

Preliminary Pharmacognostical and Phytochemical Evaluation of *Prashasta* and *Aprashasta Haritaki* (*Terminalia Chebula* Retz.)

Research Article

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Abstract

Introduction: *Haritaki* (*Terminalia chebula* Retz.) of Combretaceae family is well known drug often used in Ayurvedic therapeutics. Ayurvedic texts advised to consider the fruits that sink in stable water in therapeutics. As tannin is an important constituent of *Haritaki*, an attempt has been made to determine tannin content and other differences in acceptable and unacceptable varieties of *Haritaki*. **Materials and methods:** *Haritaki* (*Terminalia chebula* Retz.) was collected from pharmacy, Gujarat Ayurved University, Jamnagar and another sample has been collected from Indore market. These fruits were categorized into five groups, (very big, big, small, brown and *Baal Haritaki*) and again sub grouped into two on the basis of accepted and rejected qualities. Free hand selections and organoleptic examination was done of all samples, out of them three samples (sink big *Haritaki* (SBH), floating big *Haritaki* (FBH), very big *Haritaki* (VBH)) are selected for TS examination, microscopic characters, ash values, LOD, extractive values, H. P. T. L. C. , Tannin content & other physico-chemical analysis. **Results and conclusion:** The pH of 10% aqueous solution of SBH, FBH and VBH was 3. 0, 3. 2, 2. 8 and tannin content 44. 78%, 50. 22%, 33. 9% respectively. Microscopically the SBH showed abundant scleroids and rosette crystals. All the data of three samples were compared with Ayurvedic Pharmacopeia of India (API), SBH was more accepted than among three samples.

Key words: Ayurveda, *Aprashasta*, *Haritaki*, *Prashasta*, Pharmacognocny, *Terminalia chebula*,

Introduction:

Haritaki (*Terminalia chebula* Retz.) of Combretaceae family is commonly known as Chebulic myrobalan

in English and *Haritaki* in Sanskrit. It is widely distributed in India, Malaysia, Srilanka and other part of the world. (1) Myrobalan tree has 15to25 m height and 1. 5-2. 5 m diameter of trunk. Colour of fruit is yellowish – brown, odourless, astringent taste and sweetish at the end. *Haritaki* has been extensively used in Ayurveda, for its properties like *Sarvadosaprasamana* (useful in all diseases), *Rasayana* (Rejuvenator), *Chakshusya* (useful for eye), *Deepana* (stimulate the digestive function), *Anulomana* (purgative), *Hrdya* (cardio tonic) and *Medhya* (brain tonic).

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(2) *Terminalia chebula* Retz possesses a wide variety of activities like antimicrobial, (3) antioxidant, (4) antiviral, (5) anticarcinogenic, (6) hypocholesterolemic, (7) radioprotective, (8) antispasmodic, (9) and purgative. (10) In Ayurveda, seven varieties of *Haritaki* fruits, namely, *Vijaya*, *Rohini*, *Putana*, *Amrita*, *Abhaya*, *Jivanti* and *Chetaki* have been described, (11) depending upon the place of occurrence (12) and shape of the fruits. (13) and uses according to disease. (14) Few examining methods have been elaborated in classics of Ayurveda, based on which one can accept or reject *Haritaki*. A fruit of *Haritaki* which doesn't float in stable water, which is fresh, smooth, bulky, round in shape are said to be ideal for medicinal purposes. (15) There are different type of *Haritaki* fruits are available which can be categorized such as brown coloured, fully matured (very big), *Baal Haritaki*, big and small size *Haritaki* etc. Till date no work has been found regarding acceptable quality of different varieties of *Haritaki* based on pharmacognostic and analytical characters. Therefore in present study attempts have been made to evaluate differences between acceptable and unacceptable varieties of *Haritaki* based on Ayurvedic parameters as well as pharmacognostic and analytical levels.

Materials and Methods:

Collection:

One sample of *Haritaki* (very big) was collected from Indore market and other samples were collected from Pharmacy, Gujarat Ayurved University, Jamnagar. Pharmacy samples were categorized into four groups (big, small, brown and *Baal Haritaki*) and again sub grouped into two based on accepted and rejected qualities.

