



**Research Article**

**DETAILED COMPARATIVE PHARMACOGNOSTICAL AND PHYSICOCHEMICAL EVALUATION OF CHIRABILVADIYOGA W.S.R. TO BHAVANA (TRITURATION)**

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Trituration.

**ABSTRACT**

*Chirabilvadi Yoga* is a combination of four drugs *Chirabilva*, *Arjuna*, *Jyotishmati* and *Kakanasa* in powder form, each of these ingredients has solid base of previous experimental studies for their individual analgesic activity. *Bhavana* (trituration) is an important *Samskara* (process) mentioned in Ayurvedic classics by which drug may be made very potent to produce a positive results. Till date no data available on this combination *Bhavita Chirabilvadi Yoga* triturated seven times with *Chirabilva Patra Swarasa* and made dried powder. Present study is to screen the differences in pharmacognostical and pharmaceutical profile of the *Chirabilvadi Yoga* before and after *Bhavana*. After *Bhavana* significant changes were found in the pharmacognostical parameters like deformed rosette crystals and cluster crystal of *Arjuna* and broken stone cells of *Arjuna*, which are rarely found before *Bhavana* of the drug and also evaluate the differences in the pharmaceutical profile before and after *Bhavana* like loss on drying, ash value, acid insoluble ash, water soluble extract, methanol soluble extract, pH and HPTLC.

**INTRODUCTION**

Ayurveda advocates the use of single or combined drugs of herbal, mineral and animal origin. Because of their simple method of administration, single herbal drugs have their own importance in Ayurvedic therapeutics. Conversion of undesired properties of the raw material in to desirable properties is an important skill of the physician. *Bhavana* is process in which trituration of the solid matter with a liquid media for the particular time with sufficient pressure. As mentioned in *Rasatarangini*, volumetrically or gravimetrically equal to the amount of solid or loose enough to the consistency of dough. As the *Bhavana Dravya* changes particle size is also changes. Reduced particle size enhances the absorption<sup>[1,2]</sup>

Hence the bioavailability and thus potency of the drug increases resulting have more effect. In this study *Chirabilvadi yoga* is *Anubhuta Yoga* with the combination of four dried herbs i.e., leaf of *Chirabilva*, *Arjuna* bark, leaf of *Jyotishmati*, leaf of *Kaknaasa* in powder form which is administered in patients of *Sandhivata* (OA) for the management of pain. The analgesic and anti-inflammatory activities of leaf of *Chirabilva*<sup>[3]</sup>, bark of *Arjuna*<sup>[4]</sup>, leaf of *Jyotishmati*<sup>[5]</sup> and leaf of *Kakanasa*<sup>[6]</sup>, have already proved experimentally on animals. *Chirabilvadi Yoga* than triturated with the three times *Bhavana* of *Chirabilva patra swarasa* to increase its potency. The present study is carries out to authenticate the genuineness of the samples of powder form of *Chirabilva*, *Jyotishmati* and *Kakanasa* leaves and *Arjuna* bark microscopically and to differentiate the macroscopic and microscopic characters of "*Chirabilvadiyoga*" before and after three *Bhavna* of *Chirabilva* leave's juice.

**MATERIALS AND METHOD**

**Collection of the Drug**

Ingredients of *Chirabilvadi* compound viz., *Arjuna* bark was procured from the institutional pharmacy and leaf of *Chirabilva* and leaf of *Jyotishmati*

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from the campus of I.P.G.T & R.A, Jamnagar and *Kakanasa* leaves were collected from local area of Jamnagar, India. (Table 1)

Their characteristics were confirmed by correlating their morphological and microscopic features with relevant literature.

#### Preparation of the Drug

Collected leaves and bark were shade dried and made into fine powder separately with the help of mechanical grinder and sieved through 80# sieve mesh. The equal quantities of the obtained powders mixed together mechanically to get homogenous mixture.

#### Preparation of *Chirabilvadi yoga* with three *Bhavana*

The prepared powder of *Chirabilvadi yoga* was triturated with juice of *Chirabilva* leaves, three times in end runner. In each *Bhavana* sufficient amount of juice made from leaves of *Chirabilva* was added to the powder of *Chirabilvadi yoga* as it is very well soaked and then triturated for 6-8 hours daily till the *Bhavana* given to the powder was completely absorbed. On completing the three *Bhavana*, the obtained powder was dried and filtered through 80# sieve mesh.

