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MONITORING OF SEASONAL VARIATION IN LATEX OF *Calotropis procera* AIT. AND *Calotropis gigantea* L.R.Br USING FTIR SPECTROSCOPY

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Abstract: ***Background:*** *Calotropis procera* Ait and *Calotropis gigantea* L.R.Br of Family Asclepiadaceae are xerophytic plants which are found abundantly in arid zones of India. Their latex has been attributed with many pharmaceutical and therapeutic values and is being included either as a single drug or as an ingredient of many compound Ayurvedic formulations. Classical texts of *Ayurveda* recommend a specific collection protocol for crude drug collection depending on the parts used and *rutu* (season). Collection of latex has been advocated in *Sharada* (autumn) *rutu* (season). ***Objective:*** The objective of the present study was to assess the influence of six seasons, according to Ayurveda, on the organic profile of latex of *C procera* and *C gigantea* by using FTIR. ***Material and Methods:*** The crude latex of both species of Calotropis 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) from a single plant and then packed in autoclaved appendorfs and sent for FTIR analysis. ***Observations and results:*** The differentiation in FTIR was remarkable in *Grishma rutu* and thus supports the theory that seasons do influence the organic profile of the latex.

Keywords: *Arka, C. gigantean, C. procera,* FTIR, Latex, Rutu, Seasonal variation.

INTRODUCTION

Medicinal plants play an inevitable role in pharmaceutical products. They possess various bioactive compounds which are responsible for various therapeutic actions. To obtain good quality crude drugs of herbal origin with potent active principles, Ayurveda recommends to look after various factors like *Desha* (habitat), *Kala* (time) and *Guna* (properties), before their selection. **[1]**

WHO, while recommending Good Agriculture and Collection Practices (GACP), **[2]** has also advocated that medicinal plants should be harvested during the optimal season or time period. Many studies have been carried out to prove the relevance of time factor in deciding the quality of drugs. **[3]** Ayurveda categorizes medicinal plants with harmful effects, if not used judiciously, under *Upavisha* (semi-poisonous) group of plants. **[4]** *Arka* is one among such plants whose *kshira* (latex) has been extensively attributed with many pharmaceutical (*Bhasma* preparation) **[5]** and has been indicated internally in management of *Jvara* (Pyrexia), *Kushtha* (Skin disorders), Liver disorders, etc.**[6]** and advocated externally for management of *Kushtha* (skin disorders), *Vrana* (wound), *Arsha* (Hemorrhoids),etc.**[7]** *Ayurveda*, basing upon colour of the flowers of the plant, describe two variety of *Arka* **[8]** and the botanical equivalent of *Arka* and *Alarka* are considered as *Calotropis procera* Ait. & *Calotropis gigantea* L.R.Br respectively.**[9]** Both the species have a wide usage in ethnomedicine in forty four different conditions by almost fifty tribes in India.**[10]** *Ayurveda* delineates the time of collection of various parts of medicinal plants and the period for collection of latex has been quoted to be carried out in *Sharada rutu* (September- October)**[11]**. The

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Table-1: The six Rutus and their corresponding Hindu lunar months along with Gregorian month

Sr. No.	Ṛutu	Season	Hindu Lunar month	Gregorian month
1.	Vasanta	Spring	Caitra & Vaiśākha	March-April
2.	Grīṣma	Summer	Jyeṣṭha & Āṣāḍha	May-July
3.	Varṣā	Rainy season	Śrāvaṇa & Bhādrapada	August-September
4.	Śarada	Autumn	Aśvina & Kārtika	October-November
5.	Hemanta	Pre-winter	Mārgaśīrṣa & Pauṣa	December-January
6.	Śiśira	Winter	Māgha & Phālguna	February - March

six *rutu* and their corresponding Gregorian months have been denoted in **Table-1**.

The seasonal variations in *Calotropis* procera have been reported before in terms of qualitative and quantitative differences in protein, tannin, carbohydrate, phenol, fixed oil and essential oil content. **[12]** Infrared spectroscopy (IR)**[13]** has the potential to provide biochemical information without disturbing the biological sample. It targets the measurement of vibrations of bonds within chemical functional groups by generating spectrum. It can be helpful in assessing changes in primary and secondary metabolites which are responsible for medicinal properties of plants.**[14];[15]** Thus, the concept of whole drug analysis (as per Ayurvedic consideration) could be carried out with Fourier transformed Infrared spectroscopy. Till date, no FT-IR analyses have been reported to adjudicate the seasonal variation occurring in latex bearing plants.

Photoplate-1: Natural habitat

In present study, the FT-IR spectrum was used to identify the functional groups of active components present in the latex samples of *Calotropis procera* and *Calotropis gigantea* obtained in six *rutu* (seasons) based on the peak value in the region of infrared radiation.

MATERIAL AND METHODS:

Identification and authentication of plant:

The plant *Arka* with different coloured flowers i.e. purple and white, growing naturally in abundance in the peripheral areas of Gujarat Ayurveda university,

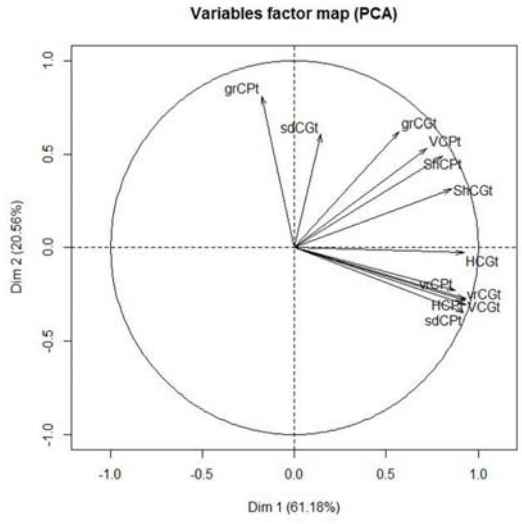

Fig-1: PCA of transmittance of both species in six rutu

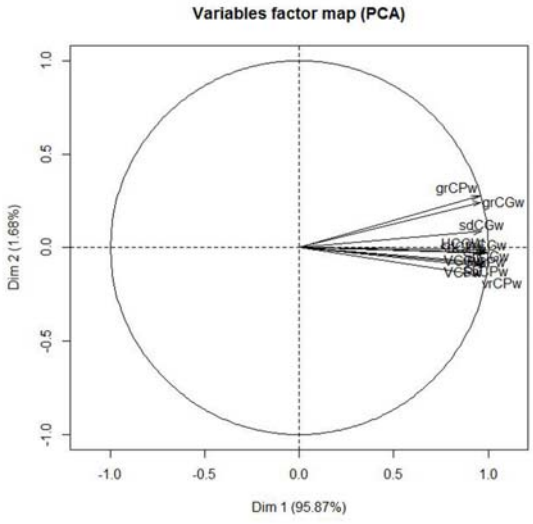

Fig-2: PCA of wavenumber of both species in six rutu

Valkeshwarinagari, Sapada road, Jamnagar, Gujarat were identified by local Vaidya and taxonomist. Their respective botanical names i.e. *Calotropis procera* Ait. and *Calotropis gigantea* L.RBr. were confirmed and authenticated by pharmacognosist of Gujarat Ayurved University, Jamnagar by studying the morphological characters of various parts with thos described in different floras and books. The herbarium of each sample were deposited to institute's pharmacognosy museum *Calotropis procera* Ait (Phm/6149) and white variety of *Calotropis gigantea* Linn. (Phm/6147) for future reference. **(Photoplate-1)**

Collection of plant material:

The fresh crude latex of both *C.gigantea* (CG) white variety and *C.procera* (CP) was collected in clean glass vials regularly in all the six rutus i.e *Vasanta* (March-April), *Grishma* (May-June), *Varsha* (July- August), *Sharada* (September- October), *Hemanta* (November- December) and *Shishira* (January- February) from the same plant respectively for both the species.

