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PHARMACOGNOSTICAL EVALUATION OF CHATURTHAMALAKA RASAYANA – AN AYURVEDA COMPOUND

Gajarmal Amit Ashok*[1] Gaurav Sharma[2], Sudipt Rath[3]

[1]PhD (Sch), Research Officer (*Ayu.*), *Central Council for Research in Ayurvedic Sciences* (CCRAS), Ministry of AYUSH, Govt. of India, New Delhi 110058, India
[2]Pharmacologist; [3]Assistant Professor;
Post-Graduation Department of *Dravyaguna*, National Institute of *Ayurveda* (NIA), Jaipur, Rajasthan, India

Email: dgalaxy78@gmail.com

ABSTRACT

Chaturthamalaka Rasayana (CR) is a unique formulation mentioned just after *Chyavanprasha Avaleha* in *Charaka Samhita*. It is one of the best and easiest of *Rasayana Kalpa* (Formulation) to prepare, which may be an alternative option for *Chyavanprasha Avaleha* in the sense of adulteration free as well as cost effectiveness. The modern medical world is seeking for an alternative class of immunomodulatory drugs which is nothing but the category of *Rasayana* mentioned in *Ayurveda*. CR consists of four formulations with simple permutation and combination of *Amalaki* (*Emblica officinalis* Gaertn.), *Bibhitaki* (*Terminalia bellerica* Roxb.) and *Haritaki* (*Terminalia chebula* Retz.) collectively called as *Triphala*. The present study was planned to evaluate a standard pharmacognostical profile of CR included collection, authentication, organoleptic study, macroscopic study and microscopic study as per standard protocols for all four formulations under CR along with main three raw drugs. CR was prepared according to the classical standard operative procedure (SOP) mentioned in *Charaka Samhita*. As the CR has not been studied yet before, it will be more helpful to evaluate the pharmacognostical screening of these four formulations, as an initial step towards standardization.

Keywords: *Chaturthamalaka, Rasayana, Ayurveda, Triphala,* Pharmacognostical

INTRODUCTION

Ayurveda, the oldest healing science, focuses on treating different ailments through balancing the three pillars of life, *Vata, Pitta* and *Kapha*.[1] The main target of *Ayurveda* is to maintain the health of healthy people.[2] One of the guiding principle of *Ayurveda* is to use herbs for improving the overall resistance of body i.e. health against common infection and pathogens.[3] Immunity is a biological term that describes a state of having sufficient biological defence to avoid infection, diseases or other unwanted biological invasion.[4] The term 'immunomodulation' [5] is used for describing the effect of various chemical mediators, hormones and drugs on the immune system. In present era, modern medicine is also looking forward to find out an alternative class of immunomodulatory drugs which are having minimal adverse effects, cost effectiveness and maximum benefits to

an individual. The *Rasayana* therapy of *Ayurveda* is basically covers the above all aspect.

One of the important *Rasayana Dravya* in *Ayurveda* is *Triphala*, consisting of *Amalaki* (*Emblica officinalis* Gaertn.), *Bibhitaki* (*Terminalia bellerica* Roxb.) and *Haritaki* (*Terminalia chebula* Retz.). This three *Dravyas* are widely used in combination or separately due easily availability and, and well established safety aspects. The immunomodulatory activity of *Amalaki*[6,7], *Haritaki* [8,9] and *Bibhitaki* [10,11] was proved by experimental study so that it is the main ingredient used in various *Ayurveda* preparations., The *Chyavanprasha Avaleha* is the most popular *Ayurveda Kalpa* (formulation) used as immunity booster supplement.[12] But all the contents of *Chyavanprasha Avaleha* are difficult to collect, having issues of adulteration and also not as much cost effective. On the other hand CR mentioned in *Charaka Samhita*, may be one of the suitable option for *Chyavanprasha Avaleha.*[13] However, before conducting the pharmacological and clinical study it is necessary to conduct the pharmacognostical study for authentication and standardization. As the CR has not been studied yet before, it will be more helpful to evaluate the pharmacognostical screening of these four formulations, as an initial step towards experimental studies.

Pharmacognosy of a plant gives a comprehensive knowledge regarding its method of identification and determination of quality and purity of the raw drugs. The species of same genus or the family shows somewhat identical morphological characters and it becomes difficult to establish authenticity of a particular drug. Every species has its own characteristic features which determine the authenticity of that particular drug. So it becomes helpful to differentiate closely related species of the same genus or the same family. Based upon pharmacognostical study, any drug is to be get standardize is an essential step. In *Ayurveda* literature, identification and standardization of the drugs has been indicated.[14] For this, *Raja Nighantu* has given seven methods for identification of drug.[15] It can be traced back to *Atharvaveda* as pharmacognostical identification of drug with classification[16], Identification according to their gross morphology[17] and in *Vishanu Purana,* morphology of the drug were described.[18] The determination of taste and taste threshold will help to determine *Panchbhautika* components and there by *Vipaka, Guna & Virya*. The parameters of standardization were described in *Charaka Vimana Sthana.*[19]

Standardization starts right from the collection of raw materials up to their clinical application. In case of *Ayurveda* medicines, the therapeutic efficacy is also related to its chemical constituents. The quality and purity refers to the total profile of the drug rather than any of its character. Therefore, a multidimensional approach is essential for standardizing of *Ayurveda* drug.

