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The Analytical Study of *Kaanda Twak* of *Vata* (*Ficus Benghalensis* Linn.) In *Shadrutu* Vis - À -Vis *Dravyasangraha Kala*

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ABSTRACT

Background: *Ayurveda* an ancient science, addresses all aspects of life by emphasizing both disease treatment and illness prevention through medicinal plants. Each plant part contains active principles at peak concentrations during specific seasons, with classical texts like *Charaka Samhita, Sushruta Samhita* and *Rajanigantu* providing guidelines for their collection. In the 21st century, modern techniques allow us to access the potency of crude drugs, making it essential to collect plant materials during the season that ensures optimal potency. **Aim and objectives:** Total pharmacognostic, phytochemical study of *Vata* (*Ficus benghalensis* Linn.) bark in each *Rutu* and compare the values in different *Rutu.* **Materials and methods:** An analytical study of bark of *Vata* (*Ficus benghalensis* Linn.) collected in six different *Rutus* was carried out. The bark was collected in six different *Rutus* and were subjected to pharmacognostic and physicochemical analysis. The aqueous and alcoholic extracts were prepared and the individual extracts were subjected to quantitative and qualitative analysis. The results were compared in between the samples and with the standard. **Results:** The present analytical study of both *Vata* (*Ficus benghalensis* Linn.) carried on 6 different *Rutus* revealed that the number of phyto-constituents and extractive values varies significantly across the 6 different *Rutus* and maximum during *Shishira Rutu.* **Conclusion:** The results suggest that there is significant difference in quantity of phytoconstituents of *Vata* bark in each *Rutu.*

Key words: *Vata, Banyan tree, Indian banyan, Sangraha Kaala, Traditional medicine, Ayurveda*

INTRODUCTION

Ayurveda emphasizes the entire process of drug utility, including collection, preservation, purification, and formulation. To achieve the desired medicinal effects, it is essential to gather plants according to Ayurvedic principles, focusing on their optimum properties. Experts indicate the importance of *Desha, Kala, Rutu* and suitable *Bhoomi* for collecting specific plant parts.[1] The timing of collection is crucial for maximizing drug potency, ensuring formulations meet quality standards. Each Ayurvedic texts outlines when to collect various plant parts such as roots, stems, bark, leaves, fruits, flowers. In Charaka Samhita, for *Kaanda Twak*, *Sharad Rutu* is the proper time of collection.[2]

This research examines the significance of the collection timing of *Kaanda Twak* of *Vata* (*Ficus benghalensis* Linn.) across all six seasons to establish its superior potency. Due to high demand for raw herbal drugs, collection often ignore optimal timings, negatively impacting product quality and leading to inconsistent effects in patients. This study aims across this issue through experimental analysis of *Kaanda Twak* of *Vata* (*Ficus benghalensis* Linn.). *Vata* having

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Kashaya Rasa, Guru Guna, Sheeta Virya, Kapha Pitta Nashaka, helpful in *Visarpa, Daha* and *Yoni Dosha*.[3]

Materials and Methods

Source of data

The study was conducted in the Research and Development department of Muniyal Institute of Ayurveda Medical Sciences, Manipal, Karnataka, India.

Method of collection of data

Twak of *Vata* (*Ficus benghalensis* Linn.) was collected from the garden. Bark was collected in 6 different *Rutus. Rutus* were followed according to Hindu calendar (*Panchanga*). The bark is collected from same tree throughout the study. The time of collection of bark as per Acharya Charaka is followed i.e., *Sharad Rutu.* The time of collection of bark of *Vata* (*Ficus benghalensis* Linn.) is according to table. [Table 1]

Table 1: Showing collection time of Bark of *Vata* (*Ficus benghalensis* Linn.)

SN	*Rutu*	Collection of Bark
1.	*Greeshma Rutu*	June 19
2.	*Varsha Rutu*	August 30
3.	*Sharad Rutu*	November 13
4.	*Hemantha Rutu*	January 11
5.	*Shishira Rutu*	March 9
6.	*Vasata Rutu*	May 8

Preparation of the trial drug

Bark of *Vata* (*Ficus benghalensis* Linn.) washed thoroughly with water to remove physical impurities like mud. It is then dried under sun until they were completely dry. After drying, 30gm of the drug was kept apart for macroscopic studies. Then the drug made into a coarse powder and kept preserved in air tight container for phytochemical and physicochemical analysis.