Packing and storage:

One ml crude latex samples was packed in an airtight appendorf and sent for analysis at

Table 2: Observed frequency, corresponding functional groups and remarks of CG latex in Vasant rutu

CG Vvasant rutu			
Observed frequency	**Reference Frequency(cm-1)**	**Group assignment or functional group**	**Remarks (CG vasant rutu)**
3429.36	3200-3550; 3400-3500 (dil. soln.)	str. O-H (H-bonded), usually broad ;wk.N-H (1°-amines), 2 bands	Alcohols and Phenols ; Amines
2928.34	2850-3000; 2500-3300 (acids) overlap C-H	str. CH3, CH2 & CH 2or 3 bands; str. O-H (very broad)	Carboxylic Acids & Derivatives, alkanes
2855.26	2850-3000; 2500-3300 (acids) overlap C-H	str. CH3, CH2 & CH 2or 3 bands; str. O-H (very broad)	Alkanes; Carboxylic Acids & Derivatives
1732.49	1720-1740;	str. C=O (saturated aldehyde)	Aldehydes & Ketones
1642.98	1630-1680; 1630-1695 (amides)	var. C=C (symmetry reduces intensity) ; str. C=O (amide I band)	Alkenes; Carboxylic Acids & Derivatives
1245.94	1000-1250 ; 1210-1320 (acids); 970-1250	med. C-N ; med-str.O-C (sometimes 2-peaks); str. C-O ;	amines ; Carboxylic Acids & Derivatives; Alcohols & Phenols
1118.37	970-1250; 1000-1250; 1000-1300	str. C-O ; med. C-N ; str.O-C (2-bands)	Alcohols & Phenols; amines ; Carboxylic Acids & Derivatives
1027.87	970-1250; 1000-1250; 1000-1300	str. C-O; med.C-N ; str.O-C (2-bands) ;	Alcohols & Phenols; amines; Carboxylic Acids & Derivatives
983.37	970-1250 ;	str. C-O;	Alcohols & Phenols;
BENDING VIBRATION			
1550.93	1550-1650 ; 1500-1560	med-str. NH2 scissoring (1°-amines) ; N-H (2¡-amide) II band	Amines ; Carboxylic Acids & Derivatives
1454.07	1350-1470	med. CH2 & CH3 deformation	Alkanes
1383.99	1350-1470 ; 1370-1390 ; 1330-1430;	med. CH2 & CH3 deformation ; med.CH3 deformation ; med.O-H bending (in-plane);	Alkanes ; Alkanes; Alcohols &Phenols;
983.37	880-995	str. =C-H & =CH2 ;	Alkenes
879.05	690-900; 660-900	str. Med C-H bending & ring puckering ; var. NH2 & N-H wagging (shifts on H-bonding)	Arenes ; Amines
779.77	690-900; 660-900	str. Med C-H bending & ring puckering; var. NH2 & N-H wagging (shifts on H-bonding)	Arenes; Amines
620.37	600-700	str.C-H deformation	Alkynes

Table-3: Observed frequency, corresponding functional groups and remarks of CP latex in Vasant rutu

CP Vasant rutu			
Observed frequency	**Reference Frequency(cm-1)**	**Group assignment or Functional group**	**Remarks (CP vasant rutu)**
3417.89	3200-3550;3400-3500 (dil. soln.)	str.O-H (H-bonded), usually broad; wk.N-H (1°-amines), 2 bands	Alcohols And Phenols; Amines
2929.81	2850-3000; 2500-3300 (acids) overlap C-H	str. CH3, CH2 & CH 2or 3 bands; str. O-H (very broad)	Alkanes, Carboxylic Acids & Derivatives,
2872.21	2850-3000; 2500-3300 (acids) overlap C-H	str. CH3, CH2 & CH 2or 3 bands; str. O-H (very broad)	Alkanes, Carboxylic Acids & Derivatives,
1736.76	1720-1740; 1735-1750 (esters)	str. C=O (saturated aldehyde); str.C=O	Aldehydes & Ketones; Carboxylic Acids & Derivatives
1643.71	1630-1680; 1630-1695 (amides)	; var. C=C (symmetry reduces intensity) ; str. C=O (amide I band)	Alkenes; Carboxylic Acids & Derivatives
1245.38	1000-1250 ; 1210-1320 (acids); 970-1250	med. C-N ; med-str.O-C (sometimes 2-peaks); str. C-O ;	Amines ; Carboxylic Acids & Derivatives; Alcohols & Phenols
1115.64	970-1250; 1000-1250; 1000-1300	str. C-O ; med. C-N ; str.O-C (2-bands)	Alcohols & Phenols; Amines; Carboxylic Acids & Derivatives
1035.5	970-1250; 1000-1250; 1000-1300	str. C-O; med.C-N ; str.O-C (2-bands);	Alcohols & Phenols; Amines; Carboxylic Acids & Derivatives
983.6	970-1250 ;	str. C-O; str.	Alcohols & Phenols;
BENDING VIBRATIONS			
1643.71	1590-1650;	med.N-H (1¡-amide) II band	Carboxylic Acids & Derivatives
1453.65	1350-1470	med. CH2 & CH3 deformation	Alkanes
1383.25	1350-1470 ; 1370-1390 ; 1330-1430;	med. CH2 & CH3 deformation ; med.CH3 deformation ; med.O-H bending (in-plane);	Alkanes ; Alkanes; Alcohols &Phenols;
1322.17	1325+/-25	S=O	Sulfone
983.6	880-995	=C-H & =CH2 ;	Alkenes
900.63	880-995,900-1050 (str)	=C-H & =CH2 ; P-OR	Alkenes,Esters
878.05	690-900; 660-900	str. Med C-H bending & ring puckering ; var. NH2 & N-H wagging (shifts on H-bonding)	Arenes ; Amines
779.68	780-850; 690-900; 660-900	=C-H & =CH2 (out-of-plane bending); str. Med C-H bending & ring puckering; var. NH2 & N-H wagging (shifts on H-bonding)	Alkenes ; Arenes; Amines
618.83	600-700	str.C-H deformation	Alkynes

Sophisticated Instrumentation Centre for Applied Research and Testing (SICART), Vallabh Vidyanagar, Gujarat, India.

Procedure:

Accurately weighted 2mg crude latex samples were ground to dry semisolid form and mixed in 200mg Kbr (FTIR grade) to make pellets. The sample pellets were placed into the sample holder and FTIR spectra were recorded in range from 4000 – 450 cm–1.

Instrument: Perkin Elmer spectrophotometer, Spectrum Instrument (Germany) with FTIR paragon 1000 PC software [Sophisticated Instrumentation Centre for Applied Research and Testing (SICART), Vallabh Vidyanagar, Gujarat.]

Observations and Results:

FTIR study of both the species of *Arka* i.e *C.gigantea* and *C. procera* in the six *rutu* depicted altogether similar pattern of wavemumbers (cm-1). By applying PCA, (Fig 1 and 2) it was observed that the samples of *Grishma* and *Sharada rutu* were different from other samples and both showed similar spectral patterns. Major functional groups found in all 12 samples were alcohols, phenols, amines,

Table-4: Observed frequency, corresponding functional groups and remarks of CG latex in Grishma rutu.

