



Exposition of Role of Diurnal and Seasonal Variation on Latex of *Calotropis procera* Ait. and *Calotropis gigantea* L.R.BR.

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AR and RA conceptualized, designed the study and executed it. They also contributed in drafting of the article. Author VS performed the analyses of the study. All authors contributed in logical interpretation. They further read and approved the final manuscript.

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ABSTRACT

Background: Time (Kala) of collection is an important criterion responsible for the quality of crude drugs of herbal origin. According to their parts used, the guidelines for rutewise (seasonal) collection of crude plant drugs has been well documented in various treatise of Ayurveda. Among various parts used of medicinal plants, Kshira (latex) of Arka and Shwetarka finds a wide usage in various pharmaceutical processes and formulations in classical literature. The latex has been quoted to be procured in Sharada rutu (September- October) but the scientific reason behind this still remains unexplored.

Materials and Methods: The fresh crude latex of both *C. gigantea* (CG) and *C. procera* (CP) were collected in clean glass vials regularly in all the six rutu i.e Vasanta (March-April), Grishma (May-June), Varsha (July- August), Sharada (September- October), Hemanta (November-December) and Shishira (January- February). The collection was done for 4 days in each rutu and

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3 times in a day i.e. before sunrise (BS), 3 hrs. after sunrise (3AS) and 6 hrs. after sunrise (6AS) from three different tender and fresh parts of the plant i.e. twig, petiole and peduncle. The observations regarding volume, girth of plant parts, pH, viscosity, specific gravity along with preliminary phytochemical analysis of latex was carried out in samples of crude latex collected in six seasons.

Results: Grishma rutu was very well differentiated from other rutus in terms of volume and girth. Also, a peculiar presence of alkaloids and proteins was observed in Sharada rutu in both the species supports the classical collection protocol in Agneya period.

Keywords: *Calotropis gigantea*; *Calotropis procera*; seasonal variation; rutu; time.

1. INTRODUCTION

Among the Indian system of medicines, Ayurveda takes a leading role and advocates to use drugs of herbal, mineral and metallic origin. Among them, drugs of herbal origin contribute a lion's share i.e. more than 90% [1].

For obtaining good quality crude drugs of herbal origin, Ayurveda recommends many factors to consider before their selection like Desha (habitat), Kala (time), Guna (properties) and Bhajana sampata (apt storage area) [2]. Among these, Kala (time) plays a major role. Various authoritative texts have focussed this necessity and have recommended a protocol namely 'Dravya sangraha Kala' wherein the collection of crude plant drug has been classified according to the parts used and rutu (season) [3,4]. The qualitative, quantitative changes in phytoconstituents and therapeutic efficacy of various plants owing to seasonal, diurnal and lunar influence has been documented [5]. Among the various plants used as an ingredient of various formulations and purification techniques of Loha in Rasashastra [6], kshira (latex) of Arka (*Calotropis procera* Ait.) and Shwetarka (*Calotropis gigantea* L. RBr) has occupied an important place. Clinicians are of a view that the volume of latex differs before and after sunrise. In classical texts, the period for latex collection has been quoted to be carried out in Sharada rutu [7]. Recent scientific studies shows that the pharmacological properties, action of the plant parts differs when collected during different time of a day and season [8]. No scientific study regarding seasonal variation in latex has been reported. Taking this into consideration, the present study was designed to carry out the physicochemical, preliminary qualitative analysis of crude latex obtained from different parts of Arka and Shwetarka during six seasons during different time period of a day.

2. MATERIALS AND METHODS

2.1 Identification and Authentication

The two species namely *Calotropis procera* Ait. and *Calotropis gigantea* L. were procured from surrounding areas of Gujarat Ayurved university and authenticated by expert of pharmacognosy laboratory of Gujarat Ayurved University, Jamnagar and herbarium of each sample has been deposited to institute's pharmacognosy museum of *Calotropis gigantea* Linn. (Phm/6147) and purple variety of *Calotropis procera* Ait. (Phm/6149) for future reference.