Selection:

Fruits are categorized into five groups (Big, small, brown, *Baal Haritaki*,

very big) on the basis of average weight (>4g-big size, >2.5g-small, >5g-Brown, >10g-very big, <2g-*Baal Haritaki*) average diameter (>3.5x1.5cm-big size, >2.5x1.0cm-small, >3.5x2.0cm-Brown, <1.5x1.0cm-*Baal Haritaki*, >5x2.5cm -very big) and colour. These five groups were again sub-grouped into two i. e. accepted and rejected varieties. Very big *Haritaki* have only one group because all fruits of this group were sunken in stable water. Therefore in present study total 9 samples (SBH- Sink big *Haritaki*, FBH- Floating big *Haritaki*, VBH- Very big *Haritaki* (all fruits were sunken no one floating) sink small *Haritaki*, floating small *Haritaki*, sink brown *Haritaki*, floating brown *Haritaki*, sink *Baal Haritaki* (immature), floating *Baal Haritaki* were studied (Plate no. 1). One *Haritaki*, fruit randomly selected from each categories for macroscopic examination. From these three *Haritaki* samples (Sinking big, floating big, and very big) were selected for other examination (Microscopic examination, powder microscopy, analytical study) (Plate no. 2)

Pharmacognostical evaluation:

Morphological studies:

Morphological characters including shape, size, weight, ridges and grooves, fruit condition were observed. (16)

Organoleptic study:

The Colour of the drug was examined under sunlight. A small portion of the drug was taken, slowly and repeatedly inhaled and examined the odor. A small portion of drug was taste by keeping over the tongue to find out the taste. (17)

Microscopical studies:

Free hand sections of *Haritaki* were observed under the microscope for the presence of primary and secondary metabolites, like starch grains, tannin. The

sections cleared with chloral hydrate to observe various ergastic cell contents like, crystals of calcium oxalate, calcium carbonate, and silica, if present any. The sections then stained with Phloroglucinol and HCl for detecting lignified elements like fibers, sclerides, xylem vessels, trachids etc. Same methods were repeated with powdered samples. (18)

Histochemical evaluation:

Thick sections of the Samples were subjected to histochemical tests to find presence of starch grains, tannin, lignin etc. by treating various reagents. (19)

Observations and Results:

Organoleptic characters are placed in Table 1, while microscopic characters are placed in table 2. Findings of powder microscopy are mentioned in table 3. Histochemical evaluation of the three samples shows the presence of Tannin, Lignin and Starch is mentioned in table 4. Results of analytical study are depicted in table 5.

HPTLC:

Methanol extract of samples subjected to HPTLC plate at 254 and 366nm. The no. of spots obtained is depicted at table 6.

Discussion:

To ensure standardized quality of herbal drugs, proper identification is utmost essential. According to World Health Organization (WHO) the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity. Organoleptic evaluation is a technique of qualitative evaluation based on the study of morphological and sensory profiles of whole drugs. The organoleptic or macroscopic studies yielded important characteristics, such as taste, ridges, aromatic and characteristic odour of the fruits which are useful diagnostic

characters. During ancient time due to lack of sophisticated instruments characteristics of many drugs were decided on the basis of organoleptic parameters such as colour, odour, taste, touch, appearance etc. and some specially developed tests such as floating in stable water for *Haritaki*, and *Bhallataka*, (20) fire test for *Kampillaka* (21) etc. It is well understood that these tests are not only effortless and commercially cheap but also have scientific basis. Present study is an attempt to provide rationality behind floating in stable water mentioned for *Haritaki* with the help of pharmacognostic and analytical study.

However, there are different types of *Haritaki* are available. These fruits can be categorized into five groups (Big, small, brown, *Baal Haritaki*, very big) according to their macroscopic characters i. e. diameter, colour, and weight etc. Fruits which were more than 10g, average weight was near about 12g, diameter more than 5x2.5 cm, golden brown colour and fully mature can be consider as VBH that was collected from Indore market. As per classics weight of *Haritaki* is 24g. (22) Which is not available in the market. AFI considered wt. of 100 *Haritaki* is equal to 1.2kg. (23) which means wt. of one *Haritaki* is approximately equal to 12 g that may comparable to very big size *Haritaki*. Fruits more than 4g, greenish yellow colour and more than 3.5x1.5 mm in diameter were categorized in big *Haritaki*. When the fruit of *Haritaki* falls off from the tree, the seed gets hard called 'bala *Haritaki*'. Sometimes, the fruits are plucked and dried while the seeds have not hardened which are also called 'bala *Haritaki*'. These are black in colour, less than 2 g and less than 1.5x1 cm in diameter. Small *Haritaki* is an immature fruit of *Haritaki*, golden yellow in colour, weight less than 3 g, and diameter are more than 2.5x1 cm whereas dark brown colour fruits of *Haritaki* can be called brown *Haritaki*. These five groups were