MATERIAL AND METHODS: CR having nine ingredients among them Triphala is the main content. CR is nothing but the simple alternative combinations of Triphala.

Collection of raw drugs:

The visit was conducted in the month of January 2018. The plant were selected from natural habitat. Fresh fruits of *Amalaki & Bibhitaki* were collected from Amboli Ghat, Konkan Region, Dist. Sindhudurga whereas, *Haritaki* from Bhimashankara, Dist. Pune, Maharashtra state. *Tila* and other remaining ingredients were collected from Jaipur, Rajasthan state, after proper identification. Field notes of size, colour, shape, texture, maturity etc made for the selection of raw fresh material. Classical parameters were also taken for the evaluation.

Test Drug Preparation: For the analysis purpose, the quantity of raw drugs were taken in the proportions that mentioned in the classical reference.

Classical method of CR preparation:[20]

Authenticated raw drugs were added in a four different combinations A, B, C and D as per classical text.

1. First, washed & cleaned all collected fresh fruits. [***Ardra Dravya Sankalana***] Took them in the combination of **[I]** *Amalaki* + *Haritaki*, **[II]** *Amalaki* + *Bibhitaki*, **[III]** *Haritaki* + *Bibhitaki* and **[IV]** *Amalaki* + *Haritaki* + *Bibhitaki*.

2. Then all of the samples were wrapped with *Tvaga* (stem) of *Butea monosperma* Linn. and smeared with mud (***Mruttika Lepana***) up to thickness of *Anguli pramana* (approximately 2-2.5cm). [***Mrida Avalipta***]
3. Then kept in sunlight for 5-6 hrs for drying. This dried mud balls were roasted in the fire generated by 8-10 *Upala* i.e. cow-dung cakes [***Kukulake Swinna***] up to it gets hot around 45 minutes. After that it was allow to cool little bit and upper coating of balls were removed. The steamed fruits were collected. The pulp and seeds were separated and only pulp was taken to prepare a paste by mortar and pestle [***Ulukhale Sampothha***]. Then *Ghrita*, *Madhu*, *Tila* (*Sesamum indicum* L.) *Pishti*, *Tila Tailam* and *Sharkara* were added in equal quantity same as the quantity of paste prepared in mortar and pestle.[***Dadhi-Ghrita–Madhu-Palala-Taila-Sharkara Samyuktam***]. In this way four test drugs were prepared.
4. *Dadhi* is one of the ingredients of the formulations; owing to its nature mostly it will reduce the stability and self-life of the test drug formulation. Therefore it was proposed to prepare the test drugs without adding *Dadhi*, which were added only at the time of pharmacognostical examination.

Macroscopic study:

The organoleptic study of collected raw drugs and prepared four formulations were conducted with naked eye, as per the pharmacognostical procedure. Data were recorded i.e. appearance, size, shape, colour, and odour etc.

Microscopic study of raw drugs:

Fresh fruit sample of *Emblica officinalis* Gaertn, *Terminalia bellerica* Roxb., *Terminalia chebula* Retz were cut in very thin slices with the help of blade after dipping them in water for some time to make them soften. After that, staining was done with safranin. After staining, mounting was done on micro slides. In this process, sections were transferred on slides & glycerine was added on sections. Then coverslip was put on sections, excess water was wiped out & then the slides were observed in microscope & photos were taken.

Microscopy study test drugs:[21]

Microscopic inspection of test drug is indispensable for the identification of broken materials; the specimen has to be treated with chemical reagents (Table 1).

Procedure:

For examining the microscopic characters of the samples I, II, III and IV, 10gm of each was taken in 100ml of methanol and it was allowed to settle down. The basement settled sediment was taken and washes with hot water. After that, it was taken on slide and treated with different chemical reagents. Then slide was warmed over a sprit lamp with low flame for a short duration. A drop of glycerine was put on the slide, was covered with the cover slip and observed under the microscope.

Table 1: Chemical reagents used for staining of the Test drug samples

1.	Iodine	**3.**	Dilute Ferric chloride ($FeCl_3$)	**5.**	$FeCl_3 + H_2SO_4$
2.	Eosine	**4.**	Safranin	**6.**	Sudan 3

OBSERVATIONS AND RESULTS:

The collected *Amalaki* fruit found to be has wt. of an average 27gm. For *Bibhitaki*, as per mentioned in the classics average weight should be 1 *Karsha* and collected sample found to be has weight of an average 8gm. For *Haritaki*, an average weight of Haritaki should be *Dwikarshita* and the collected sample found to be has weight of an average 18gm. *Ghana* and *Gurvi* parameter of *Haritaki* was found prominent and all the samples of *Haritaki* when put down

in the water sinks at the basements (Fig.1) proved they possess *Jala Nimajjana* parameter. [22]

Fig 1: *Jala Nimajjana Parikshana* of *Haritaki*

(a) Over view | (b) Side view

The plant materials were authenticated from Dept. of Botany, University of Rajasthan, Jaipur with the authentication All raw drugs were selected in a desire quantity to prepare test sample I, II, III & IV as per mentioned in classical text.