#### Pharmacognostical Organoleptic Evaluation

Pharmacognostical analysis is based on the various organoleptic characters like colour, odour, taste and touch were recorded by using sensory organs. Powder microscopy of the finished product was done without stain and after staining with Phloroglucino 1+ HCl, to observe the characters, determined the chemical nature of the cell wall along with the form and chemical nature of the content of the cells. Micro photographs were taken under Carl- Zeiss Trinocular microscope attached with camera.

#### Physicochemical Analysis

*Chirabilvadi Yoga Bhavita* and *Abhavita* were analysed with appropriate protocols for standard physicochemical parameters such as loss on drying, ash value, water soluble extract, alcohol soluble extract etc., at the Pharmaceutical Chemistry Lab, IPGT & RA, Jamnagar, Gujarat, India. Preliminary tests were carried out on methanolic extract of test drugs for the presence or absence of phyto-constituents like alkaloids, tannin and phenolic compounds, flavonoids, saponin and anthraquinone glycosides.

#### High Performance Thin Layer Chromatography (HPTLC)

HPTLC was performed as per the guideline provided by API. Methanolic extract of drug sample was used for the spotting. HPTLC was performed using Toluene+ Ethyl acetate+ Formic acid (6:3:1) solvent system and observed under visible light. The colour and Rf values of resolved spots were noted.

## OBSERVATIONS

### Pharmacognostical Parameters

#### Organoleptic Characters

Results of various parameters such as colour, odour, taste, touch and texture of the finished products (powder) are shown in Table 2, Plate A.

The sample of powdered *Chirabilvadi Yogawas* a brownish grey powder with predominant *Kashaya* (astringent) taste and characteristic smell while the sample of powdered *Bhavita Chirabilvadi Yoga* was a dark brownish green powder with slightly aromatic smell and bitter with astringent taste (Table 2) Plate A.

#### Microscopic Characters

Powder microscopy of dried *Chirabilvadi Yoga* under the microscope showed fragment of trichome of *Chirabilva*, epidermal cell with stomata of *Chirabilva*, simple fibre of *Chirabilva*, fragment of cystolith of *Chirabilva*, presence of annular vessels of *Chirabilva*, Rosette crystal of *Arjuna*, simple and compound starch grains of *Arjuna*, cork cells with tannin content of *Arjuna*, cluster crystal of *Arjuna*, tannin content of *Arjuna*, lignified fibres of *Arjuna*, fibres of *Jyotishmati*, multicellular trichomes of *Jyotishmati*, simple trichomes of *Jyotishmati*, prismatic crystal of *Jyotishmati*, spongy paranchyma of *Jyotishmati*, spiral vessels of *Jyotishmati*, epidermal cells with stomata of *Jyotishmati*, oil globules of *Jyotishmati*, stomata and trichomes of *Kakanasa* Plate B (1-30)

Powder microscopy of dried *Bhavita* (3 *Bhavna* of *Chirabilva Patraswarasa*) *Chirabilvadi Yoga* showed deposition of cystolith is increased because we triturated with *Chirabilva patra swarasa*. Fragments and opened cystolith of *Chirabilva*, simple and warty trichomes with smoothed walls of *Chirabilva*, disturbed annular vessels of *Chirabilva*, lignin content comparatively reduced, slightly deformed rosette crystals and cluster crystal of *Arjuna* because of the pressure of *Mardana*, deformed cork cells of *Arjuna* with tannin content,, slightly deformed starch grains and reduced in concentration of *Arjuna*, tannin content is in dark coloured (dark brown) of *Arjuna*, slightly ruptured epidermal cells of *Jyotishmati*, spiral vessels of the *Jyotishmati*, fragments of multicellular trichomes of *Jyotishmati*, oil globules of *Jyotishmati*, smoothed walled of multicellular trichomes of *Jyotishmati*, ruptured and opened stomata of *Jyotishmati*, simple trichomes of *Kakanasa*. Plate C (1-27)

#### Physicochemical Analysis

Results of physicochemical analysis i.e., loss on drying, ash value, water soluble extract, alcohol soluble extract, ash value etc. are shown in Table 3.