again sub grouped into two i. e. accepted and rejected on the basis of floating and sinking in stable water. Fruits of VBH are sunken in stable water no one floated so only one group of these category and two sub grouped (floating and sinking) was categoried of other four (Big, small, brown, *Baal Haritaki*). From these nine groups one-one fruit was randomly selected for macroscopic study. *Baal Haritaki* and small size *Haritaki* were immature just because of this reason microscopic examination and powder microscopy has not done in present studied. Floating sub group of all samples were infected and porous. For comparing healthy and unhealthy fruits with a good sample (very big size) three samples were selected for microscopic examination and powder microscopy.

Phyto-chemical analysis of selected three samples showed that tannin content more in floating (50%), moderate in sinking (44%) and less in very big size *Haritaki* (33%). Floating *Haritaki* infected by worms, it may form tannin or tannin like substance from other substance. According to ICMR tannin content of *Haritaki* is 20-40%. (24)

HPTLC of three-selected *Haritaki* showed that 11 spots at 254nm and 10 spots at 366nm, 7 spots were common in both spectrums in VBH. 9 spots at 254nm and 6 spots at 366nm, 5 spots were common in both spectrums in SBH. 9 spots at 254nm and 7 spots at 366nm, 6 spots were common in both spectrums in FBH. 2 spots were common for all three varieties at 254 nm. Only one spot was common for all three varieties at 366 nm. Results showed that common chemical moieties representing in all samples.

Conclusion:

As mentioned, *Baal Haritaki* and small *Haritaki* were immature and brown *Haritaki* were more ripened and not easily available so these were excluded from studied. The SBH available everywhere

and used in the preparation of Ayurvedic proprietary medicine. As per *Ayurvedic* consideration healthy fruits should be selected. FBH were more or less infected by the worms so were excluded, Although VBH matched relatively more with standard data (API), SBH were showed all the nearby qualities as that of VBH and market prize is also lower than VBH. So it may be considered that the SBH was best sampling economically.

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Table 1: Organoleptic characters of nine samples

Organoleptic characters of <i>Haritaki</i>									
Sunk <i>Haritaki</i> (acceptable)						Floating <i>Haritaki</i> (Unacceptable)			
	Big	Small	Brown	<i>Baal Haritaki</i>	Very big size	Big	Small	Brown	<i>Baal Haritaki</i>
Colour	Greenish Yellow	Golden Yellow	Dark Brown	Black	Dark Golden Brown	Light Greenish Yellow	Light Yellow	Light Yellow Brown	Black
Taste	<i>Kashaya</i> followed by Sweet Sensation	<i>Kashaya</i>	<i>Kashaya</i>	<i>Kashaya</i>	<i>Kashaya</i>	Less <i>Kashaya</i>	Less <i>Kashaya</i>	Less <i>Kashaya</i>	Less <i>Kashaya</i>
Shape	Ovoid	Ovoid	Ovoid	Ovoid	Ovoid	Ovoid	Ovoid	Ovoid	Ovoid
Size (mm)	3.7x1.7	2.7x1.8	3.9x2.2	2.5x0.8	5x2.5	3.8x1.9	3x1.2	3.4x1.9	1.9x1.3
Wrinkles	Less	Less	Moderate	Shrink	Abundant	Moderate	Abundant	Moderate	Shrink
Weight (g)	6.2	2.6	5.4	2.0	11.8	4.2	2.2	5.0	1.8
Ridges & Grooves	5x5	6x6	6x6	insignificant	6x6	5x5	5x5	6x6	5x5
Fruit condition	Uninfected healthy	Uninfected healthy	Uninfected healthy	Uninfected healthy	Uninfected healthy	Porous	Infected	Highly porous	Young Porous

