

## A PHYSICOCHEMICAL STANDARDIZATION OF GUDUCHI GHANA VATI W.S.R. TO TIME OF COLLECTION OF GUDUCHI

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### ABSTRACT

**Objective:** To collect *Guduchi* (*Tinospora cordifolia* (Thunb.) Miers) in three rutus i.e. *Grishma Rutu* (Summer), *Varsha Rutu* (Monsoon) and *Sharad Rutu* (Autumn) to prepare *Guduchi Ghana Vati* as per SOP in three batches each, respectively, to establish effect of *Sangrahana Kala* (time of collection) on finished product by analysing and comparing the different batches. **Method:** *Guduchi* was collected in three different seasons, i.e., *Grishma Rutu*, *Varsha Rutu* and *Sharad Rutu*. *Guduchi Ghana Vati* was prepared by classical method as per *Siddha Yoga Sangraha*. This is subjected to analysis. **Results:** IR spectra showed presence of aldehydes, ketones, carbonyl group, OH group and cyclic ring stretching. The quantitative yield was 3.15% in *Sharad rutu*, 2.97% in *Varsha rutu* and 2.09% in *Grishma rutu*. The quantitative yield of starch was 30.20% in *Sharad rutu*, 18.86% in *Varsha Rutu* and 14.27% in *Grishma rutu* and alkaloid was 9.09% in *Grishma rutu*, 4.25% in *Varsha Rutu* and 1.643% in *Sharad rutu*. **Conclusion:** The yield and starch content was maximum in *Sharad Rutu* whereas alkaloid content was least. Yield and starch content was least in *Grishma Rutu* whereas alkaloid was maximum. IR spectroscopy indicated presence of ketones, aldehydes, OH and carbonyl functional groups.

**Keywords:** *Sangrahana kaala*, *Tinospora cordifolia*, *Samshamani Vati*, *Grishma Rutu*, *Varsha rutu*, *Sharad Rutu*

### INTRODUCTION

*Aushadha Sangrahana Kala* (time of collection of drug) is one of the fundamental principles of *Bhaishajya Kalpana*. *Sangrahana kala* or time of collection of drugs is a very important point to consider as the physiology of plants differs in various seasons, thus, changing the chemical constituents and

properties of the plants<sup>[1]</sup>. To establish whether the seasons and time of collection of drug can affect the formulation, in the present study, *Guduchi* (*Tinospora cordifolia* (Thunb) Miers) is drug of choice, the single drug formulation *Guduchi Ghana vati* was selected for the study as *Guduchi* is a perennial twiner<sup>[2]</sup>, and its

collection time especially with reference to this formulation can be a controversy.

The aim and objectives are to collect *Guduchi* (*Tinospora cordifolia* (Thunb.) Miers) in three different season, *Grishma* (Summer), *Varsha* (Monsoon) and *Sharad* (Autumn), to prepare *Guduchi Ghana Vati* as per Standard Operating Procedures and to establish effect of *Sangrahana Kala* on finished product by analysing and comparing the samples as per Standard Operating Procedures.

**MATERIAL AND METHOD:** The study was carried out in two phases, pharmaceutical and analytical.

**Pharmaceutical study:**

**1. Grishma Rutu (Mid April- Mid May)**

- Batch 1: GGV(GR)1 where it stands for *Guduchi Ghana Vati (Grishma Rutu)* Batch 1 Similarly,
- Batch 2: GGV(GR)2
- Batch 3: GGV(GR)3

**2. Varsha Rutu (Mid July- Mid August)**

- Batch 1: GGV(VA)1 where it stands for *Guduchi Ghana Vati (Varsha Rutu)* Batch 1 Similarly,
- Batch 2: GGV(VA)2
- Batch 3: GGV(VA)3

**3. Sharad Rutu (October)**

- Batch 1: GGV(SH)1 where it stands for *Guduchi Ghana Vati (Sharad Rutu)* Batch 1 Similarly,
- Batch 2: GGV(SH)2
- Batch 3: GGV(SH)3

Each of these batches followed the following steps:

- Procurement of Raw materials
- Preparation of *Guduchi Ghana Vati*

**Procurement of Raw Materials:** Fresh, mature stem of those *Guduchi* (*Tinospora cordifolia* (Thunb.) Miers) were collected which had Mango (*Mangifera indica* L.) as host and were growing in Maniplal region of Udupi, Karnataka.