#### High Performance Thin Layer Chromatography (HPTLC)

The colour and Rf values of resolved spots of HPTLC were noted. (Table-4) (Plate no. D)

## DISCUSSION

*Bhavana* is a traditional wet grinding process which includes hydration (or soaking) with liquids, breakdown of the compound molecules, interaction of organic/inorganic compounds and drying that may leads to distinctive changes in physicochemical changes in the drug.<sup>[7]</sup>

In study of organoleptic characters of powdered drugs, there was a slight colour and taste variation between CY and BCY. Colour of CY was brownish grey which was changed as dark brownish green which indicates that the colour change owes to prolong trituration of the compound. Odour of CY was characteristic which was changed as slightly aromatic in BCY finished product.

Taste of CY is *Kashaya* (astringent) *Rasa* (taste) and *Kashaya Anurasa* which was changed as mild *Tikta* (bitter) and *Kashaya* due to three times lavigated and triturated with of *Chirabilva patra swarasa*. The alteration in taste is might be due to the effect of addition process carried out during *Bhavana Samskara* of the drugs.

Touch and texture of both the powder compound was coarse but fineness was increased in BCY might be due to breakdown of the hard cellular structures and the exposed cellular contents by prolonged trituration of the drugs. One more reason is constant wet grinding in liquid media which turns the hard material soft.<sup>[8]</sup> The addition of liquid media in *Bhavana* is suggested to further amplify the size reduction process.<sup>[9,10,11,12]</sup>

As per "Attrition theory," rubbing of the materials between liquid media and the surfaces of pestle and mortar results in PSR.<sup>[13,14]</sup>

### Microscopical Characters

While comparison of microscopic features of both the compound drug it was found that cystolith of *Chirabilva* leaf was opened and its deposition was also increased and disturbed annular vessels respectively. Deformed rosette and cluster crystal of *Arjuna*, ruptured stone cells with wide lumen, slightly deformed starch grains of *Arjuna*, smooth walled fibres and trichomes of *Jyotishmati*, opened spiral vessels and ruptured epidermal cells and opened stomata of *Jyotishmati* were also noted which the result of prolonged trituration. These can be explained by "Griffith theory," which states that all solids contain flaws (structural weakness) that may develop into a microscopic crack under stress/strain-like pressure applied during *Bhavana*.<sup>[15]</sup> During *Bhavana*, drug particles are subjected to various stresses, leading to breaking of chemical bonds to create new surfaces and retard rejoining of the broken surfaces.<sup>[16]</sup> As an outcome contents in the cellular compartment are freed which might results in increased and quick absorption as well increased assimilation and

bioavailability of the drugs. Thus, trituration process might potentiate the medicine in some contexts.

### Pharmaceutical Study

The various Physicochemical and qualitative parameters were performed as per recommended procedures of API. The data showed that a considerable difference was found in the values of some physico-chemical parameters of BCY.

There was not much variation in the pH, which ranged from 6.0 to 6.5. This slightly increased pH may be due to the "*Chirabilvapatra Swarasa*" having basic pH. This reveals that a more concentrated form or more water and methanol soluble content may not affect the pH of the samples.

Results of physicochemical analysis like loss on drying at 110°C is the major factor for the stability of the drugs. Loss on drying of BCY was higher than CY which indicates that increase amount of moisture was due to the *Bhavana* process with *Chirabilva patra Swarasa*.

The ash value indicates the presence of inorganic contents in the sample.<sup>[17]</sup> Total ash value of CY and BCY was 11.35% (w/w) and 15.75% (w/w), respectively. After the process, ash value was on higher side in *BCY* which indicates increase in organic component of processed drug and there was not much variation in the acid insoluble ash value between two samples.

Extractive values indicate the respective solubility or metabolites in referential solvent and gives probable idea of chemical constituents in the sample.<sup>[18]</sup> Water soluble extract value of CY and *BCY* were 12.28% (w/w) and 24.73% (w/w) and methanol extractive value were 8.45% (w/w) and 14.64% (w/w), respectively. In terms of extractive values, again *BCY* showed maximum value indicating the more amount of chemical constituent present in it which is soluble in respective solvents.

HPTLC study was carried out on methanolic extract of *CY* and *BCY* using Toluene: ethyl acetate (9:1) as mobile phase. There is no difference in HPTLC profile of the methanolic extract of the drugs in 254 and 366nm, 3 spots at Rf 0.02, 0.39, 0.95 were observed in 254nm UV light spectrum and 3 spots also at Rf 0.02, 0.09, 0.96 were observed in 366nm UV light spectrum. HPTLC finger printing technique showed that when the plate was scanned at 254nm and 366nm common spots were found at same Rf value which also indicates the presence of specific quality compound that may be of same nature.