Table 2: T. S. of three selected *Haritaki*

Character	SBH	FBH	VBH
Epicarp	Single layer (A1)	Single layer (B1)	Single layer (C1)
Mesocarp	Multilayer filled with starch grain & some rosettes crystal with large quantity of tannin material (A1)	Loosely arranged most of the cell empty, less no. of starch grain & rosettes crystals (B1)	Multilayer along with many rosettes crystal & starch grain (C1)
Scleroid	Abundant, pitted scleroid	Moderately less as	Moderately less as

	present (A2)	compare to SBH (B2)	compare to SBH (C2)
Vascular bundle sheath	Well defined, Xylem Phloem well defined (A2)	Not well defined (B2)	Not cleared (C2)
Tannin	Abundant (A3)	Moderately distributed (B3)	Moderately high as compare to SBH (C3)
Rosettes crystals	Abundant (A2)	Moderately less as compare to SBH (B2)	Moderately high as compare to SBH (C2)

Table 3: Powder Microscopy of three selected *Haritaki*

Character	SBH	FBH	VBH
Colour	Dark Yellow	Light Yellow	Dark Yellow
Taste	Initially less <i>Kashaya</i> strongly <i>Kashaya</i> at middle after that sweet sensation at end	After <i>Kashaya</i> followed by Sweet Sensation	<i>Kashaya</i>
Scleroid	without lumen	with wide lumen	Group of pitted Scleroids with wide lumen
Mesocarp cell	Fragment of Mesocarp & Endocarp cell compactly arranged (IA)	Loosely arranged Mesocarp cells (IIA)	Compactly arranged (IIIA)
Rosette Crystal	Present (IF)	Present (IIF)	Present (IIIF)
Tannin	Abundant (IE)	Moderately distributed (IIE)	Moderately high as compare to SBH (IIIE)
Stone cells	Pitted stone cell without lumen	Isolated Pitted stone cell with wide lumen	Pitted stone cell stone cell in group
Fibres	Moderate (ID)	Less (IID)	Large (IIID)

Table 4: Histo –chemical tests of three selected *Haritaki*

Sr. no	Reagent	Observation	Characteristics	SBH	FBH	VBH
1.	Phloroglucinol+Conc. HCl	Red	Lignified cells	present	present	present
2.	Iodine	Blue	Starch grains	present	present	present
3.	Phloroglucinol+Conc. HCl	Dissolved	Calcium-oxalate crystals	present	present	present
4.	FeCl ₃ solution	Dark blue to black	Tannin cells	present	present	present

Table 5: Analytical value of three selected *Haritaki*

Parameter	SBH	FBH	VBH	Value in API
LOD	9. 76	7. 46	12. 54	
Ash Value	2. 61	1. 39	3. 25	Not more than 5 per cent
P ^H of aqueous solution	3. 0	3. 2	2. 8	
Water Soluble Content W/W%	54. 5	69. 5	52. 3	Not less than 60 per cent
Alcohol Soluble Content W/W%	66. 4	63. 7	60. 9	Not less than 40 per cent
Tannin%	44. 78	50. 22	33. 9	