**Preparation of *Guduchi Ghana Vati*:** The method of preparation was according to the reference given in *Siddha Yoga Sangraha*<sup>[3]</sup>. The fresh, mature stem of *Guduchi* was collected and crushed. *Kwatha* of the crushed stem was prepared by adding 4 times water and reducing to one fourth the quantity on mild fire with temperature not exceeding 100 degrees Celsius. The *kwatha* was filtered The collected liquid was further heated using a water bath at temperature between 60-80 degrees Celsius until it was reduced to a thick consistency or *Ghana*. The *Ghana* was dried at less than 50 degrees Celsius until it could be rolled into pills. It was then rolled into pills. Stainless steel equipment was used throughout the procedure. The yield was calculated in terms of percentage and average percentage of yield per season was also calculated.

**Analytical Study:** Organoleptic analysis, physicochemical analysis<sup>[4]</sup>, qualitative analysis for phytoconstituents<sup>[5]</sup>, quantitative estimation of starch<sup>[6]</sup> and alkaloid<sup>[7]</sup>, Thin Layer Chromatography<sup>[8]</sup> and Infrared Spectroscopy<sup>[9]</sup> were carried out.

**RESULTS:**

Results of Pharmaceutical Study

**TABLE 1:** Comparative yield of *Guduchi Ghana*

SAMPLE	Qty of Drug (in Kg)	Qty of Water (in L)	Qty of Kwatha (in L)	Qty of Ghana Obt (in g)	% Of Ghana Obt	Avg. % Per Rutu
GGV (GR)1	1.5	6	1.6	25	1.65	2.09
GGV(GR)2	1.75	7	1.8	40	2.22	
GGV(GR)3	1.3	5.2	1.25	30	2.4	
GGV (VA)1	2.8	11.2	2.9	91	3.15	2.97
GGV(VA)2	2.9	11.6	2.9	85	2.93	
GGV (VA)3	2.3	9.2	2.4	65	2.83	
GGV (SH)1	2.7	10.8	2.8	96	3.5	3.15

GGV (SH)2	2.7	10.8	2.8	91	3.37
GGV (SH)3	2.7	10.8	2.9	70	2.59

Avg= Average, Obt= Obtained, Qty= Quantity

### Results of Analytical Study

**Table 2:** Organoleptic Parameters of *Guduchi Ghana Vati*

	GGV (GR)1	GGV (GR)2	GGV (GR)3	GGV (VA)1	GGV (VA)2	GGV (VA)3	GGV (SH)1	GGV (SH)2	GGV (SH)3
Colour	Black	Black	Black	Black	Black	Black	Black	Black	Black
Odour	Sweet	Sweet	Sweet	Sweet	Sweet	Sweet	Sweet	Sweet	Sweet
Taste	Bitter	Bitter	Bitter	Bitter	Bitter	Bitter	Bitter	Bitter	Bitter
Appearance	Sticky mass	Sticky mass	Sticky mass	Sticky mass	Sticky mass	Sticky mass	Sticky mass	Sticky mass	Sticky mass