### CONCLUSION

While preparation of *Chirabilvadi yoga* and *Bhavita Chirabilvadi yoga* pharmagnostical evaluation proved without presence of all the ingredients in *Yoga* showed that genuinity of the finished product. Thus,

*Bhavana* (trituration) is an important process during drug preparation, affecting the physicochemical and biological properties of a dosage form and increase the

bioavailability of drug. The three *Bhavana* of *Chirabilvadi yoga* profile can also be used for standardization and in future references.

**Table 1: Ingredients of *Chirabilvadi yoga***

S. No	Name of ingredients	Botanical name	Proportion
1.	<i>Chirabilva</i>	<i>Holoptelea integrifolia</i> Planch.	1part
2.	<i>Arjuna</i>	<i>Terminalia arjuna</i> Roxb.	1part
3.	<i>Jyotishmati</i>	<i>Celastrus paniculatus</i> Willd.	1part
4.	<i>Kakanasa</i>	<i>Pentatropis capensis</i> L.F.	1part

**Table 2: Organoleptic characters of *Chirabilvadi yoga***

S. No	Various Parameters	Results	
		<i>Chirabilvadi yoga</i> (CY)	<i>Bhavita Chirabilvadi Yoga</i> (BCY)
1.	Colour	Brownish grey	Dark brownish green
2.	Odour	Characteristic	Slightly aromatic
3.	Taste	Astringent	Astringent, bitter
4.	Touch	Rough	Soft
5.	Texture	Coarse	Fine

**Table 3: Physico-chemical parameters of *ChirabilvadiYoga* with and without *Bhavana***

S. No	Various Parameters	Results	
		<i>Chirabilvadi yoga</i> (CY)	<i>Bhavita Chirabilvadi Yoga</i> (BCY)
1.	pH	6.0	6.5
2.	Loss on drying	7.91% (w/w)	11.25% (w/w)
3.	Ash value	11.35 % (w/w)	15.75 % (w/w)
4.	Acid insoluble ash	1.25 % (w/w)	1% (w/w)
5.	Water soluble extractive value	12.28% (w/w)	24.73% (w/w)
6.	Methanol soluble extractive value	8.45%(w/w)	14.64% (w/w)

**High Performance Thin Layer Chromatography (HPTLC)**

**Table 4:  $R_f$  values obtained by HPTLC**

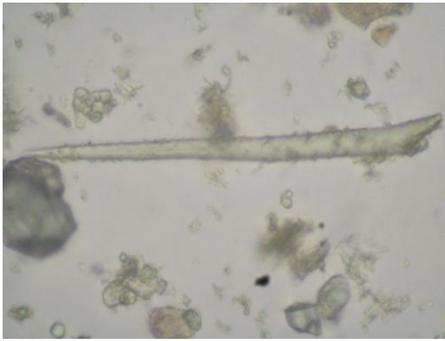
Sample	Visualize under short UV (254 nm)		Visualize under short UV (366 nm)	
	No. of spots	$R_f$ value	No. of spots	$R_f$ value
<i>Chirabilvadi yoga</i> powder without <i>Bhavana</i>	3	0.02,0.39,0.95	3	0.02,0.09,0.96
<i>Chirabilvadi yoga</i> with three <i>Bhavana</i>	3	0.02,0.39,0.95	3	0.02,0.09,0.96

HPTLC = High Performance Thin-Layer Chromatography;  $hR_f = (R_f \text{ value}) \times (100)$ .

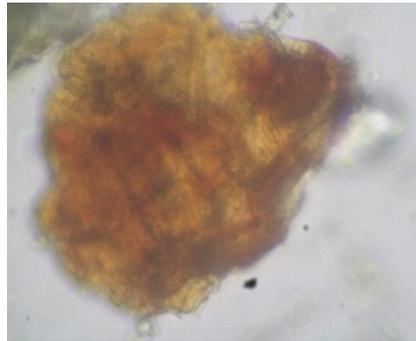
**Plate A: *Chirabilvadi Yoga* Powder**



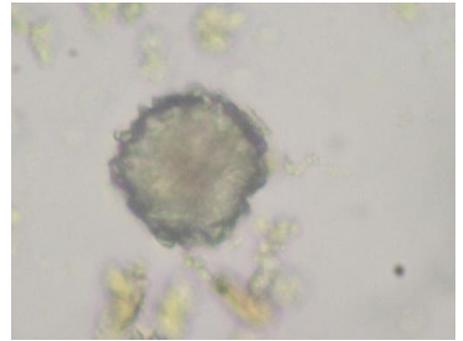
**Plate B: Powder characters of *Chirabilvadi yoga* without *Bhavna***