Pharmacognistical evaluation of the trial drug

Organoleptic study

Organoleptic characters of the stem bark of *Vata* (*Ficus benghalensis* Linn.) like colour, odour, taste and texture is observed and noted.

Macroscopic evaluation

The macroscopic features were compared to local flora for authentication. The plant parts were observed for macroscopic features by placing on a white paper surface. The external features of the test sample were documented using Canon IXUS digital camera.

Fig. 1: Collection of Bark

Microscopic examination

Sample was preserved in fixative solution. The fixative used was FAA (Formalin-5ml + Acetic acid-5ml + 70% Ethyl alcohol-90ml). The materials were left in FAA for more than 48 hours. The preserved specimens were cut into thin transverse section using a sharp blade and the sections were stained with saffranine. The slides were also stained with iodine in potassium iodide for detection of starch. Transverse sections were photographed using Zeiss AXIO trinocular microscope attached with Zeiss AxioCam camera under bright field light. Magnifications of the figures are indicated by the scale-bars.

Powder microscopy

Pinch of bark powder previously sieved was put on the slide and mounted in glycerine and powder characters were observed under the Zeiss AXIO trinocular microscope attached with Zeiss Axio Cam camera under bright field light.

Phytochemical Study[4]

Loss on drying

Weigh approximately 5 gm of the powdered sample and place it in a tared petridish. Dry the sample at 105°C for 5 hours in a hot air oven. Remove the

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petridish, cool it in a desiccator, and weigh it. Repeat drying for 30 minutes, cool and weigh again. Continue this process until two consecutive weights are constant. Calculate the moisture content percentage using the appropriate formula.

% of loss on drying = (W1+W2) – W3/W2 X 100

Where, W1 – Weight of petridish, W2 – Weight of the sample, W3 – Weight of dried sample with dish.

pH value

Prior to measuring pH, electrodes of pH meter were soaked in distilled water for 24 hours. Buffer solution of pH 4, 7 and 9 were prepared by dissolving buffer tablet in 100 ml of distilled water. The pH meter was calibrated using these buffer solutions at 25^0C. One gram of each coarsely powdered drug was added to 100 ml of distilled water, stirred for 5 minutes, and then filtered separately. After calibration, the electrodes were washed with distilled water and wiped, then immersed in the test drug solution to record the pH. This washing and measuring process was repeated until the difference between consecutive readings was less than 0.5.

Determination of total ash value

The clean and dry silica crucible was weighed in which 2 gms of each drugs were taken. Keep the crucible in a muffle furnace at a temperature not exceeding 600°C until free from carbon. The crucible containing ash was cooled in a desiccator and then weighed. The content of total ash in mg per gm of air - dried material was calculated. Percentage of ash content with reference to drugs were calculated using the formula.

Total Ash = [(W3-W2)/W1]x100

Where W1 – Wt. of drug, W2 – Wt. of empty dish, W3 – Wt. of ash with dish

Determination of acid insoluble ash

Transfer the ash into a beaker and add 25 ml of dilute hydrochloric acid. Wash the crucible with acid and add the washings to the beaker. Heat the mixture until it boils, then filter through ash less filter paper to separate the insoluble matter. Place the residue and filter paper in a crucible and ignite in a muffle furnace at a temperature not exceeding 450°C until it turns white ash. Cool the crucible in a desiccator and weigh it. Continue heating until a constant weight is achieved, then calculate the percentage of acid insoluble ash using the appropriate formula.

Insoluble ash = [(W3-W2)/W1] x100

Where, W1 – Wt. of the drug powder, W2 – Wt. of crucible, W3 – Wt. of ash with crucible.