**Table 3:** Physicochemical Parameters of *Guduchi Ghana Vati*

	GGV(GR)			GGV(VA)			GGV(SH)		
	1	2	3	1	2	3	1	2	3
L.o.D at 110°C	8.5% w/w	8.32% w/w	8.4% w/w	8.92%w/w	8.8%w/w	9% w/w	8.6% w/w	8.62% w/w	8.5% w/w
Avg L.o.D (each season)	8.41% w/w			8.91% w/w			8.57% w/w		
Total Ash Value	17.5% w/w	16.65% w/w	16.8% w/w	14.6% w/w	14.2% w/w	14.42% w/w	16% w/w	16.2% w/w	15.9% w/w
Avg Total Ash Value	16.98% w/w			14.41% w/w			16.04% w/w		
Acid Insoluble Ash	2.15% w/w	2.1% w/w	2.1% w/w	2.05% w/w	2.08% w/w	2% w/w	2.08% w/w	2.06% w/w	2.05%w/w
Avg Acid Insoluble Ash	2.12% w/w			2.04% w/w			2.06% w/w		
Water Soluble Extractive	52.48%	53.6%	52.8%	62.96%	62.74%	62.98%	45.44%	45.64%	45.3%
Avg W.S.E	52.96%			62.8%			45.46%		
Methanol Soluble Extractive	4.16%	4.11%	4.2%	3.92%	3.9%	3.92%	2.72%	2.77%	2.68%
Avg M.S.E	4.16%			3.913%			2.723%		
pH	5.58	5.8	5.8	5.6	5.58	5.6	5.6	5.6	5.59
Avg pH	5.73			5.59			5.597		
Avg. Weight	246 mg	250 mg	253 mg	248 mg	250.4 mg	248.8 8 mg	249.6mg	251.2 mg	250.6 mg
Avg. Weight (each season)	249.67 mg			249.209 mg			250.47 mg		
Time for disintegration	24min	22 min	22 min	20 min	20 min	20 min	25 min	30 min	25 min
Avg Time for Disintegration (each season)	22.67 min			20 min			26.67 min		

Avg= Average, L.o.D= Loss on Drying, M.S.E= methanol soluble extractive, W.S.E= water soluble extractive, w/w= weight/weight

**Table 4:** Qualitative assessment of *Guduchi Ghana Vati* for Phytoconstituents

TEST	GGV (GR)1		GGV (GR)2		GGV (GR)3		GGV (VA)1		GGV (VA)2		GGV (VA)3		GGV (SH)1		GGV (SH)2		GGV (SH)3	
	W	M	W	M	W	M	W	M	W	M	W	M	W	M	W	M	W	M
ALKALOID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SUGARS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GLYCOSIDES	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PHENOLIC COMPOUNDS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
FLAVONOIDS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AMINO ACIDS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PROTEINS	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
SAPONINS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
STEROLS	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+
TANNINS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

M= methanol soluble extract, W= water soluble extract

**Table 5:** Percentage quantity of Starch and Alkaloid content in *Guduchi Ghana Vati*

	GGV(GR)			GGV(VA)			GGV (SH)		
	1	2	3	1	2	3	1	2	3
Starch Content in %	14.62	14.6	13.59	20.92	19.16	16.50	26.45	28.39	35.77
Avg. Starch Content in %	14.27			18.86			30.20		
Alkaloid Content in %	9.12	9.04	9.04	4.24	4.26	4.25	1.66	1.63	1.64
Avg Alkaloid Content in %	9.09			4.25			1.643		

Avg= Average

**Table 6:** TLC of *Guduchi (Tinospora cordifolia (Thunb.) Miers)*

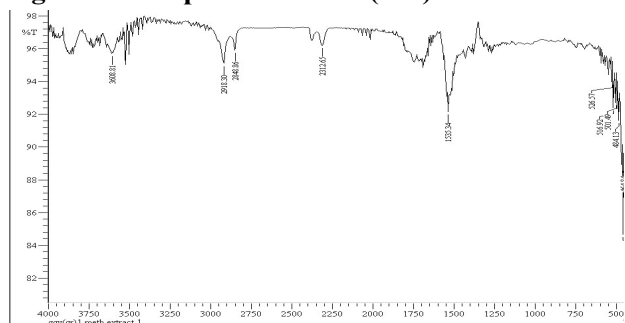
SAMPLE	NO. OF SPOTS	R <sub>f</sub> VALUE	COLOUR
GGR	3	0.29	Light Green
		0.41	Very Light green
		0.84	Dark yellow-green
GVR	3	0.29	Light Green
		0.41	Very Light green
		0.84	Dark yellow-green
GSR	3	0.29	Light Green
		0.41	Very Light green
		0.84	Dark yellow-green

GGR= *Guduchi Grishma Rutu*, GSR= *Guduchi Sharad Rutu*, GVR= *Guduchi Varsha Rutu*