4. Simple trichomes of *Jyotishmati*



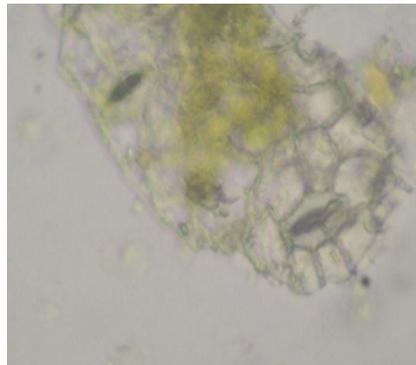
5. Cork cells with tannin content of *Arjuna*



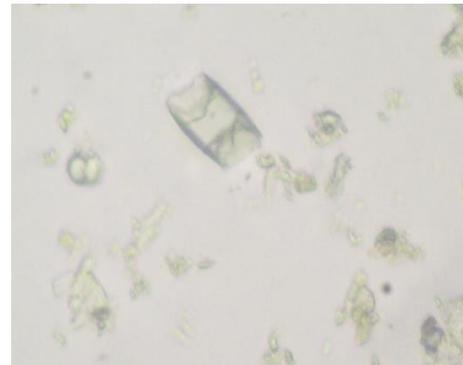
6. Cluster crystal of *Arjuna*



7. Fragment of trichome of *Chirabilva*



8. Epidermal cells with stomata of *Chirabilva*



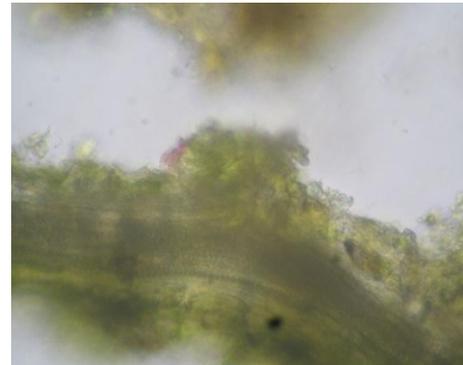
9. Prismatic crystal of *Jyotishmati*



10. Tannin content of *Arjuna*



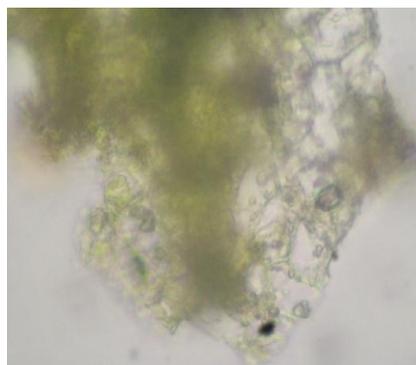
11. Spiral vessels of *Chirabilva*



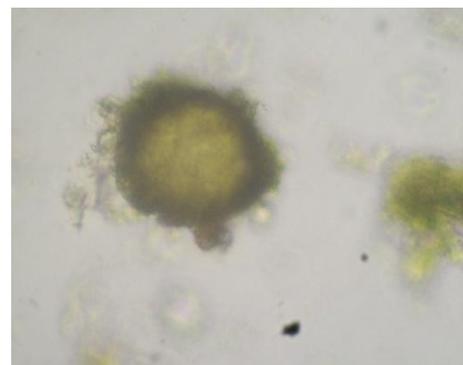
12. Spongy paranchyma of *Jyotishmati*



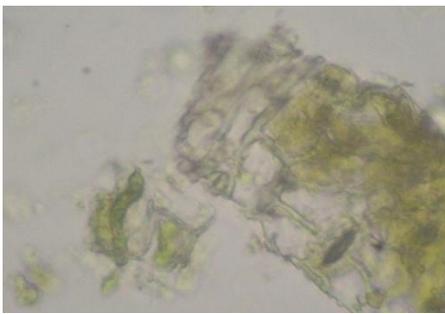
13. Multicellular trichomes of *Jyotishmati*