CG Grishma rutu			
Observed frequency	**Reference Frequency(cm-1)**	**Group assignment or functional group**	**Remarks (CG Grishma rutu)**
3412.91	3200-3550; 3400-3500 (dil. soln.)	str. O-H (H-bonded), usually broad ;wk.N-H (1°-amines), 2 bands	Alcohols And Phenols ; Amines
2930.4	2850-3000; 2500-3300 (acids) overlap C-H	str. CH3, CH2 & CH 2or 3 bands; str. O-H (very broad)	Carboxylic Acids & Derivatives,Alkanes
2872.95	2850-3000; 2500-3300 (acids) overlap C-H	str. CH3, CH2 & CH 2or 3 bands; str. O-H (very broad)	Alkanes; Carboxylic Acids & Derivatives
2856.55	2850-3000; 2500-3300 (acids) overlap C-H	str. CH3, CH2 & CH 2or 3 bands; str. O-H (very broad)	Alkanes; Carboxylic Acids & Derivatives
1735.56	1720-1740; 1735-1750 (esters)	str. C=O (saturated aldehyde); str.C=O	Aldehydes & Ketones; Carboxylic Acids & Derivatives
1650.4	1630-1680; 1630-1695 (amides)	var. C=C (symmetry reduces intensity) ; str. C=O (amide I band)	Alkenes; Carboxylic Acids & Derivatives
1244.68	1000-1250 ; 1210-1320 (acids); 970-1250	med. C-N ; med-str.O-C (sometimes 2-peaks); str. C-O ;	Amines ; Carboxylic Acids & Derivatives; Alcohols & Phenols
1117.18	970-1250; 1000-1250; 1000-1300	str. C-O ; med. C-N ; str.O-C (2-bands)	Alcohols & Phenols; Amines ; Carboxylic Acids & Derivatives
1027.33	970-1250; 1000-1250; 1000-1300	str. C-O; med.C-N ; str.O-C (2-bands) ;	Alcohols & Phenols; Amines; Carboxylic Acids & Derivatives
982.7	970-1250 ;	str. C-O;	Alcohols & Phenols;
BENDING VIBRATION			
1558.62	1550-1650 ; 1500-1560	med-str. NH2 scissoring (1°-amines) ; N-H (2¡-amide) II band	Amines ; Carboxylic Acids & Derivatives
1542.89	1500-1560	N-H (2¡-amide) II band	Carboxylic Acids & Derivatives
1455.03	1350-1470	med. CH2 & CH3 deformation	Alkanes
1383.19	1350-1470 ; 1370-1390 ; 1330-1430;	med. CH2 & CH3 deformation ; med.CH3 deformation ; med.O-H bending (in-plane);	Alkanes ; Alkanes; Alcohols &Phenols;
982.7	880-995	str. =C-H & =CH2 ;	Alkenes
878.77	690-900; 660-900	str. Med C-H bending & ring puckering ; var. NH2 & N-H wagging (shifts on H-bonding)	Arenes ; Amines
780.07	780-850; 690-900; 660-900;	=C-H & =CH2 (out-of-plane bending); str. Med C-H bending & ring puckering; var. NH2 & N-H wagging (shifts on H-bonding:);	Alkenes ; Arenes; Amines
672.52	600-700; 650-770; 660-900	str.C-H deformation; var.wk. O-H bend (out-of-plane); var. NH2 & N-H wagging (shifts on H-bonding)	Alkynes; Alcohols And Phenols; Amines
620.24	600-700	str.C-H deformation	Alkynes

carboxylic acid derivatives, aldehydes, ketones, alkynes, alkenes, arenes, etc.

Tables 2-13 give an account of the presence of functional groups in six *rutu* in *Calotropis gigantea* and *Calotropis procera.*

DISCUSSION:

Reference frequencies of all the samples were analyzed for differences according to seasons for both the species. The distinct differences as well as similarities have been noted down per species in the range of those respective frequencies a under

3200-3550; 3400-3500:

C. gigantea: One observed band was present in this reference frequency range in all six *rutu* (seasons) which suggests the presence of str.O-H (H-bonded), usually broad; wk.N-H (1°-amines), 2 bands i.e. certain alcohols, phenols and amines.

Table-5: Observed frequency, corresponding functional groups and remarks of CP latex in Grishma rutu

CP Grishma rutu			
Observed frequency	**Reference Frequency(cm-1)**	**Group assignment or functional group**	**Remarks (CP Grishma rutu)**
3419.75	3200-3550;3400-3500 (dil. soln.)	str.O-H (H-bonded), usually broad; wk.N-H (1°-amines), 2 bands	Alcohols And Phenols; Amines
2928.54	2850-3000; 2500-3300 (acids) overlap C-H	str. CH3, CH2 & CH 2or 3 bands; str. O-H (very broad)	Alkanes, Carboxylic Acids & Derivatives,
2872.95	2850-3000; 2500-3300 (acids) overlap C-H	str. CH3, CH2 & CH 2or 3 bands; str. O-H (very broad)	Alkanes, Carboxylic Acids & Derivatives,
2855.33	2850-3000; 2500-3300 (acids) overlap C-H	str. CH3, CH2 & CH 2or 3 bands; str. O-H (very broad)	Alkanes, Carboxylic Acids & Derivatives,
1736.26	1720-1740; 1735-1750 (esters)	str. C=O (saturated aldehyde); str.C=O	Aldehydes & Ketones; Carboxylic Acids & Derivatives
1643.99	1630-1680; 1630-1695 (amides)	; var. C=C (symmetry reduces intensity) ; str. C=O (amide I band)	Alkenes; Carboxylic Acids & Derivatives
1244.86	1000-1250 ; 1210-1320 (acids); 970-1250	med. C-N ; med-str.O-C (sometimes 2-peaks); str. C-O ;	Amines ; Carboxylic Acids & Derivatives; Alcohols & Phenols
1115.22	970-1250; 1000-1250; 1000-1300	str. C-O ; med. C-N ; str.O-C (2-bands)	Alcohols & Phenols; Amines ; Carboxylic Acids & Derivatives
1028.27	970-1250; 1000-1250; 1000-1300	str. C-O; med.C-N ; str.O-C (2-bands) ;	Alcohols & Phenols; Amines; Carboxylic Acids & Derivatives
982.72	970-1250 ;	str. C-O; str.	Alcohols & Phenols;
BENDING VIBRATION			
1643.99	1590-1650;	med.N-H (1¡-amide) II band	Carboxylic Acids & Derivatives
1558.62	1550-1650 ; 1500-1560	med-str. NH2 scissoring (1°-amines) ; N-H (2¡-amide) II band	Amines ; Carboxylic Acids & Derivatives
1542.28	1500-1560	N-H (2¡-amide) II band	Carboxylic Acids & Derivatives
1454.91	1350-1470	med. CH2 & CH3 deformation	Alkanes
1382.98	1350-1470 ; 1370-1390 ; 1330-1430;	med. CH2 & CH3 deformation ; med.CH3 deformation ; med.O-H bending (in-plane);	Alkanes ; Alkanes; Alcohols &Phenols;
982.72	880-995	=C-H & =CH2 ;	Alkenes
878.91	690-900; 660-900	str. Med C-H bending & ring puckering ; var. NH2 & N-H wagging (shifts on H-bonding)	Arenes ; Amines
779.64	780-850; 690-900; 660-900	=C-H & =CH2 (out-of-plane bending); str. Med C-H bending & ring puckering; var. NH2 & N-H wagging (shifts on H-bonding)	Alkenes ; Arenes; Amines
671.48	600-700; 650-770; 660-900	str.C-H deformation; var.wk. O-H bend (out-of-plane); var. NH2 & N-H wagging (shifts on H-bonding)	Alkynes; Alcohols And Phenols; Amines
619.89	600-700	str.C-H deformation	Alkynes

C. procera: One observed band was present in this reference frequency range in all six *rutu* (seasons), same as in the case of *C.gigantea.* These frequencies depict amino acids that were present in all six rutu in both the species.

2850-3000; 2500-3300:

C. gigantea: There were two bands present in all *rutu* in this reference frequency range which suggests the presence of str. CH3, CH2 & CH 2or 3 bands; str. O-H (very broad) i.e. alkanes and Carboxylic acid derivatives. There was an extra band found in the *Grishma rutu* sample suggesting the presence of some other compound too.