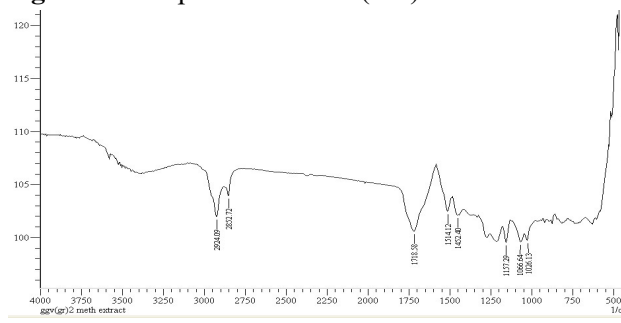
**Table 7:** TLC of *Guduchi Ghana Vati*

SAMPLE	NO. OF SPOTS	R <sub>f</sub> VALUE	COLOUR
GGV (GR)1	3	0.284	Light brown
		0.43	Cream brown
		0.8375	Dark yellow
GGV(GR)2	3	0.282	Light brown
		0.42	Cream brown
		0.8375	Dark yellow
GGV(GR)3	3	0.284	Light brown
		0.43	Cream brown
		0.8375	Dark yellow
GGV(VA)1	3	0.292	Light brown
		0.42	Cream brown
		0.8375	Dark yellow
GGV(VA)2	3	0.292	Light brown
		0.42	Cream brown
		0.8375	Dark yellow
GGV(VA)3	3	0.292	Light brown
		0.42	Cream brown
		0.8375	Dark yellow
GGV(SH)1	3	0.282	Light brown
		0.44	Cream brown
		0.8375	Dark yellow
GGV(SH)2	3	0.282	Light brown
		0.44	Cream brown
		0.8375	Dark yellow
GGV(SH)3	3	0.282	Light brown
		0.44	Cream brown
		0.8375	Dark yellow