Macroscopic Study:

The description of macroscopic study of main raw drugs was mentioned in Table 2 along with Fig. 2 and that of all four test drugs were mentioned in Table 3 with Fig.3.

Fig 2: Raw Drugs

Amalaki (*Emblica officinalis*)	*Bibhitaki* (*Terminalia bellerica*)	*Haritaki* (*Terminalia chebula*)
		

Table 2: Macroscopy observations of raw drugs

	Amalaki (*Emblica officinalis*)	*Bibhitaki* (*Terminalia bellerica*)	*Haritaki* (*Terminalia chebula*)
Size	2.5-3.5 cm in diameter	2.5-4.0 cm in diameter	20-30 mm long, 15-25 mm wide
Shape	Globose	Spherical to ovoid	Elongated ovoid
Colour	Light yellowish or pinkish with a few dark specks	Greyish brown with whitish shiny pubescent surface	Yellowish-green
Texture	Fleshy, smooth with six prominent lines	wrinkled appearance	Wrinkled and ribbed longitudinally
Odour	Citrus	Aromatic	Fruity
Taste	Sour-astringent followed by delicately sweet.	Astringent	Astringent bitter

Fig 3: Test Drugs I, II, III and IV

Test drug I	Test drug II	Test drug III	Test drug IV
			

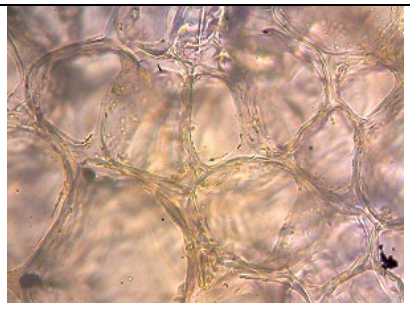
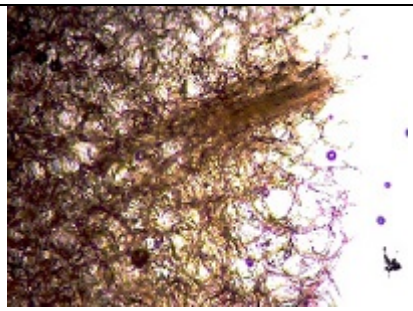
Table 3: Macroscopy observations of Test drugs

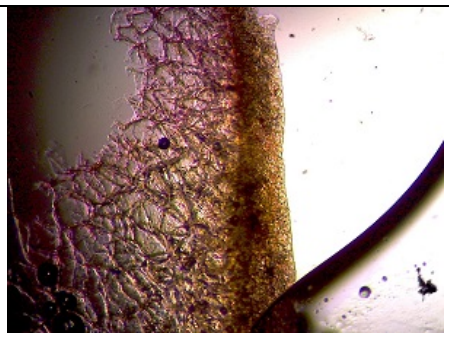
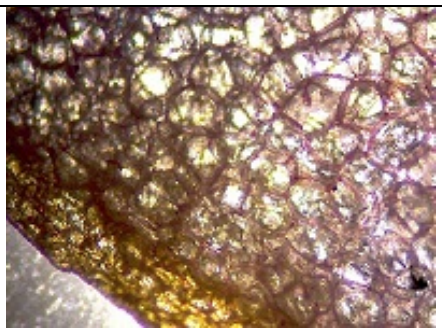
	Test drug I	Test drug II	Test drug III	Test drug IV
Colour	Greenish Brown	Brownish Black	Greenish brown	Greenish brown
Odour	Non-specific unpleasant odour	Odour of Ghrita	Non-specific odour	Non-specific unpleasant odour
Taste	Sour-sweet followed by Bitter	Sour-sweet followed by Astringent	Sour-sweet followed by Bitter	Sour-sweet followed by Bitter
Consistency	Semisolid	Semisolid	Semisolid	Semisolid
Texture	Sticky paste	Sticky paste	Sticky paste	Sticky paste

Microscopic Study:

i) ***Amalaki*** (*Emblica officinalis* Gaertn.): Transverse section of mature fruit shows an epicarp consisting of single layer of epidermis and 2-4 layers of hypodermis; epidermal cell, tabular in shape, covered externally with a thick cuticle and appear in surface view as polygonal; hypodermal cells tangentially elongated, thick-walled, smaller in dimension than epidermal cells; mesocarp forms bulk of fruit, consisting of thin-walled parenchymatous cells with intercellular spaces, peripheral 6-9 layers smaller, ovoid or tangentially elongated while rest of cells larger in size, isodiametric and radially elongated; several collateral fibro vascular bundles scattered throughout mesocarp consisting of xylem and phloem; xylem composed of tracheal elements, fibre tracheids and xylem fibres; tracheal elements show reticulate scalariform and spiral thickenings; xylem fibres elongated with narrow lumen and pointed end; mesocarp contains large aggregates of numerous irregular silica crystals (Fig.4).