Table-6: Observed frequency, corresponding functional groups and remarks of CG latex in Varsha rutu

CG Varsha rutu			
Observed frequency	**Reference Frequency(cm-1)**	**Group assignment or functional group**	**Remarks (CG Varsha rutu)**
3394.02	3200-3550; 3400-3500 (dil. soln.)	str. O-H (H-bonded), usually broad ;wk.N-H (1°-amines), 2 bands	Alcohols And Phenols ; Amines
2932.31	2850-3000; 2500-3300 (acids) overlap C-H	str. CH3, CH2 & CH 2or 3 bands; str. O-H (very broad)	Carboxylic Acids & Derivatives,Alkanes
2872.95	2850-3000; 2500-3300 (acids) overlap C-H	str. CH3, CH2 & CH 2or 3 bands; str. O-H (very broad)	Alkanes; Carboxylic Acids & Derivatives
1735.1	1720-1740;	str. C=O (saturated aldehyde)	Aldehydes & Ketones
1648.9	1630-1680; 1630-1695 (amides)	var. C=C (symmetry reduces intensity) ; str. C=O (amide I band)	Alkenes; Carboxylic Acids & Derivatives
1245.1	1000-1250 ; 1210-1320 (acids); 970-1250	med. C-N ; med-str.O-C (sometimes 2-peaks); str. C-O ;	Amines ; Carboxylic Acids & Derivatives; Alcohols & Phenols
1121.41	970-1250; 1000-1250; 1000-1300	str. C-O ; med. C-N ; str.O-C (2-bands)	Alcohols & Phenols; Amines ; Carboxylic Acids & Derivatives
1031.41	970-1250; 1000-1250; 1000-1300	str. C-O; med.C-N ; str.O-C (2-bands) ;	Alcohols & Phenols; Amines; Carboxylic Acids & Derivatives
982.37	970-1250 ;	str. C-O;	Alcohols & Phenols;
BENDING VIBRATIONS			
1556.57	1550-1650 ; 1500-1560	med-str. NH2 scissoring (1°-amines) ; N-H (2¡-amide) II band	Amines ; Carboxylic Acids & Derivatives
1453.2	1350-1470	med. CH2 & CH3 deformation	Alkanes
1383.86	1350-1470 ; 1370-1390 ; 1330-1430;	med. CH2 & CH3 deformation ; med.CH3 deformation ; med.O-H bending (in-plane);	Alkanes ; Alkanes; Alcohols &Phenols;
1324.2	1325+/-25	S=O	Sulfone
982.37	880-995	str. =C-H & =CH2 ;	Alkenes
877.43	690-900; 660-900	str. Med C-H bending & ring puckering ; var. NH2 & N-H wagging (shifts on H-bonding)	Arenes ; Amines
616.38	600-700	str.C-H deformation	Alkynes

C. procera: There were two bands present in *Sharada rutu, Shishira rutu , Hemanta rutu* and *Vasanta rutu* suggesting the presence of str. CH3, CH2 & CH 2or 3 bands; str. O-H (very broad) i.e. alkanes and Carboxylic acid derivatives. There was an extra band found in *Grishma rutu* whereas only one band was found in *Varsha rutu* .

1720-1740:

C. gigantea: One band was present commonly in all *rutu* suggestive of presence of str. C=O (saturated aldehyde) i.e aldehydes and ketones. But one additional band was noticed in *Sharada rutu* sample falling in 1710-1720 that differentiated the saturated aldehyde and ketone. 1769.09, an additional unknown band was also observed.

C. procera: One band was present commonly in all *rutu* suggestive of presence of str. C=O (saturated aldehyde) i.e aldehydes and ketones.

1735-1750:

C. gigantea: One band co-inciding this frequency was obtained only in *Grishma* sample which suggests presence of aliphatic esters.

C. procera: One band was present commonly in all *rutu* suggestive of presence of str.C=O i.e

Carboxylic acid derivatives mainly esters group which is very peculiar for *C.procera* species.

1630-1680; 1630-1695:

C. gigantea: One band was present commonly in all *rutu* suggestive of presence of var. C=C (symmetry reduces intensity); str. C=O (amide I band) i.e alkenes and carboxylic acids.

C. procera: One band was present commonly in all *rutu* suggestive of presence of var. C=C (symmetry reduces intensity); str. C=O (amide I band) i.e alkenes and carboxylic acids.

Table-7: Observed frequency, corresponding functional groups and remarks of CP latex in Varsha rutu

CP Varsha rutu			
Observed frequency	**Reference Frequency(cm-1)**	**Group assignment or functional group**	**Remarks (CP Shishira rutu)**
3408.59	3200-3550; 3400-3500 (dil. soln.)	str. O-H (H-bonded), usually broad ; wk.N-H (1°-amines), 2 bands	Alcohols And Phenols ; Amines
2954.83	2850-3000; 2500-3300 (acids) overlap C-H	str. CH3, CH2 & CH 2or 3 bands; str. O-H (very broad)	Alkanes, Carboxylic Acids & Derivatives,
1735.26	1720-1740; 1735-1750 (esters)	str. C=O (saturated aldehyde); str.C=O	Aldehydes & Ketones; Carboxylic Acids & Derivatives
1644.64	1630-1680; 1630-1695 (amides)	; var. C=C (symmetry reduces intensity) ; str. C=O (amide I band)	Alkenes; Carboxylic Acids & Derivatives
1245.06	1000-1250 ; 1210-1320 (acids); 970-1250	med. C-N ; med-str.O-C (sometimes 2-peaks); str. C-O ;	Amines ; Carboxylic Acids & Derivatives; Alcohols & Phenols
1121.34	970-1250; 1000-1250; 1000-1300	str. C-O ; med. C-N ; str.O-C (2-bands)	Alcohols & Phenols; Amines ; Carboxylic Acids & Derivatives
1024.76	970-1250; 1000-1250; 1000-1300	str. C-O; med.C-N ; str.O-C (2-bands) ;	Alcohols & Phenols; Amines; Carboxylic Acids & Derivatives
982.37	970-1250 ;	str. C-O; str.	Alcohols & Phenols;
BENDING VIBRATIONS			
1454.29	1350-1470	med. CH2 & CH3 deformation	Alkanes
1382.15	1350-1470 ; 1370-1390 ; 1330-1430;	med. CH2 & CH3 deformation ; med.CH3 deformation ; med.O-H bending (in-plane);	Alkanes ; Alkanes; Alcohols &Phenols;
1323.2	1325+/-25	S=O	Sulfone
982.37	880-995	=C-H & =CH2 ;	Alkenes
876.11	690-900; 660-900	str. Med C-H bending & ring puckering ; var. NH2 & N-H wagging (shifts on H-bonding)	Arenes ; Amines
671.77	600-700; 650-770	str.C-H deformation; var-wk O-H bend (out-of-plane)	Alkynes ;Alcohols And Phenols
619.48	600-700	str.C-H deformation	Alkynes
467.13	-	-	-

1210-1320:

C. gigantea: One band was present commonly in all *rutu* suggestive of presence of med-str.O-C (sometimes 2-peaks) i.e. carboxylic acids.

C.procera: The findings were similar to *C.gigantea.*

1000-1250:

C.gigantea: Three bands were present commonly in all *rutu* except two bands in *Shishira rutu* suggestive of presence of med. C-N i.e. amines.

C. procera: The findings were similar to *C.gigantea.*

970-1250

C. gigantean: Four bands were present commonly in all *rutu* except three bands in *Shishira rutu* suggestive of presence of str. C-O i.e alcohols and phenols.

C. procera: The findings were similar to *C.gigantea.*

1000-1300:

C. gigantea: Two bands were present commonly in all *rutu* except one band in *Shishira rutu* suggestive of presence of str. O-C (2-bands) i.e carboxylic acids.

C. procera: The findings were similar to *C.gigantea.*

The bands between 1000-1100 cm-1 depict presence of carbohydrates.