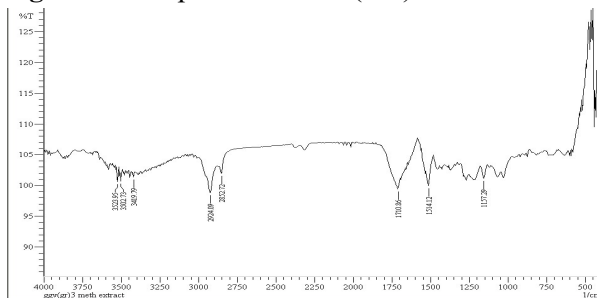
**Figure 1:** IR Spectra of GGV(GR)1



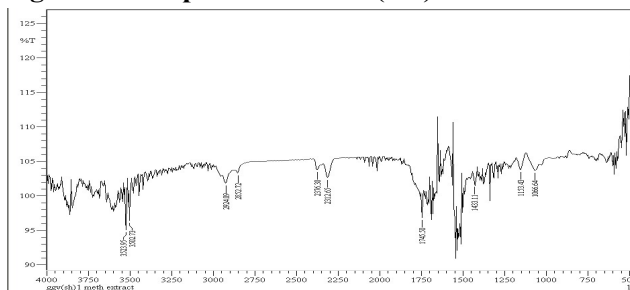
**Figure 2:** IR Spectra of GGV (GR)2



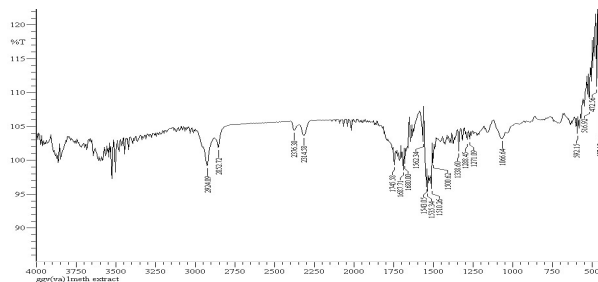
**Figure 3: IR Spectra of GGV(GR)3**



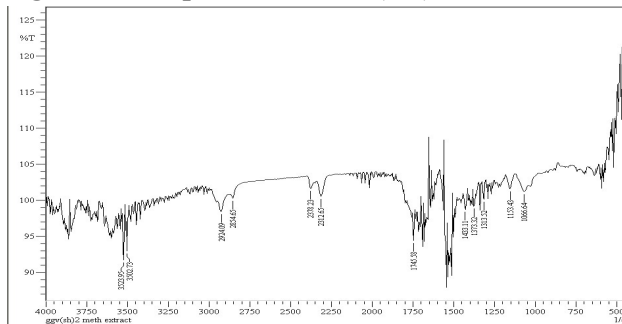
**Figure 7: IR Spectra of GGV (SH)1**



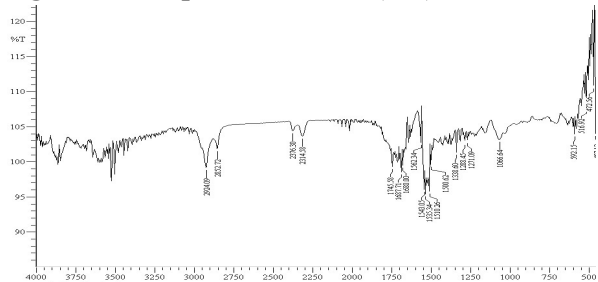
**Figure 4: IR Spectra of GGV (VA)1**



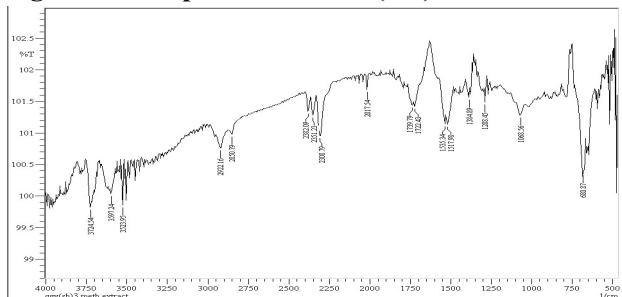
**Figure 8: IR Spectra of GGV (SH)2**



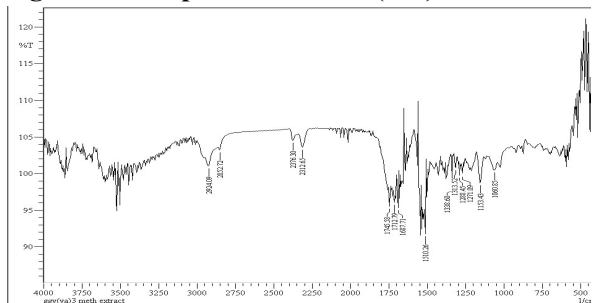
**Figure 5: IR Spectra of GGV (VA)2**



**Figure 9: IR Spectra of GGV (SH)3**



**Figure 6: IR Spectra of GGV (VA)3**



**Table 8:** IR chart comparing raw *Guduchi (Tinospora cordifolia (Thunb.) Miers)* and *Guduchi Ghana Vati*

Guduchi	GGV (GR)1	GGV (GR)2	GGV (GR)3	GGV (VA)1	GGV (VA)2	GGV (VA)3	GGV (SH)1	GGV (SH)2	GGV (SH)3
3523.95			3523.95				3523.95	3523.95	3523.95
1462.04				1562.34	1562.34				
		1452.40		1543.05	1543.05				
				1535.34	1535.34				1535.34
	1535.34			1510.26	1510.26	1510.26			
				1500.62	1500.62		1433.11	1433.11	
				1338.60	1338.60	1338.60		1373.32	
				1288.45	1288.45	1288.45			1288.45
1269.16				1271.09	1271.09	1271.09			
1161.15		1157.29	1157.29						
		1066.64		1066.64	1066.64	1060.85	1066.64	1066.64	1068.56
1029.99		1026.13							