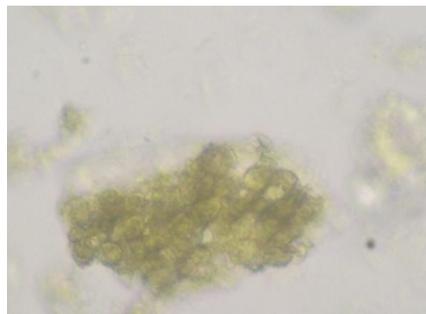
14. stomata of *Kakanasa*



15. Fragment of cytolith of *Chirabilva*



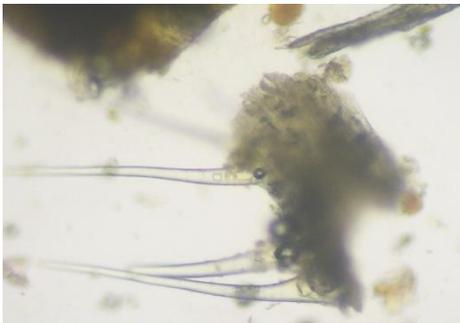
16. Epidermal cells with stomata of *Jyotishmati*



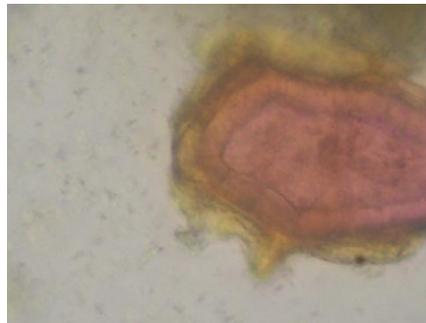
17. Spongy paranchyma of *Jyotishmati*



18. lignified fibres of *Arjuna*

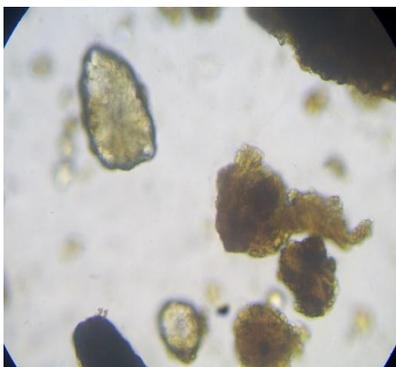


19. Trichomes of *Kakanasa*

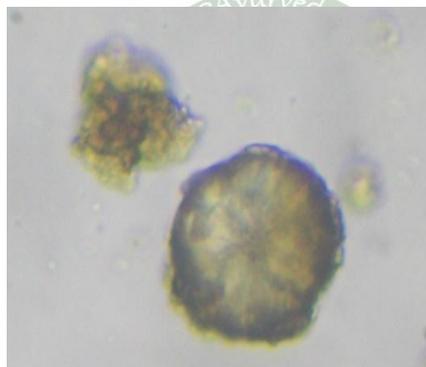


20. Stone cells of *Arjuna*

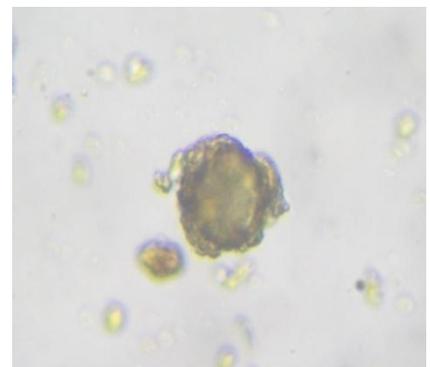
**Plate C: Powder characters with *Bhavna***



1. Deposition of cystolith is increased.



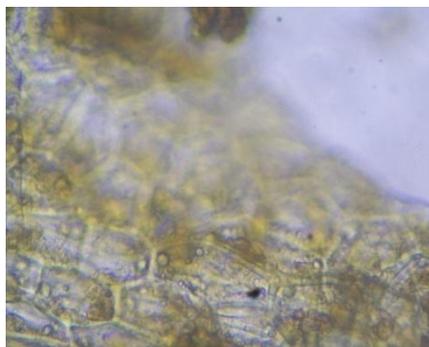
2. Fragment of cystolith of *Chirabilva*