1550-1650:

C. gigantea: One band was found common in four *rutu* except *Sharada* and *Hemanta rutu*

Table-8: Observed frequency, corresponding functional groups and remarks of CG latex in Sharada rutu

CG Sharada rutu			
Observed frequency	**Reference Frequency (cm-1)**	**Group assignment or functional group**	**Remarks (CG Sharada rutu)**
3389.7	3200-3550	str.O-H (H-bonded), usually broad	Alcohols And Phenols
2938.28	2850-3000; 2500-3300 (acids) overlap C-H	str. CH3, CH2 & CH 2or 3 bands; str. O-H (very broad)	Carboxylic Acids & Derivatives,Alkanes
2856.55	2850-3000; 2500-3300 (acids) overlap C-H	str. CH3, CH2 & CH 2or 3 bands; str. O-H (very broad)	Alkanes; Carboxylic Acids & Derivatives
1769.09	-	-	-
1730.46	1720-1740;	str. C=O (saturated aldehyde)	Aldehydes & Ketones
1718	1710-1720	str C=O (saturated ketone)	Aldehydes & Ketones
1642.82	1630-1680; 1630-1695 (amides)	var. C=C (symmetry reduces intensity) ; str. C=O (amide I band)	Alkenes; Carboxylic Acids & Derivatives
1246.48	1000-1250 ; 1210-1320 (acids); 970-1250	med. C-N ; med-str.O-C (sometimes 2-peaks); str. C-O ;	Amines ; Carboxylic Acids & Derivatives; Alcohols & Phenols
1121.19	970-1250; 1000-1250; 1000-1300	str. C-O ; med. C-N ; str.O-C (2-bands)	Alcohols & Phenols; Amines ; Carboxylic Acids & Derivatives
1029.37	970-1250; 1000-1250; 1000-1300	str. C-O; med.C-N ; str.O-C (2-bands) ;	Alcohols & Phenols; Amines; Carboxylic Acids & Derivatives
982.37	970-1250 ;	str. C-O;	Alcohols & Phenols;
BENDING VIBRATIONS			
1515.7	1500-1560	med N-H (2¡-amide) II band	Carboxylic Acids & Derivatives
1452.96	1350-1470	med. CH2 & CH3 deformation	Alkanes
1384.92	1350-1470 ; 1370-1390 ; 1330-1430;	med. CH2 & CH3 deformation ; med.CH3 deformation ; med.O-H bending (in-plane);	Alkanes ; Alkanes; Alcohols &Phenols;
1323.11	1325+/-25	S=O	Sulfone
982.37	880-995	str. =C-H & =CH2 ;	Alkenes
879.25	690-900; 660-900	str. Med C-H bending & ring puckering ; var. NH2 & N-H wagging (shifts on H-bonding)	Arenes ; Amines
749.6	690-900; 660-900,650-770	str. Med C-H bending & ring puckering ; var. NH2 & N-H wagging (shifts on H-bonding); var-wkO-H bend (out-of-plane)	Arenes ; Amines;Alcohols And Phenols
718.77	675-730;600-700; 690-900; 650-770	med cis-RCH=CHR; str C-H deformation; str-med C-H bending & ring puckering ; O-H bend (out-of-plane)	Alkenes; Alkynes; Arenes; Alcohols And Phenols
657.47	600-700; 650-770;	str.C-H deformation; var.wk. O-H bend (out-of-plane);	Alkynes; Alcohols And Phenols
624.86	600-700	str.C-H deformation	Alkynes

suggestive of presence of med-str. NH2 scissoring (1°-amines) i.e. amines.

C. procera: One band was found in *Shishira, Grishma* and *Hemanta rutu* only.

1500-1560:

C. gigantea: One band was found common in all *rutu* suggestive of presence of N-H (2¡-amide) II band i.e carboxylic acids.

C. procera: Two bands were present in *Shishira and Grishma* rutu while only one band was present in Hemanta rutu and absent in Varsha, Sharada and Vasanta rutu .

1325 +/- 25:

C. gigantea: One band was found common in *Sharada, Hemanta and Shishira* rutu suggestive of S=O i.e sulfone.

Table-9: Observed frequency, corresponding functional groups and remarks of CP latex in Sharada rutu

CP Sharada rutu			
Observed frequency	**Reference Frequency(cm-1)**	**Group assighnment or functional group**	**Remarks (CP Sharada rutu)**
3410.57	3200-3550;3400-3500 (dil. soln.)	str.O-H (H-bonded), usually broad; wk.N-H (1°-amines), 2 bands	Alcohols And Phenols; Amines
2939.98	2850-3000; 2500-3300 (acids) overlap C-H	str. CH3, CH2 & CH 2or 3 bands; str. O-H (very broad)	Alkanes, Carboxylic Acids & Derivatives,
2856.55	2850-3000; 2500-3300 (acids) overlap C-H	str. CH3, CH2 & CH 2or 3 bands; str. O-H (very broad)	Alkanes, Carboxylic Acids & Derivatives,
1735.48	1720-1740; 1735-1750 (esters)	str. C=O (saturated aldehyde); str.C=O	Aldehydes & Ketones; Carboxylic Acids & Derivatives
1644.97	1630-1680; 1630-1695 (amides)	; var. C=C (symmetry reduces intensity) ; str. C=O (amide I band)	Alkenes; Carboxylic Acids & Derivatives
1245.39	1000-1250 ; 1210-1320 (acids); 970-1250	med. C-N ; med-str.O-C (sometimes 2-peaks); str. C-O ;	Amines ; Carboxylic Acids & Derivatives; Alcohols & Phenols
1113.34	970-1250; 1000-1250; 1000-1300	str. C-O ; med. C-N ; str.O-C (2-bands)	Alcohols & Phenols; amines ; Carboxylic Acids & Derivatives
1029.37	970-1250; 1000-1250; 1000-1300	str. C-O; med.C-N ; str.O-C (2-bands) ;	Alcohols & Phenols; amines; Carboxylic Acids & Derivatives
982.61	970-1250 ;	str. C-O; str.	Alcohols & Phenols;
BENDING VIBRATIONS			
1453.73	1350-1470	med. CH2 & CH3 deformation	Alkanes
1382.96	1350-1470 ; 1370-1390 ; 1330-1430;	med. CH2 & CH3 deformation ; med.CH3 deformation ; med.O-H bending (in-plane);	Alkanes ; Alkanes; Alcohols & Phenols;
1323.2	1325+/-25	S=O	Sulfone
982.61	880-995	=C-H & =CH2 ;	Alkenes
878.16	690-900; 660-900	str. Med C-H bending & ring puckering ; var. NH2 & N-H wagging (shifts on H-bonding)	Arenes ; Amines
778.03	780-850; 690-900; 660-900	=C-H & =CH2 (out-of-plane bending); str. Med C-H bending & ring puckering; var. NH2 & N-H wagging (shifts on H-bonding)	Alkenes ; Arenes; Amines
625.94	600-700	str.C-H deformation	Alkynes

C. procera: One band was found common in all *rutu* except *Grishma rutu* .

1350-1470:

C. gigantea: Two bands were found common in all *rutu* suggestive of presence of med. CH2 & CH3 deformation i.e. alkanes.

C.procera: The findings were similar to *C.gigantea.*

1370-1390; 1330-1430:

C. gigantea: One band was found common in all *rutu* suggestive of presence of med.CH3 deformation; med.O-H bending (in-plane) i.e alkanes and alcohols & phenols.