Determination of water-soluble extractive value

Accurately weighed 10gm of coarsely powdered drug was placed in a glass Stoppard conical flask. Flask was filled with 100ml distilled water and macerate. The lid was closed for 24 hr. Frequently shaking was done during first 6 hours and allowed to stand for 18 more hours without shaking. After 24 hours, it was filtered to remove insoluble matter. 25 ml of the filtrate was taken in a weighed silica dish and kept on water bath for evaporation. It is taken out and cooled in a desiccator and weighed. Percentage of water-soluble extractive value was calculated.

Water soluble extract = [(W2-W1) x 100/W x 25] x 100

Where, W – Wt. of drug powder, W1 – Wt. of crucible, W2 – constant weight

Determination of the alcohol soluble extractive value

Accurately weighed 10gm of coarsely powdered drug was placed in a glass Stoppard conical flask. Flask was filled with 100ml alcohol and macerate. The lid was closed for 24 hours. Frequently shaking was done during first 6 hours and allowed to stand for 18 more hours without shaking. After 24 hours, it was filtered to remove insoluble matter. 25 ml of the filtrate was taken in a weighed silica dish and kept on water bath for evaporation. It is taken out and cooled in a desiccator and weighed. Percentage of alcohol soluble extractive value was calculated.

Water soluble extractive = [(W2 – W1) x 100/ W x 25] x 100

Where, W – wt. of drug powder, W1 – wt. of crucible, W2 – Constant weight

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Determination of Organic Chemical Constituents

Tests for detection of alkaloid

Mayer's test

Mayer's reagent was used for this test. The test filtrate was treated with few drops of this reagent. Formation of cream colour precipitates indicate the presence of alkaloids.

Tests for detection of flavanoids

Alkaline reagent test

To the test filtrate, add few drops of dilute Sodium hydroxide solution. Appearance of Dark yellow colour which turns colourless on addition of few drops of dil. Hydrochloric acid indicates the presence of flavonoids.

Tests for detection of tannins and phenolic compounds

Ferric chloride reagent

A 5 percent w/v solution of ferric chloride in 90 percent alcohol was prepared. Few drops of this solution were added to a little of the above filtrate. Appearance of dark green or dark blue indicate the presence of tannins.

Tests for detection of glycosides

Kellar-killani test

The solution was dissolved in 1 ml of glacial acetic acid containing traces of ferric chloride and transferred to 1 ml of concentrated sulphuric acid, to get the reddish-brown colour at junction indicate the presence of glycosides.

Tests for detection of steroids

Salkowaski test

To the extract, 2 ml of chloroform was mixed with few drops of conc. H_2SO_4 and shaked well and allowed to stand for some time, red colour appears at the lower end indicate the presence of steroid.

Tests for detection of carbohydrates

Fehling's test

To the test filtrate, 1 ml of Fehling's A and 1 ml of Fehling's B solutions were added and boiled in water bath for 5-10 minutes. First a yellow, then a brick red precipitate if obtained, indicates the presence of carbohydrates.

Tests for detection of proteins

Biuret test

To the test filtrate, 1 ml of 4% sodium hydroxide solution was added to it, followed by a drop of 1% solution of copper sulphate is added. Appearance of violet or pink color indicate the presence of proteins.

Tests for detection of saponins

Foam test

To the small quantity of filtrate, add few ml of distilled water and shake well. Appearance of froth indicate the presence of saponins.

Determination Of Total Phenolic Content[5]

Reference: Singleton & Rossi 1965; Mahdavi *et al*., 2010 and Wern K. H., *et al*., 2016.