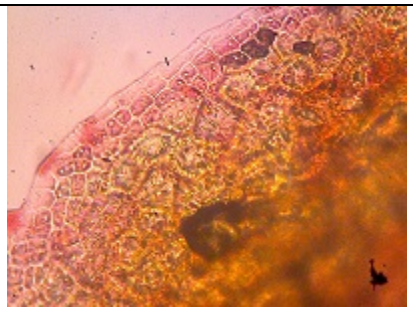
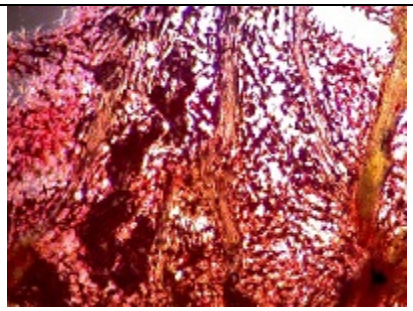
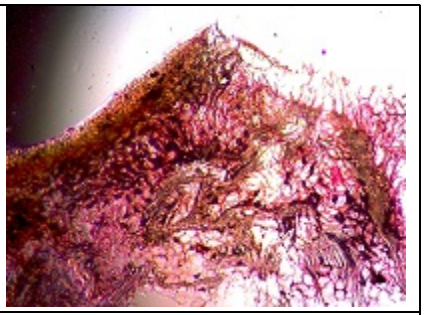
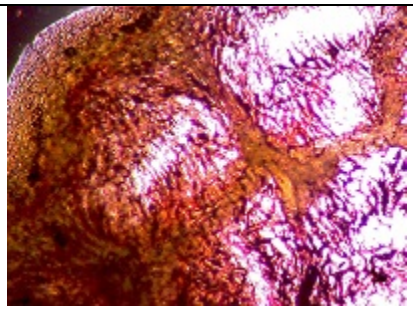
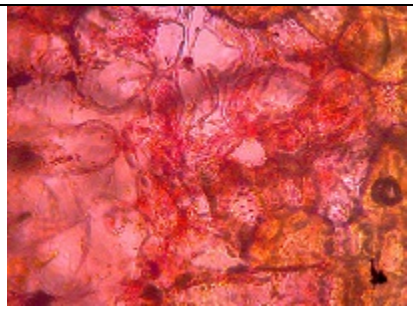
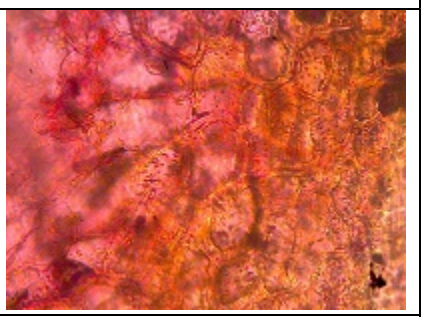
Fig. 4: Microscopy of fruit of *Embelica officinalis* Garten

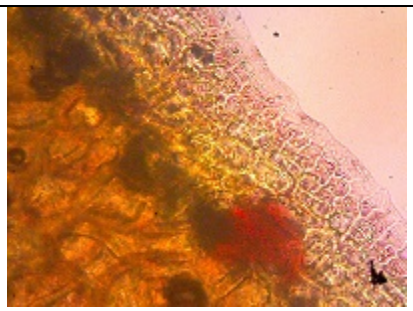
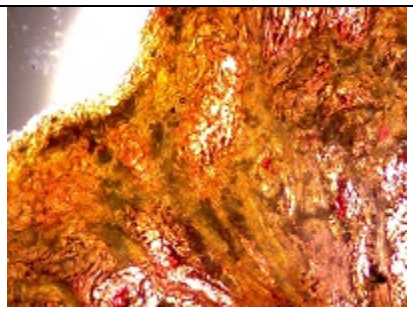
	
Parenchymal cells with intercellular space	Collateral fibro vascular bundle consisting xylem and phloem

	
Epidermis with thick cuticle	Hypodermis layer (polygonal cells), Aggregates of silica crystals

iii) ***Haritaki*** (*Terminalia chebula* Retz): Transverse section of pericarp shows epicarp consisting of one layer of epidermal cells inner tangential and upper portions of radial wall thick, mesocarp, 2-3 layers of collenchyma, followed by a broad zone of parenchyma in which fibres and sc1ereids in group and vascular bundles scattered, fibres with peg like out growth and simple pitted walls, sclereids of various shapes and sizes but mostly elongated, tannins and raphides in parenchyma, endocarp consists of thick-walled sclereids of various shapes and sizes, mostly elongated, epidermal surface view reveal polygonal cells, uniformly thick walled, several of them divided into two by a thin septa, starch grains simple rounded or oval in shape, measuring 2-7 µ in diameter, found in plenty in almost all cells of mesocarp (Fig.5).