C. procera: The findings were similar to *C.gigantea.*

880-995:

C. gigantea: One band was found common in all *rutu* suggestive of presence of str. =C-H & =CH2 i.e. alkenes.

C. procera: Two bands were observed in *Shishira rutu* and *Vasanta rutu* while one band each in rest of the *rutu* .

660-900:

C. gigantea: Three bands were found in *Shishira* and *Grishma* while two bands were found in *Sharada rutu , Vasanta rutu* and *Hemanta rutu*

Table-10: Observed frequency, corresponding functional groups and remarks of CG latex in Hemanta rutu

CG Hemanta rutu			
Observed frequency	**Reference Frequency(cm-1)**	**Group assignment or functional group**	**Remarks (CG Hemanta rutu)**
3402.29	3400-3500(dil. soln.); 3200-3550	str.O-H (H-bonded), usually broad; wk.N-H (1°-amines), 2 bands	Alcohols And Phenols; Amines
2931.99	2850-3000; 2500-3300 (acids) overlap C-H	str. CH3, CH2 & CH 2or 3 bands; str. O-H (very broad)	Carboxylic Acids & Derivatives,alkanes
2872.95	2850-3000; 2500-3300 (acids) overlap C-H	str. CH3, CH2 & CH 2or 3 bands; str. O-H (very broad)	alkanes; Carboxylic Acids & Derivatives
1734.79	1720-1740;	str. C=O (saturated aldehyde);	Aldehydes & Ketones;
1649.85	1630-1680; 1630-1695 (amides)	var. C=C (symmetry reduces intensity) ; str. C=O (amide I band)	Alkenes; Carboxylic Acids & Derivatives
1245.05	1000-1250 ; 1210-1320 (acids); 970-1250; 1000-1300	med. C-N ; med-str.O-C (sometimes 2-peaks); str. C-O ; str.O-C (2-bands)	amines ; Carboxylic Acids & Derivatives; Alcohols & Phenols; Carboxylic Acids & Derivatives
1119.9	970-1250; 1000-1250; 1000-1300	str. C-O ; med. C-N ; str.O-C (2-bands)	Alcohols & Phenols; amines ; Carboxylic Acids & Derivatives
1031.41	970-1250; 1000-1250; 1000-1300	str. C-O; med.C-N ; str.O-C (2-bands) ;	Alcohols & Phenols; amines; Carboxylic Acids & Derivatives
984.41	970-1250 ;	str. C-O; str. ;	Alcohols & Phenols;
BENDING VIBRATION			
1548.4	1500-1560	N-H (2¡-amide) II band	Carboxylic Acids & Derivatives
1455.98	1350-1470	med. CH2 & CH3 deformation	Alkanes
1323.91	1325+/-25	S=O	Sulfone
1384.12	1350-1470 ; 1370-1390 ; 1330-1430;	med. CH2 & CH3 deformation ; med.CH3 deformation ; med.O-H bending (in-plane);	med. CH2 & CH3 deformation ; med.CH3 deformation ; med.O-H bending (in-plane);
984.41	880-995	=C-H & =CH2	Alkenes
878.16	690-900; 660-900	str. Med C-H bending & ring puckering ; var. NH2 & N-H wagging (shifts on H-bonding)	Arenes ; Amines
675.86	600-700; 650-770; 660-900	str.C-H deformation; var.wk. O-H bend (out-of-plane); var. NH2 & N-H wagging (shifts on H-bonding)	Alkynes; Alcohols And Phenols; Amines
619.33	600-700	str.C-H deformation	Alkynes

while only one band was present in *Varsha rutu* which is suggestive that amines were found more in *Shishira* and *Grishma*.

C. procera: Three bands were found in *Grishma* while two bands were found in *Shishira, Sharada rutu , Vasanta rutu* and *Hemanta rutu* while only one band was present in *Varsha rutu* which is suggestive that amines were found more in *Grishma rutu* .

690-900:

C. gigantea: Three bands were found in *Sharada rutu* and *Shishira rutu* while two bands were found in *Grishma* and *Vasanta rutu* while only one band in *Varsha* and *Hemanta rutu* which probably denoted large amount of arenes present in *Sharada* and *Shishira rutu* .

C. procera: Two bands were observed in all *rutu* except *Varsha rutu* .

The absorption bands at 837 to 721cm-1 are indicative of benzene rings.

650-770:

C. gigantea: Three bands were found in *Sharada rutu* while one band each in *Hemanta rutu* and *Grishma rutu* which indicates presence of alcohol and phenol groups whereas no bands were observed in rest of the *rutu* .

C. procera: Only one band was found in *Varsha* and *Grishma* rutu only.

Table-11: Observed frequency, corresponding functional groups and remarks of CP latex in Hemanta rutu

CP Hemanta rutu			
Observed frequency	**Reference Frequency(cm-1)**	**Group assignment or functional group**	**Remarks (CP hemanta rutu)**
3409.45	3200-3550;3400-3500 (dil. soln.)	str.O-H (H-bonded), usually broad; wk.N-H (1°-amines), 2 bands	Alcohols And Phenols; Amines
2931.84	2850-3000; 2500-3300 (acids) overlap C-H	str. CH3, CH2 & CH 2or 3 bands; str. O-H (very broad)	Alkanes, Carboxylic Acids & Derivatives,
2856.55	2850-3000; 2500-3300 (acids) overlap C-H	str. CH3, CH2 & CH 2or 3 bands; str. O-H (very broad)	Alkanes, Carboxylic Acids & Derivatives,
1735.07	1720-1740; 1735-1750 (esters)	str. C=O (saturated aldehyde); str.C=O	Aldehydes & Ketones; Carboxylic Acids & Derivatives
1644.92	1630-1680; 1630-1695 (amides)	; var. C=C (symmetry reduces intensity) ; str. C=O (amide I band)	Alkenes; Carboxylic Acids & Derivatives
1245.28	1000-1250 ; 1210-1320 (acids); 970-1250	med. C-N ; med-str.O-C (sometimes 2-peaks); str. C-O ;	Amines ; Carboxylic Acids & Derivatives; Alcohols & Phenols
1123.46	970-1250; 1000-1250; 1000-1300	str. C-O ; med. C-N ; str.O-C (2-bands)	Alcohols & Phenols; Amines ; Carboxylic Acids & Derivatives
1027.33	970-1250; 1000-1250; 1000-1300	str. C-O; med.C-N ; str.O-C (2-bands) ;	Alcohols & Phenols; Amines; Carboxylic Acids & Derivatives
982.37	970-1250 ;	str. C-O; str.	Alcohols & Phenols;
BENDING VIBRATION			
1548.28	1550-1650 ; 1500-1560	med-str. NH2 scissoring (1°-amines) ; N-H (2¡-amide) II band	Amines ; Carboxylic Acids & Derivatives
1454.8	1350-1470	med. CH2 & CH3 deformation	Alkanes
1384.1	1350-1470 ; 1370-1390 ; 1330-1430;	med. CH2 & CH3 deformation ; med.CH3 deformation ; med.O-H bending (in-plane);	Alkanes ; Alkanes; Alcohols &Phenols;
1321.55	1325+/-25	S=O	Sulfone
982.37	880-995	=C-H & =CH2 ;	Alkenes
878.85	690-900; 660-900	str. Med C-H bending & ring puckering ; var. NH2 & N-H wagging (shifts on H-bonding)	Arenes ; Amines
778.03	780-850; 690-900; 660-900	=C-H & =CH2 (out-of-plane bending); str. Med C-H bending & ring puckering; var. NH2 & N-H wagging (shifts on H-bonding)	Alkenes ; Arenes; Amines
619.21	600-700	str.C-H deformation	Alkynes

600-700:

C. gigantea: Three bands were found in *Sharada rutu* while two bands in *Grishma rutu* one band each in *Vasanta, Shishira rutu* & *Varsha rutu* which indicates presence of alkynes more pronounced in *Sharada rutu* .

C. procera: Two bands were observed in *Grishma* and *Varsha rutu* while one band was present in rest of the *rutu.*

The bands at 553-633 cm-1 depict aromatic compounds (phosphates).

675-730:

C. gigantea: One band from this range was exclusively present in *Sharada* sample which indicates presence of alkenes group.