Procedure

Take different concentrations of 0.4 ml of samples, standard (Gallic acid) and blank sample separately. Add 3.6 ml of distilled water to all the samples. Add 0.4 ml of Folin-Ciocalteu reagent to all the samples. Add 4 ml of 7 % sodium carbonate to all the samples. All the samples are made up to the volume 10 ml with distilled water, followed by thorough mixing and allowed to incubation for 90 minutes at room temperature. The absorbance was measured at 765 nm. Calibration curve will be plotted using Gallic acid standard. The result will be expressed as Gallic acid equivalent (mg GAE / 100ml)

RESULT

Macroscopic study of *Vata*

Fresh bark is typically smooth and light gray to greenish in colour. The bark is rough with fissures and relatively thick. Fresh cut surface exudates plenty of latex. Dried Outer surface of bark is brown in colour while the inner surface is cream in colour with lot of fibres. [Fig. 2-3]

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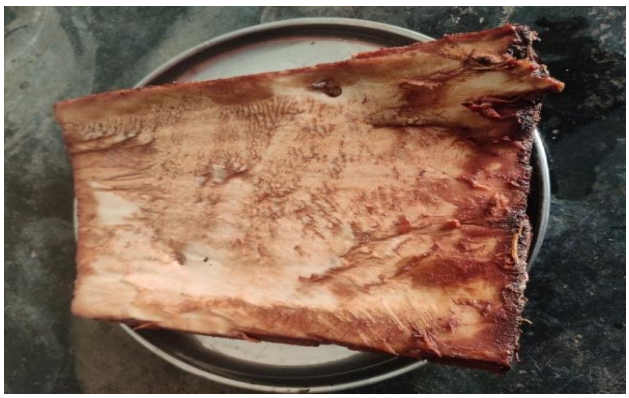

Fig. 2: Fresh bark of *Vata*

Fig. 3: Dried bark of *Vata*

Microscopic study of *Vata*

Macroscopic study of bark of *Ficus benghalensis* Linn. Shows the following parts – cork, lactiferous tubes, stone cells, sclereids, phloem, prismatic crystals of calcium oxalate. [Fig 4-8]