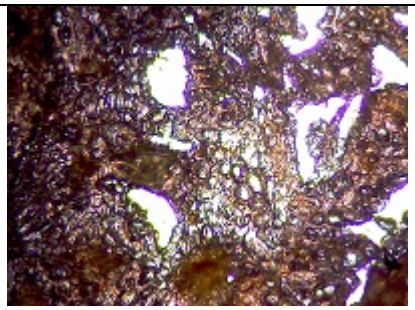
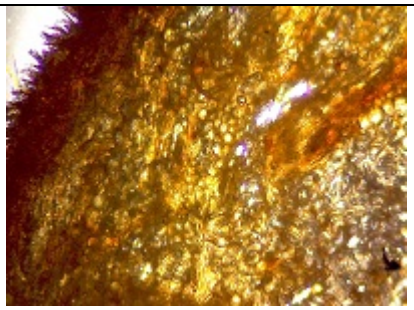
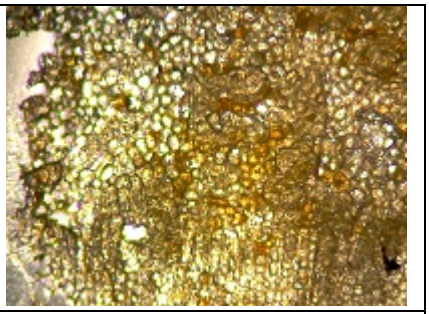
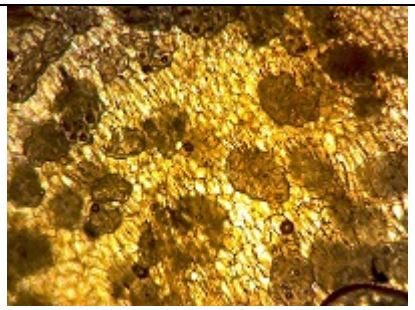
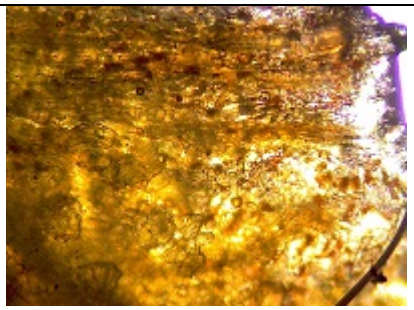
Fig. 5: Microscopy of fruit of *Terminalia chebula* Retz

		
Epicarp consisting of single layer epidermal cell	Long fibres	Calcium oxalate crystal
		
Endocarp consist of vascular part with scleroid fibres,	Mesocarp consisting of parenchymatous cells	Starch grains

	
Mesocarp consisting of 2-3 layers collenchyma cells	Thick elongated scleroid cells, containing tannins

ii) ***Bibhitaki*** (*Terminalia bellerica* Roxb): Transverse section of fruit shows an outer epicarp consisting of a layer of epidermis, most of epidermal cells elongate to form hair like protuberance with swollen base, composed of a zone of parenchymatous cells, slightly tangentially elongated and irregularly arranged, intermingled with stone cells of varying shape and size, elongated stone cells found towards periphery and spherical in the inner zone of mesocarp in groups of 3-10, mesocarp traversed in various directions by numerous vascular strands, bundles collateral, endarch, simple starch grains and some stone cells found in most of mesocarp cells, few peripheral layers devoid of starch grains, rosettes of calcium oxalate and stone cells present in parenchymatous cells, endosperm composed of stone cells running longitudinally as well as transversely (Fig.6).