C. procera: It was not observed in this species.

780-850:

C. procera: One band was present in each rutu except in *Varsha rutu* .

900-1050:

C. procera: One band has been exclusively observed in *Vasanta rutu* .

Table-11: Observed frequency, corresponding functional groups and remarks of CP latex in Hemanta rutu

CP Hemanta rutu			
Observed frequency	**Reference Frequency(cm-1)**	**Group assignment or functional group**	**Remarks (CP hemanta rutu)**
3409.45	3200-3550;3400-3500 (dil. soln.)	str.O-H (H-bonded), usually broad; wk.N-H (1°-amines), 2 bands	Alcohols And Phenols; Amines
2931.84	2850-3000; 2500-3300 (acids) overlap C-H	str. CH3, CH2 & CH 2or 3 bands; str. O-H (very broad)	Alkanes, Carboxylic Acids & Derivatives,
2856.55	2850-3000; 2500-3300 (acids) overlap C-H	str. CH3, CH2 & CH 2or 3 bands; str. O-H (very broad)	Alkanes, Carboxylic Acids & Derivatives,
1735.07	1720-1740; 1735-1750 (esters)	str. C=O (saturated aldehyde); str.C=O	Aldehydes & Ketones; Carboxylic Acids & Derivatives
1644.92	1630-1680; 1630-1695 (amides)	; var. C=C (symmetry reduces intensity) ; str. C=O (amide I band)	Alkenes; Carboxylic Acids & Derivatives
1245.28	1000-1250 ; 1210-1320 (acids); 970-1250	med. C-N ; med-str.O-C (sometimes 2-peaks); str. C-O ;	Amines ; Carboxylic Acids & Derivatives; Alcohols & Phenols
1123.46	970-1250; 1000-1250; 1000-1300	str. C-O ; med. C-N ; str.O-C (2-bands)	Alcohols & Phenols; Amines ; Carboxylic Acids & Derivatives
1027.33	970-1250; 1000-1250; 1000-1300	str. C-O; med.C-N ; str.O-C (2-bands) ;	Alcohols & Phenols; Amines; Carboxylic Acids & Derivatives
982.37	970-1250 ;	str. C-O; str.	Alcohols & Phenols;
BENDING VIBRATION			
1548.28	1550-1650 ; 1500-1560	med-str. NH2 scissoring (1°-amines) ; N-H (2¡-amide) II band	Amines ; Carboxylic Acids & Derivatives
1454.8	1350-1470	med. CH2 & CH3 deformation	Alkanes
1384.1	1350-1470 ; 1370-1390 ; 1330-1430;	med. CH2 & CH3 deformation ; med.CH3 deformation ; med.O-H bending (in-plane);	Alkanes ; Alkanes; Alcohols &Phenols;
1321.55	1325+/-25	S=O	Sulfone
982.37	880-995	=C-H & =CH2 ;	Alkenes
878.85	690-900; 660-900	str. Med C-H bending & ring puckering ; var. NH2 & N-H wagging (shifts on H-bonding)	Arenes ; Amines
778.03	780-850; 690-900; 660-900	=C-H & =CH2 (out-of-plane bending); str. Med C-H bending & ring puckering; var. NH2 & N-H wagging (shifts on H-bonding)	Alkenes ; Arenes; Amines
619.21	600-700	str.C-H deformation	Alkynes

An additional unknown band of 467.13 has been observed in *C. procera* in *Varsha rutu* which indicates presence of unknown compound.

The results in a study of FTIR **[16]** of *Calotropis gigantea* showed that the bands 3434 cm–1 VS (O-H), 2919 cm–1 S (CH3CH2), 1637 cm–1 VS (C=O), 1424 cm–1 S (C-H), 1104 cm–1 S (C-O,C-C) and 1028 cm–1 S (C-O) confirm the presence of Calotropagenin and Calotropin. The correlation of presence of amino acids, carbohydrates has also been made in this study. In the present study, bands that were close to these ranges can probably be Calotropin and Calotropagenin.

Acharya Sushruta **[17]** has classified the collection time according to seasons as *Agneya rutu i.e.(Sharada, Grishma* and *Vasanta)* and *Saumya rutu (Hemanta, Shishira* and *Varsha)*. The differentiation in FTIR bands obtained after applying PCA is more pronounced in *Grishma* and *Sharada* samples. On keen analysis of presence and absence of peaks as given above, some additional peaks have been obtained in *Grishma rutu* and *Sharad rutu* in number as compared to other samples in total.

Table 12: Observed frequency, corresponding functional groups and remarks of CG latex in Shishira rutu

CG Shishira rutu			
Observed frequency	**Reference Frequency(cm-1)**	**Group assighnment or functional group**	**Remarks (CG Shishira rutu)**
3437.39	3200-3550; 3400-3500 (dil. soln.)	str. O-H (H-bonded), usually broad ;wk.N-H (1°-amines), 2 bands	Alcohols And Phenols ; Amines
2927.12	2850-3000; 2500-3300 (acids) overlap C-H	str. CH3, CH2 & CH 2or 3 bands; str. O-H (very broad)	Carboxylic Acids & Derivatives,Alkanes
2856.55	2850-3000; 2500-3300 (acids) overlap C-H	str. CH3, CH2 & CH 2or 3 bands; str. O-H (very broad)	Alkanes; Carboxylic Acids & Derivatives
1732.26	1720-1740;	str. C=O (saturated aldehyde)	Aldehydes & Ketones
1640.83	1630-1680; 1630-1695 (amides)	var. C=C (symmetry reduces intensity) ; str. C=O (amide I band)	Alkenes; Carboxylic Acids & Derivatives
1246.23	1000-1250 ; 1210-1320 (acids); 970-1250	med. C-N ; med-str.O-C (sometimes 2-peaks); str. C-O ;	Amines ; Carboxylic Acids & Derivatives; Alcohols & Phenols
1094.19	970-1250; 1000-1250; 1000-1300	str. C-O; med.C-N ; str.O-C (2-bands) ;	Alcohols & Phenols; Amines; Carboxylic Acids & Derivatives
982.37	970-1250 ;	str. C-O;	Alcohols & Phenols;
BENDING VIBRATIONS			
1542.12	1550-1650 ; 1500-1560	med-str. NH2 scissoring (1°-amines) ; N-H (2¡-amide) II band	Amines ; Carboxylic Acids & Derivatives
1519.79	1500-1560	med N-H (2¡-amide) II band	Carboxylic Acids & Derivatives
1455.24	1350-1470	med. CH2 & CH3 deformation	Alkanes
1323.62	1325+/-25	S=O	Sulfone
1383.78	1350-1470 ; 1370-1390 ; 1330-1430;	med. CH2 & CH3 deformation ; med.CH3 deformation ; med.O-H bending (in-plane);	Alkanes ; Alkanes; Alcohols &Phenols;
982.37	880-995	str. =C-H & =CH2 ;	Alkenes
878.49	690-900; 660-900	str. Med C-H bending & ring puckering ; var. NH2 & N-H wagging (shifts on H-bonding)	Arenes ; Amines
855.68	690-900; 660-900	str. Med C-H bending & ring puckering ; var. NH2 & N-H wagging (shifts on H-bonding)	Arenes ; Amines
800.51	690-900; 660-900; 780-850	str. Med C-H bending & ring puckering ; var. NH2 & N-H wagging (shifts on H-bonding); (out-of-plane bending)	Arenes ; Amines; Alkenes
620.89	600-700	str.C-H deformation	Alkynes

These are mostly from aldehydes, ketones and amide groups.

CONCLUSION

There were significant differences noted in the bands obtained from the latex samples of species of *Arka* i.e. *Calotropis procera* and *Shwetarka* i.e white variety of *Calotropis gigantea* collected in six *rutus*. Thus, the concept of *Kala* (influence of time on phytochemical profile of plant) holds true especially in case of inputs of *Sushruta* regarding *Agneya rutu* due to the distinct bands obtained in *Grishma* and *Sharada rutu.*

Further scope:

This concept of *Agneya rutu* can be more elucidated with quantification of phytoconstituents (calotropin, calotropagenin, uscharin,etc.) present in latex responsible for metabolic alterations systemically *in vivo* or irritation as local response along with FTIR.