Fig. 4: Stem bark

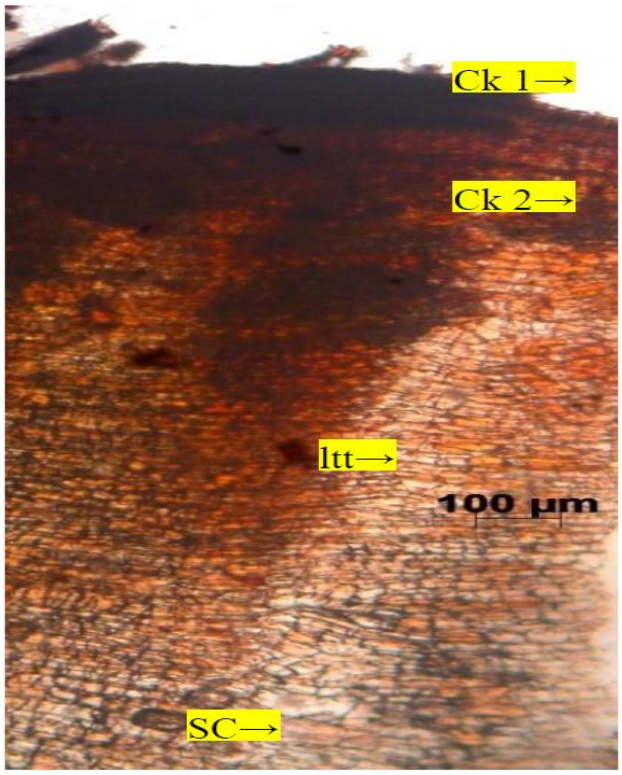

Fig. 5: Cork 1 and 2

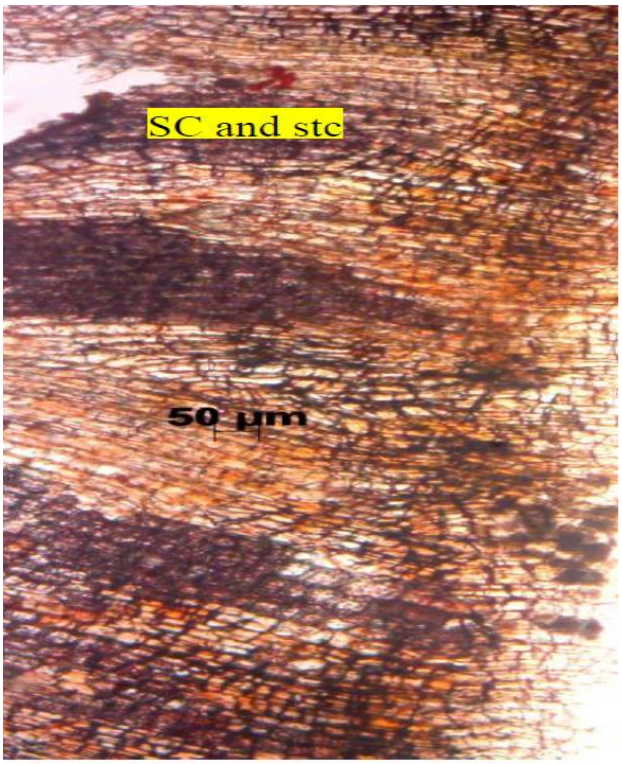

Fig. 6: Stone cells and starch

Ck - cork; **ltt -** laticiferous tubes; **SC -** stone cell; **Scl -** sclereids; **SC and stc -** stone cell and starch

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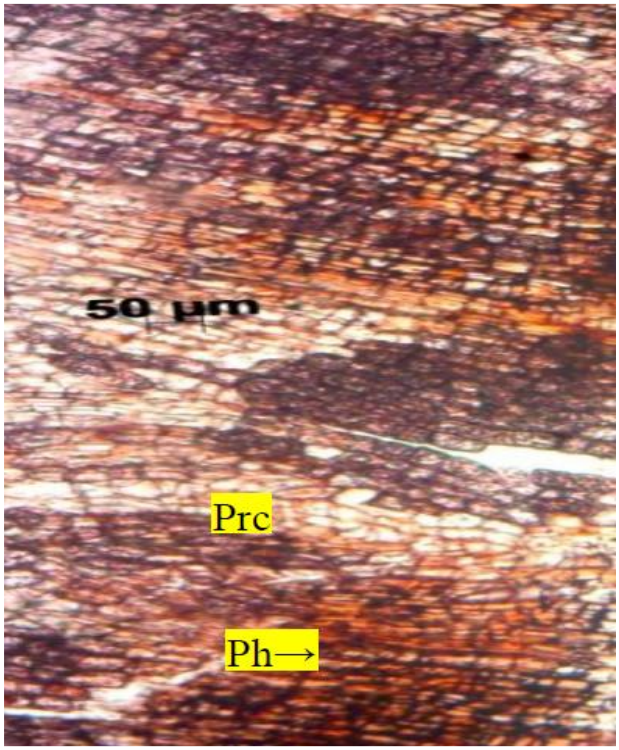

Fig. 7: Phloem region

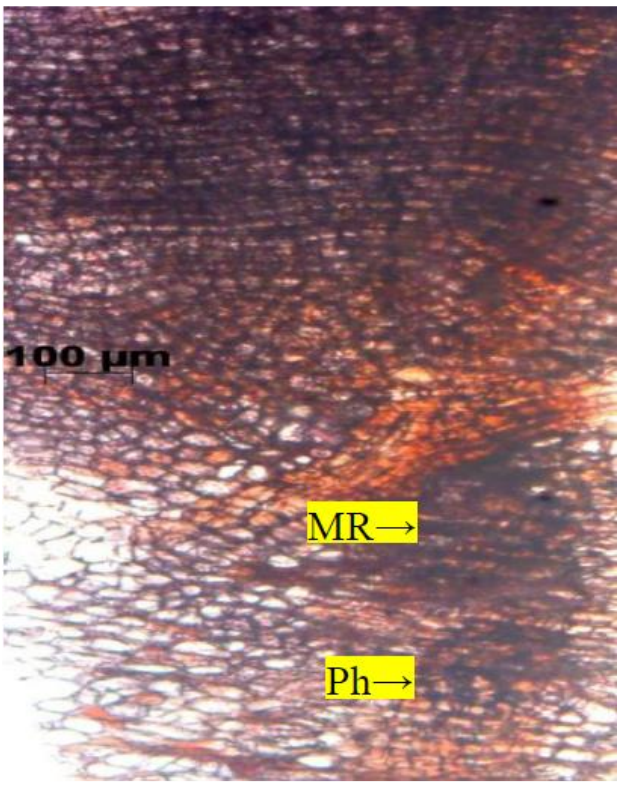

Fig. 8: Medullary rays

Prc - Prismtic crystals of calcium oxalate; **Ph -** phloem, **MR -** medullary ray