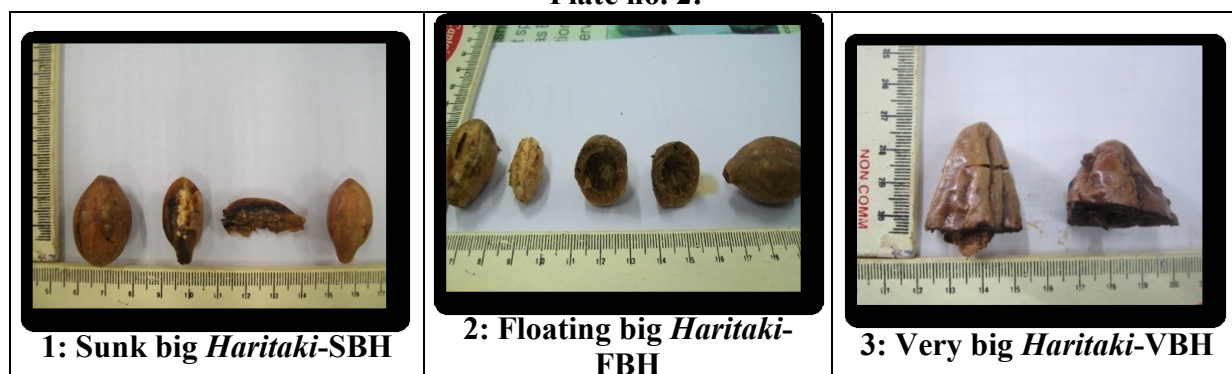
Table 6: Results of HPTLC

Sr. No.	Sample	No. of Spots	254 nm	No. of Spots	366nm
1	SBH	9	0. 02, 0. 20, 0. 27, 0. 33, 0. 60, 0. 63, 0. 76, 0. 90, 0. 95	06	0. 02, 0. 20, 0. 27, 0. 57, 0. 60, 0. 95
2	FBH	9	0. 02, 0. 06, 0. 17, 0. 21, 0. 33, 0. 62, 0. 77, 0. 90 0. 95	07	0. 02, 0. 06, 0. 17, 0. 21, 0. 59, 0. 77, 0. 95
3	VBH	11	0. 02, 0. 10, 0. 13, 0. 20, 0. 27, 0. 33, 0. 49, 0. 53, 0. 59, 0. 86, 0. 94	10	0. 02, 0. 10, 0. 13, 0. 19, 0. 27, 0. 44, 0. 49, 0. 59, 0. 87, 0. 94

Plate no. 1: Nine different Market samples of *Haritaki*



Plate no. 2:

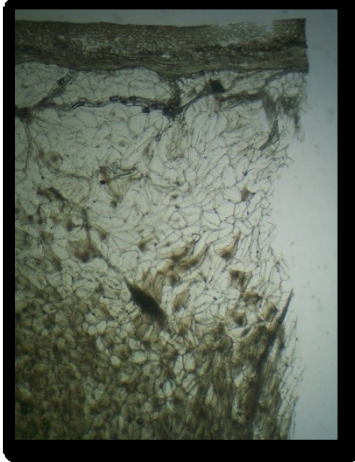


1: Sunk big *Haritaki*-SBH

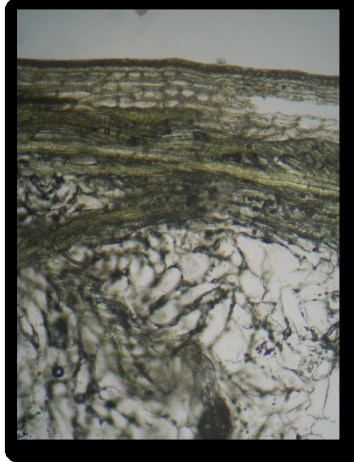
2: Floating big *Haritaki*-FBH

3: Very big *Haritaki*-VBH

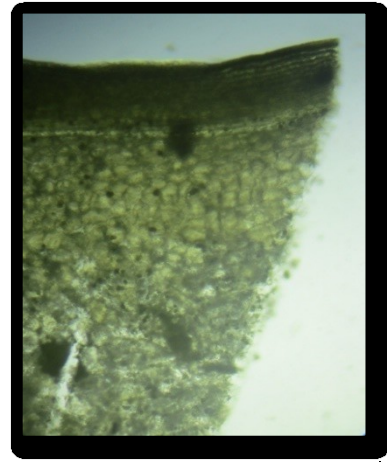
Plate no. 3: Transverse section of selected samples:



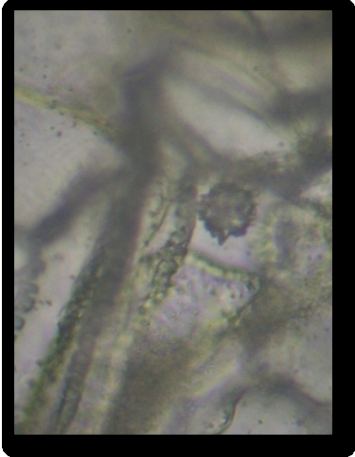
**A1:SBH:Epidermis,
Mesocarp, Tannin contant**