2.2 Physicochemical Analysis

2.2.1 Procedure for collection

The fresh crude latex of both *C. gigantea* (CG) and *C. procera* (CP) were collected in clean glass vials regularly in all the six rutu i.e Vasanta (March-April), Grishma (May-June), Varsha (July- August), Sharada (September- October), Hemanta (November- December) and Shishira (January- February). The collection was done for 4 days in each rutu and 3 times in a day i.e. before sunrise (BS), 3 hrs. after sunrise (3AS) and 6 hrs. after sunrise (6AS) from three different tender and fresh parts of the plant i.e. twig, petiole and peduncle. The observations were recorded in terms of volume and girth of each part, every time during collection in a specially designed research proforma. The latex was collected in graduated and labelled clean plastic screw capped vials for measuring the volume. The girth of plant parts was also assessed using a digital vernier calliper and recorded. The crude latex of both the species were evaluated for the following parameters following the procedures recommended by Ayurvedic pharmacopoeia of India (API).

1. pH [9]
2. Specific gravity [10]
3. Relative Viscosity [11]

2.2.2 Preliminary phytochemical analysis

The freshly collected latex of both the species were subjected individually to centrifugation at 15000 rpm for 30 min in the centrifuge SIGMA 3-30K with relative centrifugal force (RCF) 22891 at 24°C in all ruti in the morning time. The rubbery residue amounted to approx. 10-12 ml after centrifugation of 80 ml crude latex of each sample. The supernatant layer was decanted and the samples (70 ml) were further used on the same day for preliminary phytochemical analysis.

The two samples were then subjected to various qualitative tests [12,13,14] regularly in each season to assess the presence of alkaloids (using Mayer's reagent, Wagner's reagent and Dragendorff reagent), tannins (Using FeCl₃), flavonoids (Vanillin HCl), steroid & terpenoids (Salkowski reaction), cardiac glycosides (Legal's test), carbohydrates (Molisch test, Fehling's test) and proteins (Biuret test) following standard parameters. The chemicals used were of

analytical purchased from Merck chemicals Pvt. Ltd., Mumbai.

3. RESULTS AND DISCUSSION

The observations were recorded in terms of volume and girth of each part, every time during collection. The seasonal similarity and diversity was assessed by applying Principal Component Analysis (PCA) using Unscrambler software 9.7 version. PCA finds a few orthogonal linear combinations of the original variables with the largest variances; these linear combinations are the PCs that would be retained for subsequent analyses.

PCA is the most widely used multivariate analysis technique for (unsupervised dimension reduction wherein all variables equally treated without specifying a response) transforming the original measurement variables into new variables called principal components (PCs) [15].

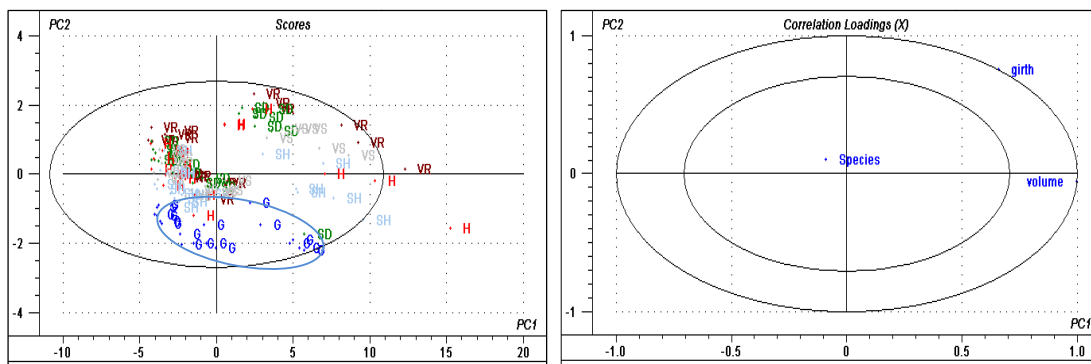


Fig. 1. PCA of seasonal variation in CG and CP latex (H-hemant, G-Grishma, VR-Varsha, VS-Vasant, SH- Shishira, SD- Sharad)