Powder microscopy of *Vata*

Powder microscopy of bark of *Ficus benghalensis* Linn. shows – stone cells, phloem with sieve tube and companion cells, sclereids, prismatic crystals. [Fig 9-15]

Fig. 9: Powder of *Vata*

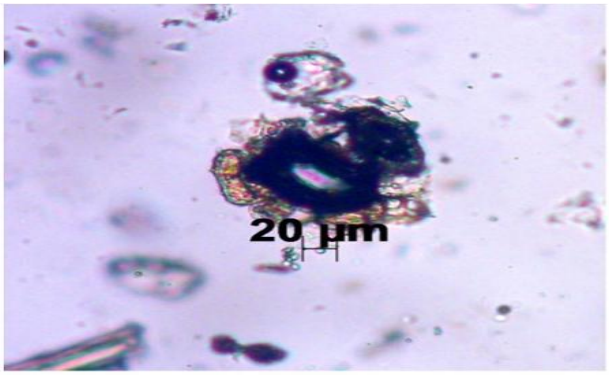

Fig. 10: Stone cells

Fig. 11: Phloem with sieve tube and Companion cells

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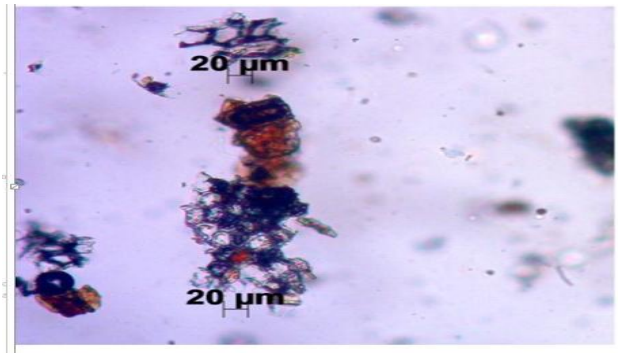

Fig. 12: Rhytidoma in surface view

Fig. 13: Sclereids

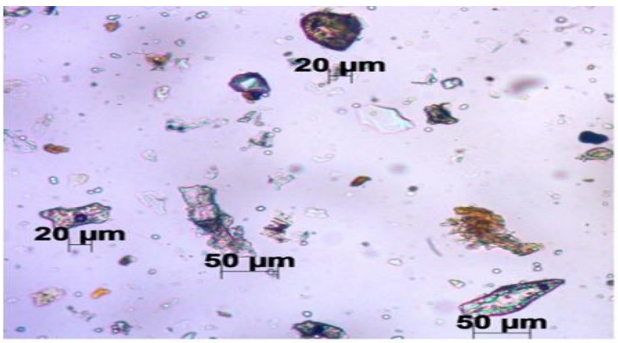

Fig. 14: Sclereids, stone cells, prismatic crystal

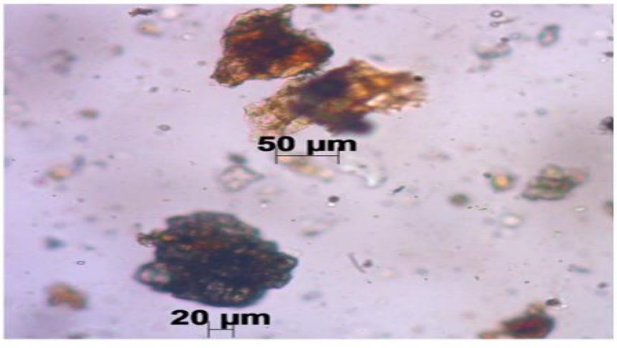

Fig. 15: Stone cells

Result of Physico-Chemical Parameters

Values of loss on drying, total ash, acid insoluble ash and extractive value of bark of *Vata* (*Ficus benghalensis* Linn.) showed varied values in different *Rutu*. [Table 2]

Table 2: Showing physico-chemical parameter of *Vata* (*Ficus benghalensis* Linn.)