**B1: FBH: Epidermis,
Mesocarp, Tannin contant**



**C1: VBH: Epidermis,
Mesocarp, Tannin contant**



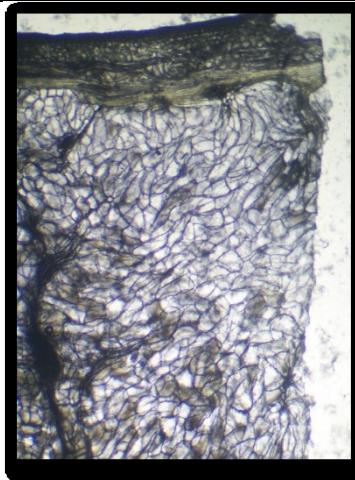
**A2: SBH: Mesocarp cell
with starch grain and
Rossette crystals**



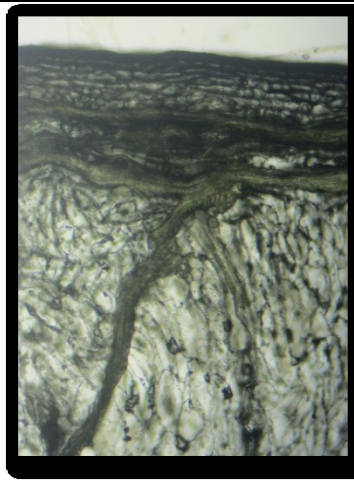
**B2: FBH: Mesocarp cell
with starch grain and
Rossette crystals**



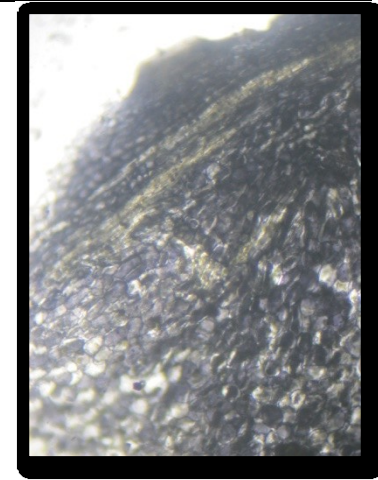
**C2: VBH: Mesocarp cell
with starch grain and
Rossette crystals**



**A3: SBH: FeCl₃ treated for
tannin location**

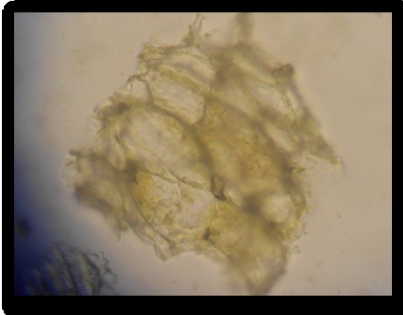
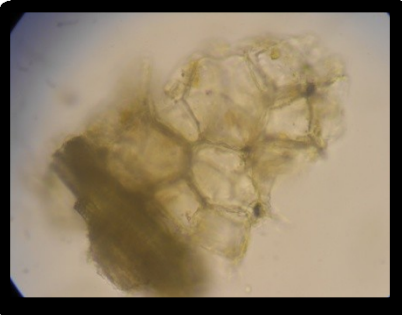


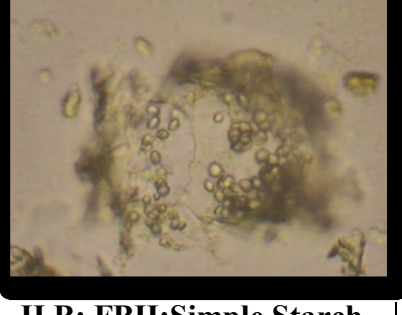
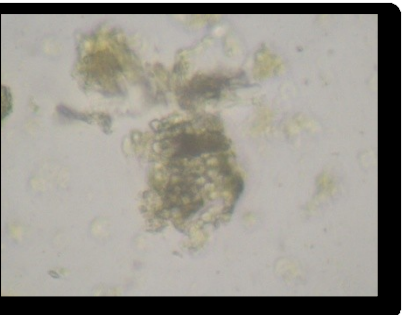
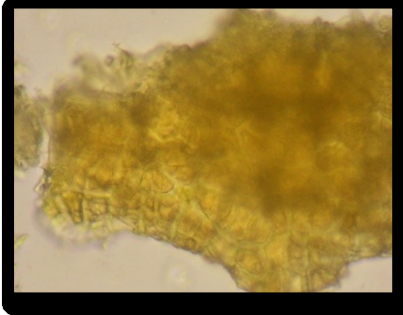
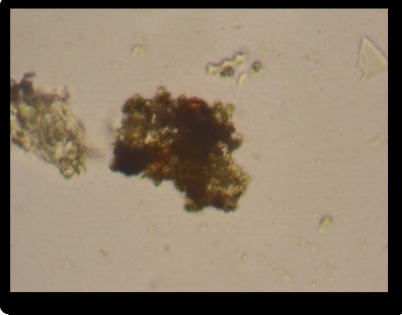