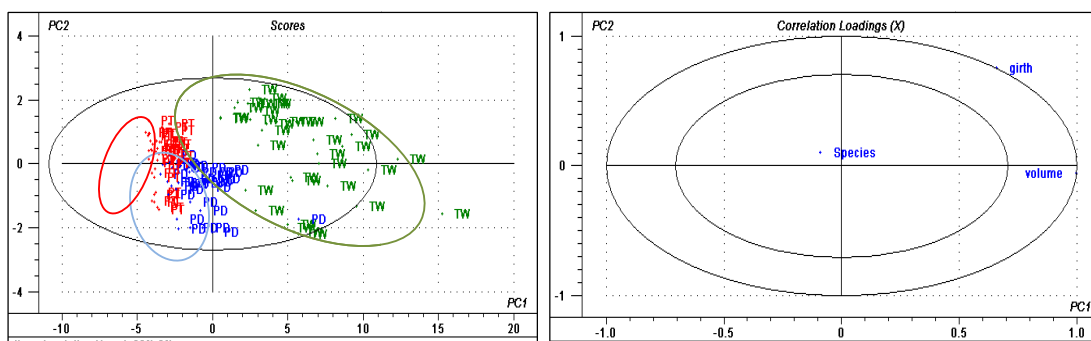


Fig. 2. PCA of partwise variation in six seasons in CG and CP latex (TW-twig, PD-peduncle, PT-petiole)

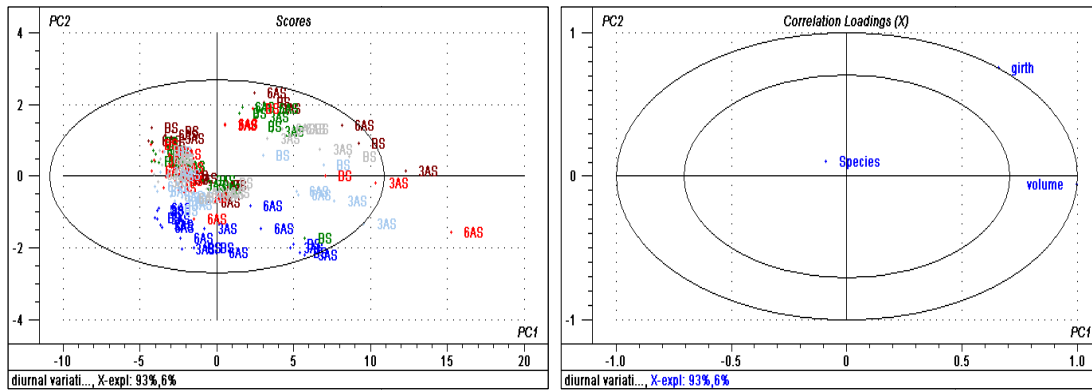


Fig. 3. PCA of Diurnal variation in CG and CP latex (BS-before sunrise, 3AS- 3 hrs. After sunrise, 6AS- 6 hrs. After sunrise)