## DISCUSSION

*Guduchi* samples were taken at random from same geographic area (Manipal, Karnataka) and same host, *Mangifera indica* L. The choice of *Mangifera indica* L. as host was due to non availability of adequate *Nimba (Azadirachta indica (A. Juss))* host in this geographic region. The selection of three seasons was because the seasons experienced in the coastal region are summer, monsoon and October heat (Autumn). It was also limited by the duration of study. GGV (SH) 1 obtained 3.5% of Ghana followed by GGV (SH) 2, which was 3.37% and GGV (VA) 1 obtained 3.1%. In GGV (GR) 1 the yield was minimal, that is, 1.6% which may be due to less quantity of drugs taken. The variation in the quantity of raw drugs taken was the issue of availability of fresh drug in each season. GGV (VA) 2, GGV (VA) 3 and GGV (SH) 3 recorded 2.93%, 2.83% and 2.59% as final yield respectively. . All the samples were black, in the form of sticky mass with sweetish odour and bitter taste. Prepared pills were spherical, smooth and sticky. All physicochemical parameters showed no appreciable difference and were within the prescribed limits. The phytochemical constituents were comparable to studies carried out in *Tinospora cordifolia* (Thunb.) Miers. When evaluated, starch content was maximum in *Guduchi Ghana Vati* prepared using *Guduchi* collected in *Sharad Rutu* (average 30.20%). The minimum was in sample collected in *Grishma Rutu* (14.27%) and it was moderate in *Guduchi Ghana Vati* prepared using sample collected in *Varsha Rutu* (18.86%). This is quite comparable with previous studies carried out [10,11]. The maximum percentage of total alkaloid content was detected in *Guduchi Ghana Vati* prepared using *Guduchi* collected in *Grishma*

*Rutu* (9.09%). It was minimum in GGV (SH) (1.643%) and moderate (4.25%) in GGV (VA). This is partially comparable with previous study where total alkaloid content was found varying in the various seasons (Aug> Oct) [12]. TLC showed similar observations in all samples as three compounds with  $R_f$  values 0.284, 0.43 and 0.8375 respectively were detected. These look to be same compounds observed in raw *Guduchi* and found to be stable despite heating. The IR spectrum of an organic compound provides a unique fingerprint, which is readily distinguished from the absorption patterns of other compounds; only optical isomers absorb in the same way. In the current study, it was interesting to note that even though there are some similarities in the peaks observed, among all the samples, there were visible differences noted. However, there were similarities in the samples among individual categories, i.e. among three samples each of individual categories like GGV (GR), GGV (VA) and GGV (SH). *Guduchi Ghana Vati* prepared with sample of *Guduchi* collected in *Grishma Rutu* showed 8-9 peaks, whereas those prepared with sample collected in *Varsha Rutu* showed 14-19 peaks and *Guduchi Ghana Vati* prepared with sample collected in *Sharad rutu* showed 10-15 peaks. The major peaks and functional groups of primary compounds were analysed and the units compared with standard Infrared chart [13].  $\alpha$ ,  $\beta$ -unsaturated aldehydes and ketones (C=O) with peak value at  $1710\text{ cm}^{-1}$  is seen in GGV (GR) 3. IR spectrum registered a broad band at  $3419.79\text{-}3724.54\text{ cm}^{-1}$  and a stretching band at  $1680\text{-}1697.71\text{ cm}^{-1}$ . This indicates presence of OH groups and Carbonyl (C=O) group. The spectrum also shows peak at  $680.87\text{ cm}^{-1}$  [GGV (SH) 3],  $1026.132\text{-}1066.64\text{ cm}^{-1}$  which indicates presence of

cyclic ring stretching. Observed peaks were then compared with previous work carried out on IR Spectroscopy of *Tinospora cordifolia*. There are several major peaks comparable with that of *Tinospora cordifolia* (Thunb.) Miers.<sup>[14]</sup>

## CONCLUSION

The percentage yield of *Guduchi Ghana* was calculated for each batch with the average yield of *Guduchi Ghana* prepared from samples procured in *Sharad Rutu* being maximum at 3.15% and *Grishma Rutu*, least at 2.09%. Additional studies of Quantitative estimation of Starch and Alkaloids were done in which the batches prepared from *Guduchi* collected in *Sharad Rutu* showed the maximum quantity of Starch at 30.20% and least alkaloid at 1.643%. In contrast, *Guduchi Ghana Vati* prepared from *Guduchi* collected in *Grishma Rutu* had the least starch content at an average of 14.27% but maximum alkaloid content at 9.09%. The Thin Layer Chromatography of the nine samples corresponded with the raw drug. The IR spectroscopy confirmed the presence of aldehydes, ketones, OH groups, carbonyl groups and indicated presence of cyclic ring stretching. This shows that there is a variation in the formulation based on season of collection. The pharmaceutical yield is best in *Sharad rutu* and there is more starch content whereas in *Grishma rutu* there is more alkaloid content. Further studies are required to effectively establish variations and possible effects of these variations.

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