**B3: FBH: FeCl₃ treated for
tannin location**



**C3: VBH: FeCl₃ treated for
tannin location**

Plate no. 4: powder microscopy of selected samples:

		
I A: SBH: Mesocarp cells	II A: FBH: Mesocarp cells	III A: VBH: Mesocarp cells
		
I B: SBH: Simple Starch grains	II B: FBH: Simple Starch grains	III B: VBH: Simple Starch grains
		
I C: SBH: Epidermal cells with Tannin content	II C: FBH: Epidermal cells with Tannin content	III C: VBH: Epidermal cells with Tannin content
		
I D: SBH: Fibres	II D: FBH: Fibres	III D: VBH: Fibres

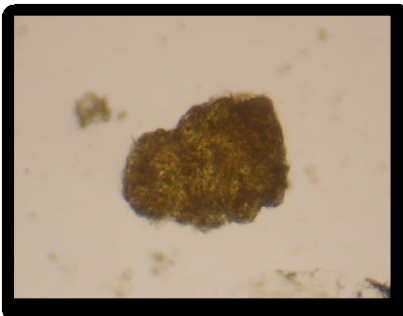
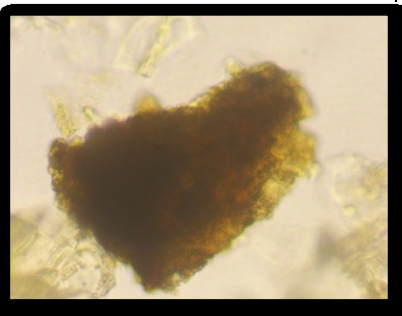
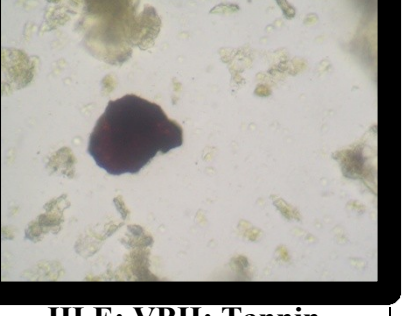
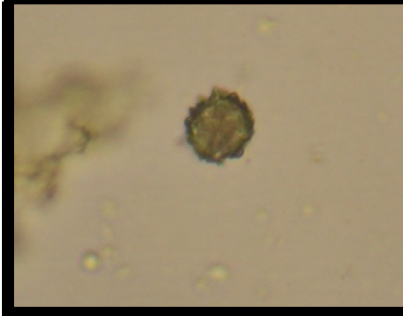
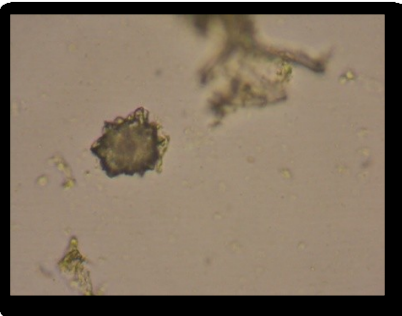