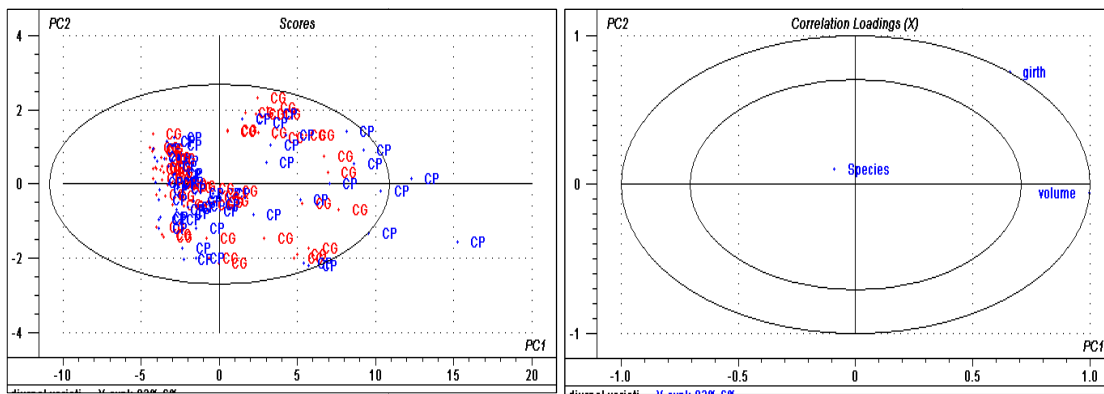


Fig. 4. PCA of species wise variation in CG and CP latex

3.1 Seasonal Variation

By applying PCA, It was observed that in case of seasonal variation, samples collected during Grishma (May and July) are well differentiated from other ritu both in terms of volume and girth. The outliers included samples from Hemanta ritu and Varsha ritu as the volumes obtained were highest in these two ritu at a particular period of time.

3.2 Partwise Variation

In case of parts of collection of latex, the latex collected from twig is seen to be clearly differentiated from other parts i.e pedicel and peduncle. Highest volume was observed in Varsha ritu and Hemanta ritu in twig as outlier. Thus, the twigs had greater girth and hence greater volume possibly due to increased area of laticiferous cells.

3.3 Diurnal and Specieswise Variation

In case of diurnal readings, all are near to similar and hence not differentiated neatly with respect to volume and girth. Thus, the diurnal variation did not affect the volume of the latex in both the species. The present study does not confirm the traditional belief that the volume of latex is obtained more before sunrise.

As regards the two different species of *Calotropis* selected for present study is concerned, it is observed that all the species readings are near to similar and hence not differentiated neatly with respect to volume and girth. Thus, it can be reported that there is no major difference in volume in six different seasons in the two botanical sources of Arka.

In case of pH, specific gravity and viscosity observed in the six seasons, A gradual decrease in pH was observed in both the species of Arka starting from Vasanta ritu to Shishira ritu from

5.24 to 4.24 (Tables 1-2) The pH being acidic confirms the Agneya guna of the plant and if thought in between the lines, the synonyms also depict the Sun God i.e Agni mahabhuta [16]. The specific gravity was highest in *C. gigantea* during

Vasanta rutu whereas it is highest in *C. procera* during Shishira rutu (Table 3). The mean viscosity was highest for *C. gigantea* in Varsha rutu whereas it is highest for *C. procera* in Shishira rutu (Tables 4-5).

Table 1. The average pH of CG and CP latex collected in Vasanta, Grishma and Varsha

CG									
Rutu	Vasanta			Grishma			Varsha		
Time	BS	3AS	6AS	BS	3AS	6AS	BS	3AS	6AS
Mean pH	5.42	5.35	5.25	5.32	5.22	5.06	5.37	5.29	5.27
CP									
Mean pH	5.48	5.51	5.53	5.45	5.43	5.23	5.37	5.44	5.39

Table 2. The average pH of CG and CP latex collected in in Sharada, Hemanta and Shishira

CG									
Rutu	Sharada			Hemanta			Shishira		
Time	BS	3AS	6AS	BS	3AS	6AS	BS	3AS	6AS
Mean pH	5.03	5.07	4.90	4.50	4.50	4.45	4.45	4.51	4.42
CP									
Mean pH	5.24	5.13	5.11	4.64	4.73	4.64	4.57	4.64	4.60