	Greeshma	*Varsha*	*Sharad*	*Hemantha*	*Shishira*	*Vasantha*
Loss on drying	13.74	9	10.33	11.03	13.74	13.98
pH	6.52	6.42	5.93	6.47	6.34	6.39
Total ash	11.98	6.45	6.53	6.06	6.09	4.89
Acid insoluble ash	1.49	1.78	1.83	0. 99	0.99	1.19
Water soluble extractive	13.58	15.76	8.06	8.04	13.59	11.67
Alcohol soluble extractive	10.61	17.34	7.91	6.31	13.58	15.73

Results of Preliminary Phytochemical Analysis

Phytochemical analysis of *Vata* (*Ficus benghalensis* Linn.) shows the presence of alkaloids, tannin, glycosides, carbohydrates, proteins, steroids, saponin. [Table 3]

Table 3: Showing tests for phytochemical analysis of *Vata* (*Ficus benghalensis* Linn.)

	Greeshma		*Varsha*		*Sharad*		*Hemanta*		*Shishira*		*Vasanta*	
	Aq	Al	Aq	Al	Aq	Al	Aq	Al	Aq	Al	Aq	Al
Flavanoids	-	-	-	-	-	-	-	-	-	-	-	-

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Alkaloids	+	+	+	+	+	+	+	+	+	+	+	+
Tannin	+	+	+	+	+	+	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+	+	+	+	+	+	+
Steroids	+	+	+	+	+	+	+	+	+	+	+	+
Carbohydrate	+	+	+	+	+	+	+	+	+	+	+	+
Protein	+	+	+	+	+	+	+	+	+	+	+	+
Saponin	+	-	+	-	+	-	+	-	+	-	+	-

Total Phenolic Content Estimation

Estimation of Total Phenolic Content (TPC) shows highest concentration of *Vata* (*Ficus benghalensis* Linn.) in *Shishira Rutu* and lowest concentration in *Greeshma Rutu*. [Table 4]

Table 4: Showing total phenolic content estimation of *Vata* (*Ficus benghalensis* Linn.)

	***Vata* 1**	***Vata* 2**	***Vata* 3**	***Vata* 4**	***Vata* 5**	***Vata* 6**
Conc.	***Greeshma***	***Varsha***	***Sharad***	***Hemantha***	***Shishira***	***Vasantha***
1	2.53	2.44	3.26	5.91	9.71	7.38
2	3.76	4.24	5.79	6.74	11.12	7.71
4	4.94	7.62	7.85	9.35	12.82	9.44
8	7.88	9.47	8.82	10.94	14.03	10.29
10	8.88	12.91	11.03	15.62	15.65	13.35
20	10.09	14.18	13.79	16.29	25.35	14.62
40	12.06	16.94	14.88	26.24	41.74	16.15
80	14.09	20.09	19.59	32.47	63.50	28.12
100	19.24	25.91	21.68	43.88	77.44	29.38
200	41.59	52.38	41.68	90.79	141.32	58.88
400	95.71	112.26	78.68	165.88	245.56	96.09
800	154.82	175.91	131.21	301.41	403.94	177.97
1000	207.15	222.21	153.85	346.24	474.15	194.35

Discussion

Phytochemical study

Loss on drying

This is the test designed to determine the moisture content and number of volatile substances in the sample. If the moisture content of the sample was within the acceptable range, suggesting that the sample can be stored for a longer period and is less likely to be affected by microbes. Moisture content of *Vata* (*Ficus benghalensis* Linn.) was highest in *Vasantha Rutu* and lowest in *Varsha Rutu*. These values fluctuate based on local conditions and harvesting methods. Highest value may be because of seasonal humidity levels.

pH

Bark samples of *Vata* (*Ficus benghalensis* Linn.) of six *Rutu* having acidic pH i.e., < 7. This variation in pH may indicate the presence of certain organic acids which may influence the chemical properties of the bark or seasonal changes like temperature, humidity and rainfall which can affect the metabolic processes of the trees, leading to variations in pH.