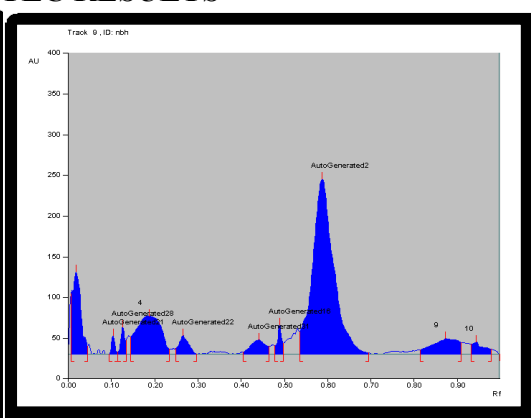
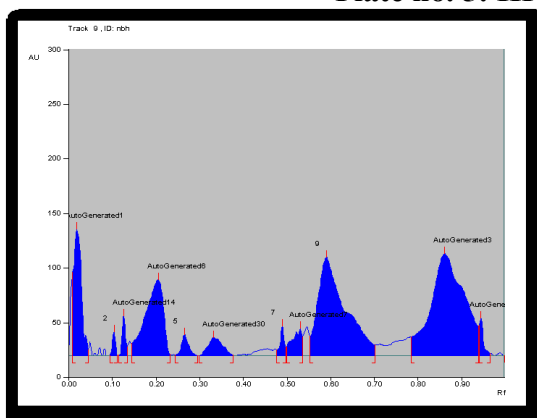
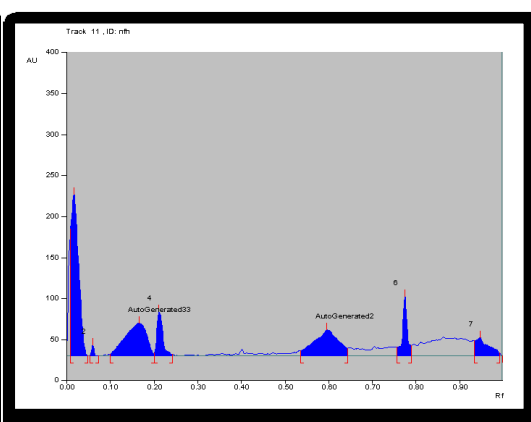
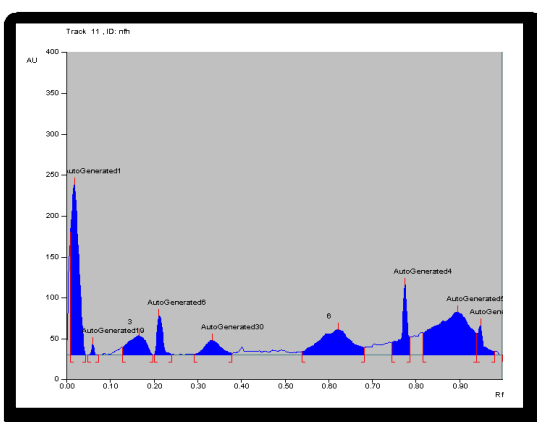
		
I E: SBH: Tannin content	II E: FBH: Tannin content	III E: VBH: Tannin content
		
I F: SBH: Rosette crystal of Calcium oxalate	II F: FBH: Rosette crystal of Calcium oxalate	III F: VBH: Rosette crystal of Calcium oxalate

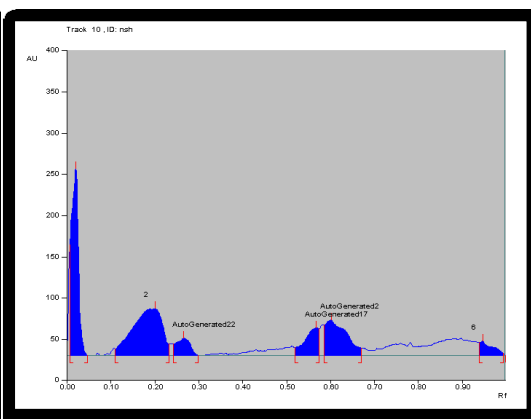
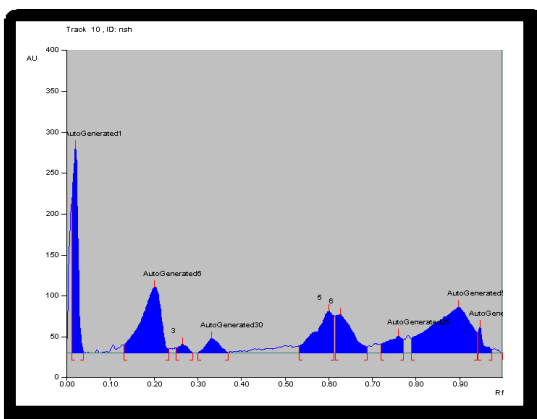
Plate no. 5: HPTLC RESULTS



VBH AT 254nm VBH AT 366nm



FBH AT 254nm FBH AT 366nm



SBH AT 254 nm SBH AT 366nm