3. Deformed cluster crystal of *Arjuna*



4. Fibres with smooth walled



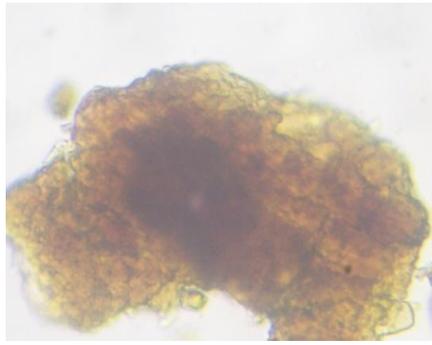
5. Slightly ruptured epidermal cells of *Jyotishmati*



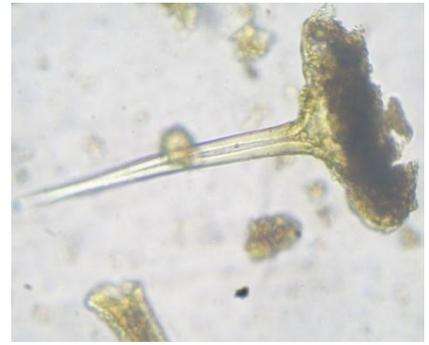
6. Simple trichomes of the *Chirabilva*



7.Spiral vessels of *Jyotishmati*



8.Deformed cork cells of *Arjuna* with tannin content



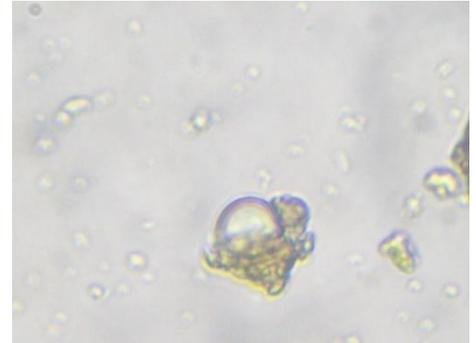
9.Simple trichomes of *Kakanasa*



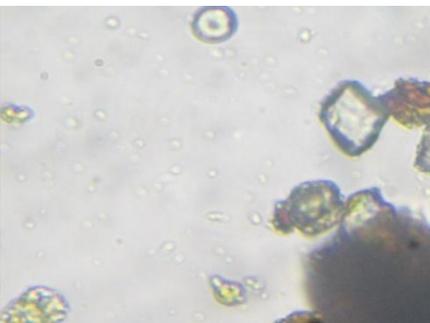
10.Warty trichome of *Chirabilva*



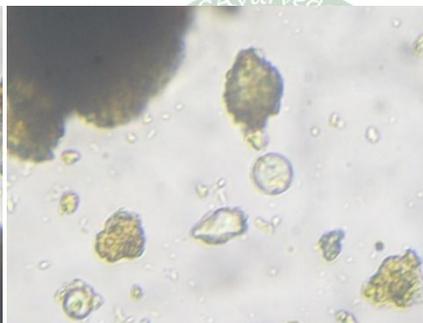
11.Fragment of multicellular trichomes of *Jyotishmati*



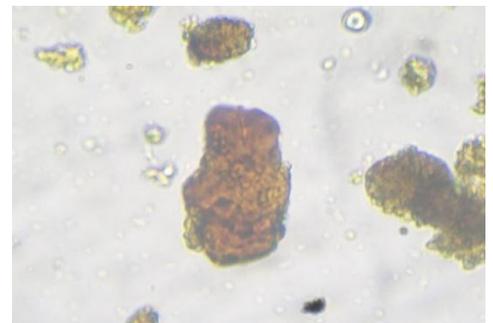
12.Oil globules of *Jyotishmati*



13.Slightly deformed starch grains and reduced in concentration of *Arjuna*



14.Aleurone grains of the *Jyotishmati*



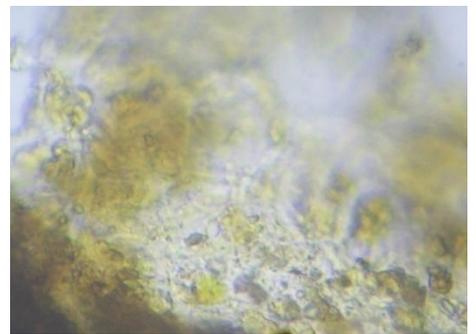
15.Tannin content is in dark coloured (dark brown) of *Arjuna*