Ash value

The ash value of crude drugs is a measure of the inorganic residue remaining after the drug is incinerated. It indicates the total amount of minerals or impurities present in the drug.

All the values of *Vata* (*Ficus benghalensis* Linn.) are within the normal range i.e., not more than 8% but in *Greeshma Rutu* it exceeds normal limit i.e., 11.98. It may be due to several factors. During this season, environmental stressors such as high temperatures and reduced moisture can influence the plants metabolic processes. This can lead to increased mineral accumulation in plant tissues, resulting in a higher ash content. If the plant absorbs more minerals to cope with the heat, this can further raise the total ash content.

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For acid insoluble ash value of *Vata* (*Ficus benghalensis* Linn.), the observations found were within the normal limit i.e., not more than 3%.

Extractive value

Extractive values of crude drugs refer to the amounts of soluble components that can be extracted from a drug using various solvents. These values help in assess the quality and concentration of active constituents among the different drugs. Water and alcohol soluble extractive values of *Vata* (*Ficus benghalensis* Linn.) are within the normal range.

Total phenolic content estimation

Total phenols content of *Vata* (*Ficus benghalensis* Linn.) is highest during *Shishira Rutu* and lowest in *Greeshma Rutu.* Total phenolic content is highest in certain seasons due to environmental factors such as temperature, humidity, and light conditions that influence phenolic synthesis. During these periods, plants might produce more phenolics as a defence mechanism against stress, pests, or UV radiation. Additionally, phenolic compounds often serve essential role in growth and development, which can vary throughout the year, leading to fluctuations in their concentrations.

Another interpretation could be that the physiological state of the plant during different *Rutus* affects phenolic content. In seasons like *Shishira*, plants may be in a phase of active growth or recovery from stress, leading to increased phenolic synthesis for protection and metabolic functions. Conversely, in *Greeshma Rutu*, the plant may prioritize survival over secondary metabolite production due to heat and drought, resulting in lower phenolic levels. Additionally, the timing of bark collection may coincide with periods of phenolic degradation or lower metabolic activity, further contributing to the observed variations in total phenolic content.

Acharya Charaka has told collection of bark in *Sharad Rutu*[6] but the current study interprets the total phenols is more in *Shishira Rutu* for *Vata* (*Ficus benghalensis* Linn.). But there are differences in phenolic estimation in each *Rutu*. The current study has interpreted that collection time of bark in case of *Vata* (*Ficus benghalensis* Linn.) more suits according to Sushruta as he told *Sowmya Dravya* is to be collected in *Sowmya Rutu*.[7] As the *Dravya* have *Kashaya Rasa* and *Sheeta Virya* which is suggestive of *Sowmya Dravya*.

CONCLUSION

All samples were subjected to thorough pharmacognostic, phytochemical and quantitative studies. The results were analysed and compared and following conclusions were drawn. Physiochemical and phytochemical study of *Vata* (*Ficus benghalensis* Linn.) were carried out and some of the results are as per standards. In physiochemical analysis, total ash value of *Vata* (*Ficus benghalensis* Linn.) is more than normal limit in *Greeshma Rutu* but values are within normal limit in *shard Rutu* and that of acid insoluble ash, all values are within normal limit. In water and alcohol soluble extractive, values in each *Rutu* showed normal limit even in *Sharad Rutu*. The phytochemical analysis revealed the presence of alkaloids, tannins, glycosides, steroids, carbohydrates, proteins, saponin. Total phenolic content estimation on aqueous extract of *Vata* (*Ficus benghalensis* Linn.) was done which quantifies the gallic acid which shows the higher results in *Shishira Rutu*. There is difference in total phenolic content in each *Rutu* but significance of collection of bark at *Sharad Rutu* according to Acharya Charaka is not met. However, Sushruta claim of *Soumya Dravya* in *Sowmya Rutu* has fulfilled satisfactorily.

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