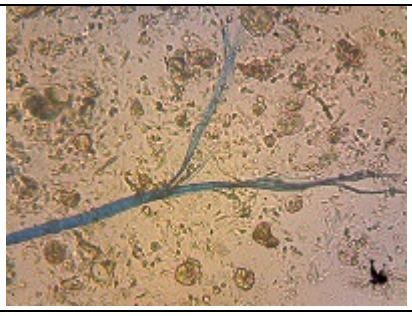
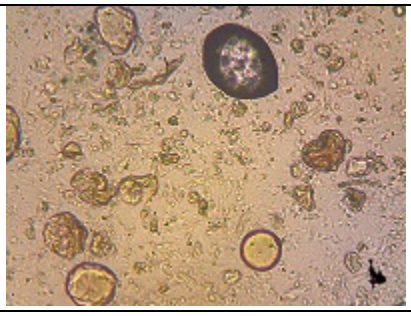
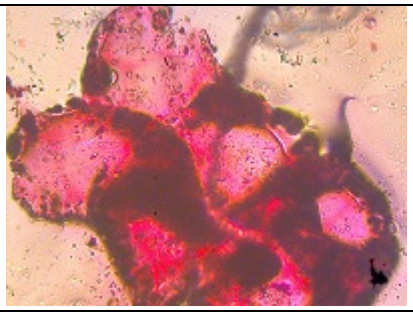
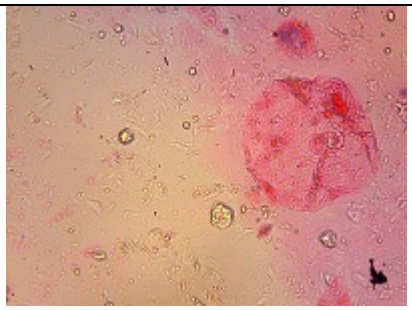
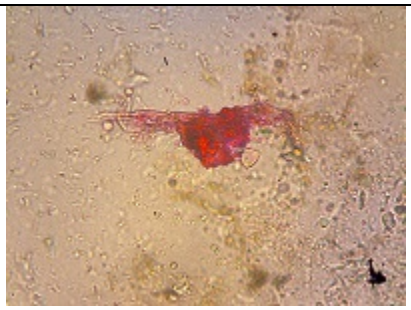
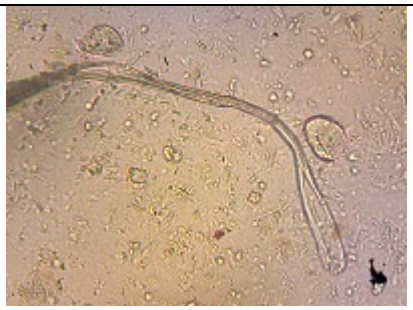
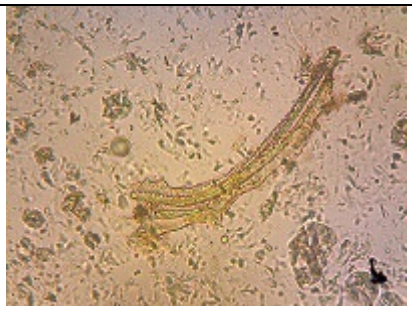
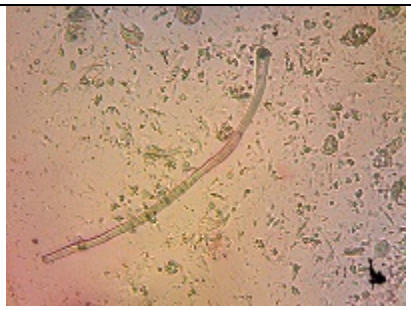
Fig. 6: Microscopy of fruit of *Terminalia bellerica* Roxb

		
Collateral vascular bundles	Crystals & Epicarp consisting of epidermal cells elongate to form hair like protuberance with swollen base	Mesocarp consist of parenchymatous cell with brown pigmentation
		
Stone cells	Schleroid cells	

Powder microscopy of Test drugs:

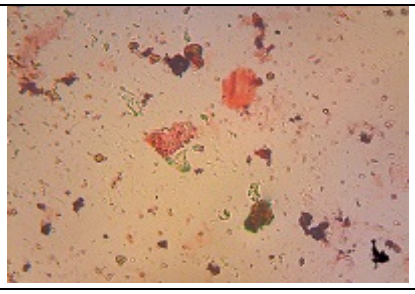
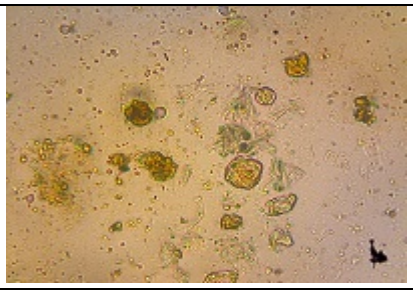
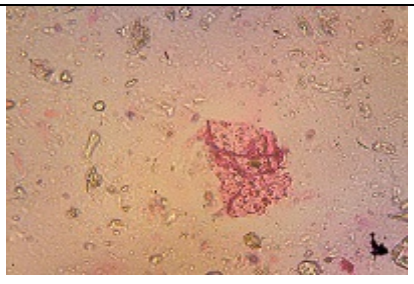
1) **Test drug I:** (Fig.7)

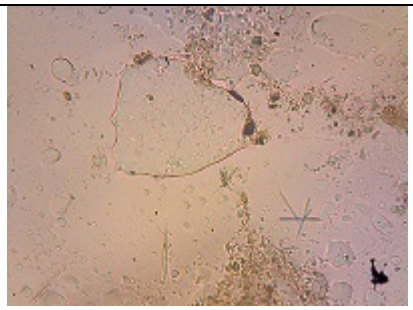
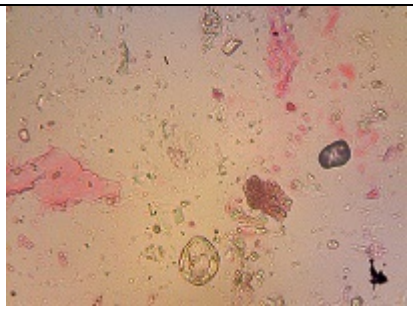
Fig. 7: Microscopy of Test drug I

		
Violet Colour of Starch (Stain: Iodine Solution)	Pale yellow Colour of Cellulose (Stain: Iodine Solution)	Red Colour of cell nuclei (Stain: Safranin)
		
Pink colour of Protein (Stain: Eosin Solution)	Red Colour of Cutin (Stain: Sudan III)	Fibers
		
Tracheids	Trichomes	

2) **Test drug II:** (Fig.8)