Table13: Observed frequency, corresponding functional groups and remarks of CP latex in Shishira rutu

CP shishir rutu			
Observed frequency	**Reference Frequency(cm-1)**	**Group assighnment or functional group**	**Remarks (CP Shishira rutu)**
3418.92	3200-3550; 3400-3500 (dil. soln.)	str. O-H (H-bonded), usually broad ;wk.N-H (1°-amines), 2 bands	Alcohols And Phenols ; Amines
2929.53	2850-3000; 2500-3300 (acids) overlap C-H	str. CH3, CH2 & CH 2or 3 bands; str. O-H (very broad)	Alkanes, Carboxylic Acids & Derivatives,
2856.55	2850-3000; 2500-3300 (acids) overlap C-H	str. CH3, CH2 & CH 2or 3 bands; str. O-H (very broad)	Alkanes, Carboxylic Acids & Derivatives,
1735.08	1720-1740; 1735-1750 (esters)	str. C=O (saturated aldehyde); str.C=O	Aldehydes & Ketones; Carboxylic Acids & Derivatives
1644.34	1630-1680; 1630-1695 (amides)	; var. C=C (symmetry reduces intensity) ; str. C=O (amide I band)	Alkenes; Carboxylic Acids & Derivatives
1246.12	1000-1250 ; 1210-1320 (acids); 970-1250	med. C-N ; med-str.O-C (sometimes 2-peaks); str. C-O ;	Amines ; Carboxylic Acids & Derivatives; Alcohols & Phenols
1111.94	970-1250; 1000-1250; 1000-1300	str. C-O ; med. C-N ; str.O-C (2-bands)	Alcohols & Phenols; Amines ; Carboxylic Acids & Derivatives
982.37	970-1250 ;	str. C-O; str.	Alcohols & Phenols;
BENDING VIBRATIONS			
1541.49	1550-1650 ; 1500-1560	med-str. NH2 scissoring (1°-amines) ; N-H (2¡-amide) II band	Amines ; Carboxylic Acids & Derivatives
1517.75	1500-1560	med N-H (2¡-amide) II band	Carboxylic Acids & Derivatives
1454.75	1350-1470	med. CH2 & CH3 deformation	Alkanes
1382.9	1350-1470 ; 1370-1390 ; 1330-1430;	med. CH2 & CH3 deformation ; med.CH3 deformation ; med.O-H bending (in-plane);	Alkanes ; Alkanes; Alcohols &Phenols;
1324.36	1325+/-25	S=O	Sulfone
982.37	880-995	=C-H & =CH2 ;	Alkenes
902.68	880-995,900-1050 (str)	=C-H & =CH2 ; P-OR	Alkenes,Esters
878.37	690-900; 660-900	str. Med C-H bending & ring puckering ; var. NH2 & N-H wagging (shifts on H-bonding)	Arenes ; Amines
806.64	690-900; 660-900; 780-850;	str. Med C-H bending & ring puckering ; var. NH2 & N-H wagging (shifts on H-bonding); =C-H & =CH2 (out-of-plane bending);	Arenes ; Amines; Alkenes
611.28	600-700	str.C-H deformation	Alkynes

Similarly, the influence of place on quality of latex collected from different area should also be studied.

Key Message

This study supports the theory that seasons do influence the organic profile of the latex thus validating concept of *Kala* (time) in *Ayurveda*.

Contribution of Authors

This work was carried out in collaboration between all authors. Anagha Ranade, Rabinarayan Acharya and Sudipta Roy conceptualized, designed the study and executed it. They also contributed in drafting of the article. Vinay Shukla, Jayantkumar Maji performed the analyses of the study. All authors contributed in logical interpretation. They further read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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References

1. Acharya JT., Editor. *Charaka Samhita.* commentary of Chakrapani. *Kalpasthana*. 1/7, 1st Ed., Chaukhambha Prakashana, Varanasi, Reprint 2011; 652.
2. Anonymous. WHO guidelines on good agricultural and collection practices (GACP) for medicinal plants (PDF). World health organisation. Geneva: 2003. 11.
3. Acharya RN, Ranade AV. Fostering the concept of Kala-The Relevance of time factor in augmenting crude drug quality. In: Dhiman KS. Padhi MM.Mangal AK. Srikanth N. Editors. Recent trends in Good agricultural and Collection practices for medicinal plants. First edition. New Delhi: CCRAS. Ministry of Ayush. pp 323-344.2015.
4. Bhide B, Acharya RN. Concept of Visha and its pharmacological basis in Ayurveda.*IJRAP* 2012; 3(2):137-140.
5. Ranade AV, Acharya RN. Arka and its pharmaceutical attributes in Indian alchemy (Rasashastra): A comprehensive review. *International Journal of Ayurvedic Medicine*. 2015; 6(4): 280-288.
6. Ranade AV, Acharya R. Pharmacotherapeutic archives of Arka. *Journal of Medicinal Plants Studies* 2016; 4(4): 94-108
7. Ranade AV, Acharya RN. Canvassing the external applications of *Arka*: A Classical Memoir. *Jour. of Ayurveda & Holistic Medicine.* 2015; 3(5): 54-81
8. Ranade Anagha, Harisha CR, Acharya Rabinarayan and Jani Switu. Micro-Morphological and Micrometric evaluation of White and Purple variety Flowers of *Calotropis gigantea* Linn. R.Br. *IJUPBS*. Sept-Oct 2014; 3(5): 289-302.
9. Ranade Anagha V, Acharya Rabinarayan. Arka and its botanical equivalents: A critical analysis. *Ayurpharm Int J Ayur Alli Sci.* 2015; 4(3): p 60-68.
10. Ranade Anagha, Acharya Rabinarayan. An Appraisal On Ethno-Medicinal Claims Of Calotropis Procera Ait And Calotropis Gigantea (Linn) R.Br. - Two Source Drugs Of Ayurvedic Medicinal Plant 'Arka'. Global *J Res. Med. Plants & Indigen. Med.* (2014); 3 (12): 475–488.
11. Acharya YT. editor. Charaka samhita (Ayurveda Dipika Vyakhya). 1st ed. Varanasi: Chaukhambha Prakashan; 2011. Vimanasthana *Kalpasthana* 1/10, 653.
12. Sheth F. Range of seasonal phytochemical variations in *Calotropis procera* (Ait.) R.Br. Int. *J.Med.Arom. Plants* 2011;1(2): 180-183.
13. Pillai LS, Nair BR. Functional group analysis of Cleome viscose L. and C. burmanni W. & A. (Cleomaceae) extracts by FT-IR. *Journal of Pharmacognosy and Phytochemistry* 2014; 2(6): 120-124.
14. Surewicz WK, Mantsch HH, Chapman D. Determination of protein secondary structure by Fourier transform infrared spectroscopy: A critical assessment. *Biochemistry* 1993; 32(2):389-3.
15. Yang J, Yen HE. Early salt stress effects on the changes in chemical composition in leaves of ice plant and Arabidopsis. A Fourier transform infrared spectroscopy study. *Plant Physiol* 2002; 130(2):1032-42.
16. Ramamurthy N. Kannan S. Fourier Transform Infrared Spectroscopic Analysis of a plant (*Calotropis Gigantea* Linn) from an industrial village, Cuddalore Dt, Tamilnadu, India. *Romanian J. Biophys.* 2007; 17(4): 269–276.
17. Sharma PV. Editor *Sushruta Samhita*. with Nibandhasangraha of Dalhana. Sutrasthana 36.verse 5. 9th edition. Chaukhambha orientalia, Varanasi, 2007; 159

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