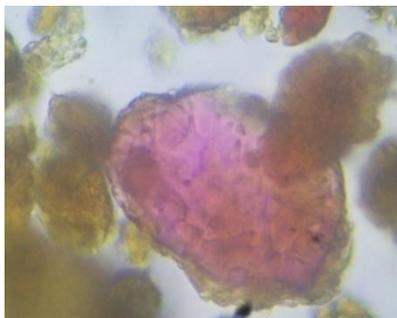
16.Cystolith of *Chirabilva* is opened



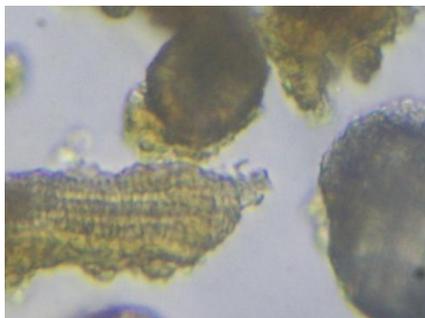
17.Smoothed walled of multicellular trichomes of *Jyotishmati*



18.Ruptured and opened stomata of *Jyotishmati*



19.Liganin content comparatively reduced

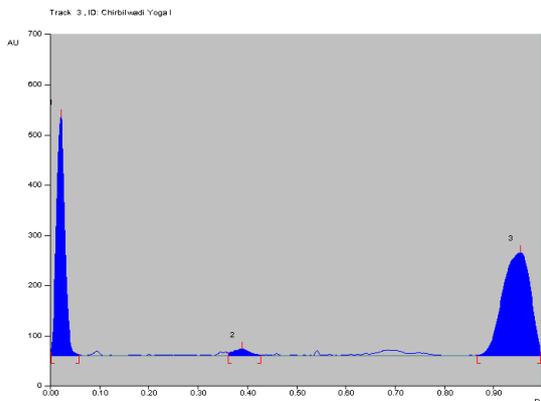


20.Disturbed annular vessels of the *Chirabilva*

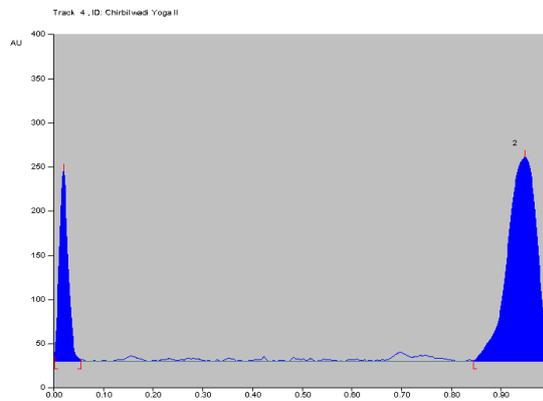


21.Unligified fibres of *Arjuna* withno lignin content

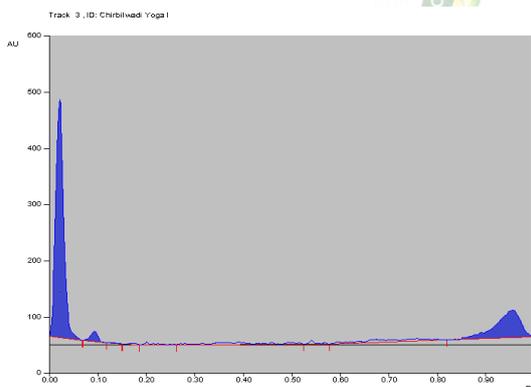
**Plate D: HPTLC of Methanolic extract of *Chirabilvadi Yoga* compound**



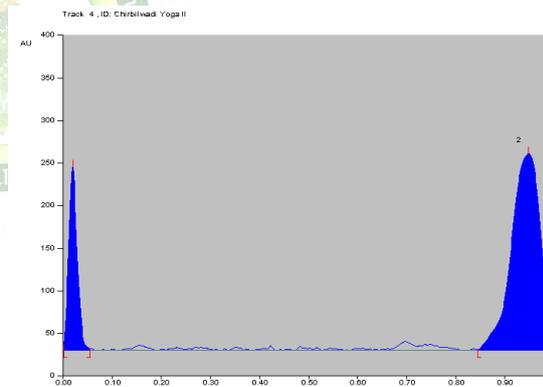
Densitogram at 254nm without *bhavna*



Densitogram at 254nm with *bhavna*



Densitogram at 366nm without *bhavna*



Densitogram at 366nm with *bhavna*

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