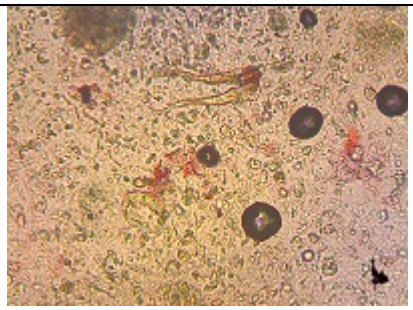
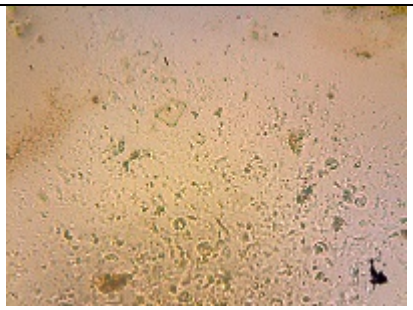
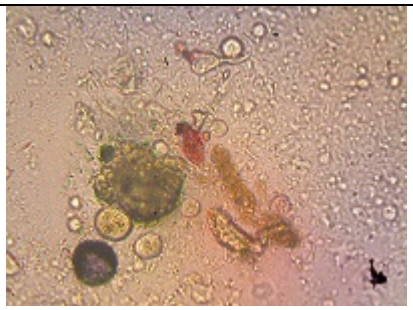
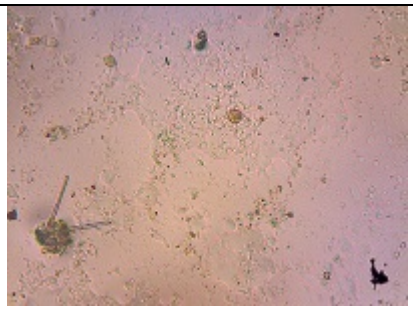
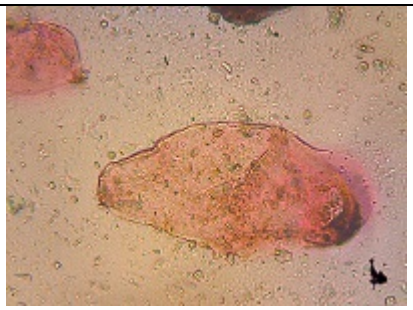
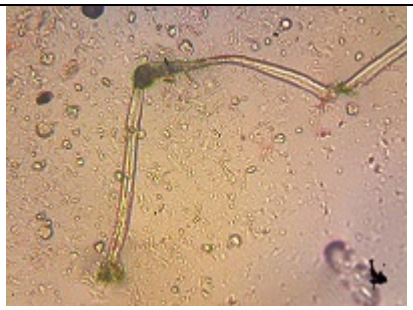
Fig. 8: Microscopy of Test drug II

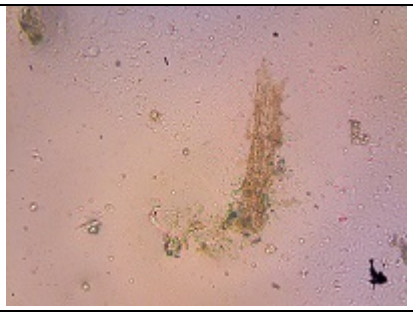
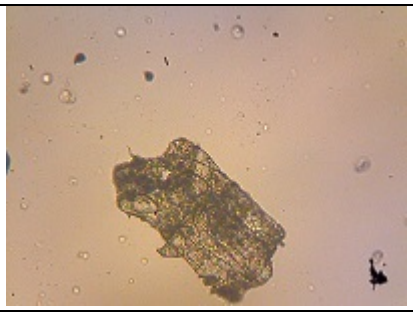
		
Red Colour of cell nuclei	Violet Colour of Starch, Pale yel-	Pink colour of Protein

(Stain: Safranin)	low Colour of Cellulose (Stain: Iodine Solution)	(Stain: Eosin Solution)
		
Green colour of Tannins (Stain: Dil. Ferric Chloride Solution)	Fibers	Vessels
		
Trichome		

3) **Test drug III:** (Fig.9)

