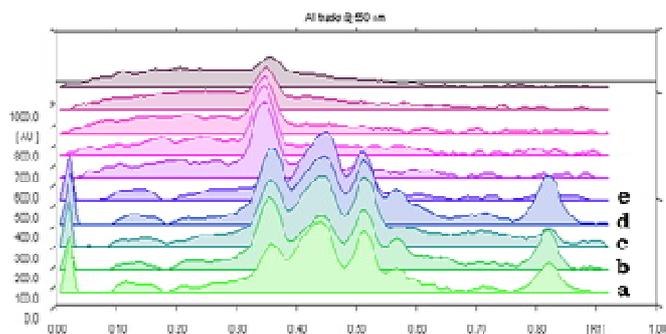


Fig 3: A&B. HPTLC densitometric quantification of lupeol in methanol extract of *D. gangeticum* root collected in various seasons. a. June; b. August; c- October; d-December; e-January.

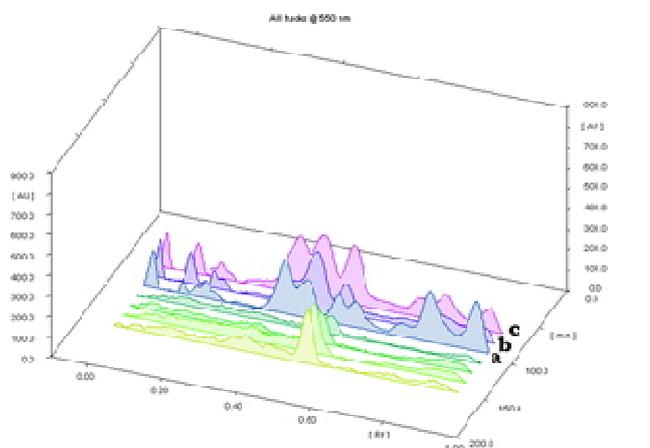


Fig 4: HPTLC densitometric quantification of lupeol in methanol extract of *D. gangeticum* root collected from different regions. A-High altitude; b-Medium altitude; c-Lower altitude.

4. Conclusions

From the study it is concluded that as the 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. In the plant, the concentration of active principles is high in full bloom period, it is the best period for collection for high percentage and this is contradictory to the statement given in classical texts, according to which the roots should be collected only after the completion of seed shedding. This might be mentioned by the *Acharyas* in the conservation point of view. So while collecting the plants it is better to keep some plants undisturbed for the seed shedding for the sustainable use of that particular plant. All the plants are perennial in nature and that may be the reason for this type of variation.

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