Table 3. The mean specific gravity of CG and CP collected during six rutu

Rutu	Sp. Gr of CG	Sp. gr. of CP
Vasanta	1.044	1.047
Grishma	1.034	1.052
Varsha	1.043	1.051
Sharada	1.040	1.048
Hemanta	1.043	1.045
Shishira	1.043	1.057

Table 4. The mean viscosity of CG in all six rutu

CG				
Rutu	D1	T1	T0	Viscosity (D1T1/T0)
Vasanta	1.044	121.75	40	3.177
Grishma	1.034	109	38.5	2.927
Varsha	1.043	146.25	45	3.389
Sharada	1.040	118.5	38.5	3.201
Hemanta	1.043	40	15.25	2.735
Shishira	1.043	97.75	38.5	2.648

Table 5. The mean viscosity of CP in all six rutu

CP				
Rutu	D1	T1	T0	Viscosity (D1T1/T0)
Vasanta	1.047	106	40	2.774
Grishma	1.052	87.75	38.5	2.397
Varsha	1.051	87	45	2.031
Sharada	1.048	115.5	38.5	3.144
Hemanta	1.045	39.25	15.25	2.689
Shishira	1.057	123	38.5	3.376

Table 6. Preliminary phytochemical analysis of CG latex and CP latex

Sr. no	Test	Vasanta		Grishma		Varsha		Sharada		Hemanta		Shishira	
		CG	CP	CG	CP	CG	CP	CG	CP	CG	CP	CG	CP
1.	Alkaloids	-	-	+	+	+	-	+	-	+	-	-	-
2.	Tannins	-	-	+	+	-	+	+	+	+	+	-	-
3.	Carbohydrates	+	+	+	+	+	+	+	+	+	+	+	+
4.	Reducing sugars	-	-	-	-	-	-	+	-	-	+	-	-
5.	Steroids	+	+	+	+	+	+	+	+	+	+	+	+
6.	Proteins	-	-	-	-	-	-	+	+	-	-	+	+
7.	Amino acids	-	-	-	-	-	-	-	-	-	-	-	-
8.	Cardiac glycosides	-	-	-	-	-	-	-	-	+	+	-	-
9.	Flavonoids	+	+	+	+	+	+	+	+	+	+	+	+
10.	Fats and oils	+	+	+	+	+	+	+	+	+	+	+	+

*+: presence; -: absence

3.4 Preliminary Phytochemical Analysis

Data pertaining to the observed result of preliminary phytochemical analysis is tabulated in table above:

- i. Preliminary phytochemical analysis of centrifuged and decanted latex samples of *C. gigantea* and *C. procera* revealed the presence of carbohydrates, steroids, flavonoids and fixed oils in all the six rutu.
- ii. Tannins were present in Grishma rutu and Hemanta rutu in both the samples. Test for alkaloid was positive in *C. gigantea* while that for tannins was positive in *C. procera* in Varsha rutu.
- iii. Alkaloids were present in *C. gigantea* in Sharada rutu whereas in both species in Sharada rutu. Also, test for reducing sugar was positive in *C. gigantea* in Sharada.
- iv. Proteins were present in both the species of Arka in Sharada and Shishira rutu. Test for cardiac glycosides was positive in both the species in Hemanta rutu.

Thus, a wide seasonal variation in presence of cardiac glycosides, alkaloids and tannins was seen in both the species. The utility of the latex can be sought according to the predominance of phytochemicals in that particular rutu. The classical collection period for kshira (latex) has been quoted to be in Sharada rutu. The preliminary findings for predominance of proteins, alkaloids, reducing sugars along with carbohydrates, steroids, flavonoids and fixed oils in the latex of the two species in Sharada rutu proves the point.

4. CONCLUSION

The overall findings support the concept of Kala (influence of time) on quality of crude latex of Arka. Time i.e diurnal and seasonal variation certainly alter the properties of Arka kshira. Thus, once the active phytochemical is ascertained and its higher concentration is adjudged, the collection at an appointed point of year can assure the best quality of the crude latex and ultimately good quality formulations.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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