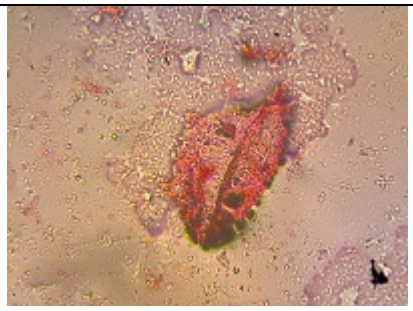
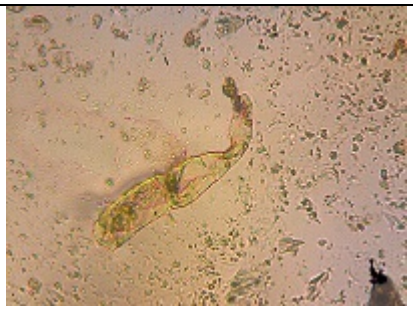
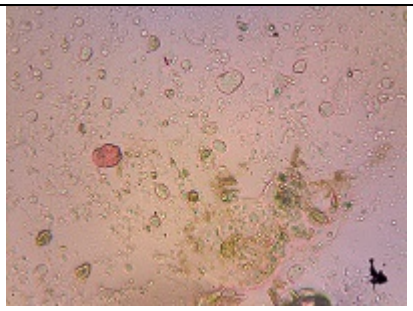
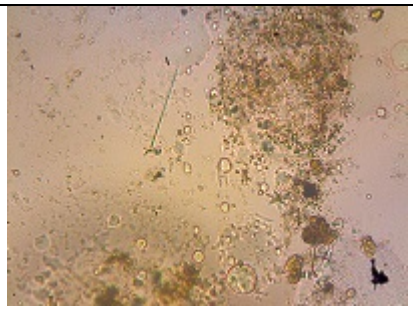
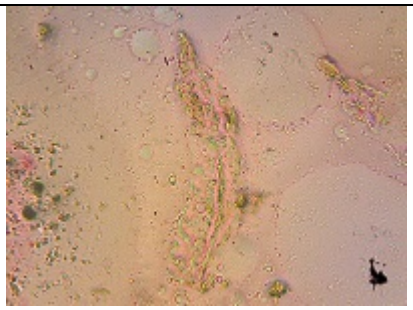
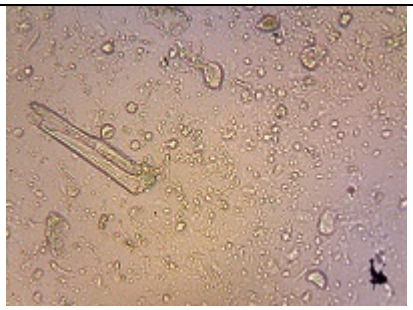
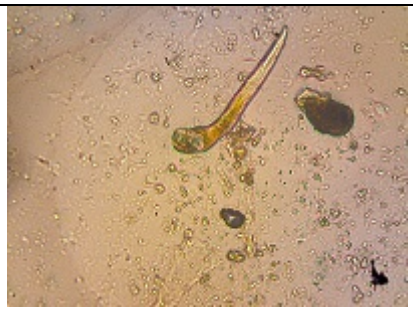
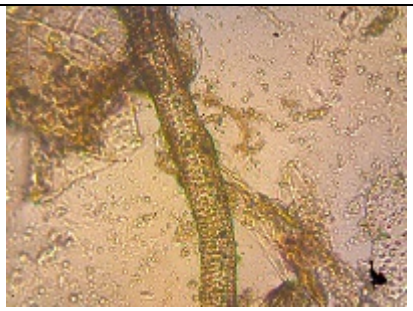
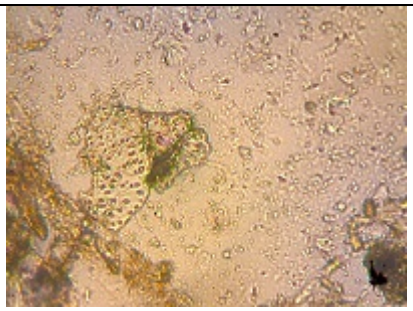
Fig. 9: Microscopy of Test drug III

		
Red Colour of cell nuclei (Stain: Safranin)	Violet Colour of Starch, Pale yellow Colour of Cellulose (Stain: Iodine Solution)	Red Colour of Cutin (Stain: Sudan III)
		
Green colour of Tannins	Pink colour of Protein	Fibers

(Stain: Dil. Ferric Chloride Solution)	(Stain: Eosin Solution)	
		
Trichomes	Tracheids	Vessels

4) **Test drug IV:** (Fig.10)

Fig. 10: Microscopy of Test drug IV

		
Red Colour of cell nuclei (Stain: Safranin)	Violet Colour of Starch, Pale yellow Colour of Cellulose (Stain: Iodine Solution)	Red Colour of Cutin (Stain: Sudan III)
		
Green colour of Tannins (Stain: Dil. Ferric Chloride Solution)	Pink colour of Protein (Stain: Eosin Solution)	Fibers
		
Trichomes	Tracheids	Vessels

DISCUSSION

This study includes pharmacognostical study of selected main raw drugs of CR i.e. *Amalaki* (*Emblica officinalis*), *Bibhitaki* (*Terminalia bellerica*) and *Haritaki* (*Terminalia chebula*) along with four test sample formulations. The macroscopic and microscopic characteristics of the fresh fruits of *Amalaki*, *Haritaki* as well *Bibhitaki* were done and found in accordance with those are given in *Ayurvedic pharmacopeia of India.*[23] This is the first time we conducted pharmacognostical study on CR hence it is not possible to compare this four test drugs with any other standard established formula. So that, the observations of these study are unique and major points to be came to known that, the macroscopic examination of Test drug I, III and IV shows greenish brown colour, non-specific unpleasant odour, sour-sweet followed by bitter in taste while test drug II shows brownish black colour, odour of *Ghrita*, sour-sweet followed by astringent taste. Hence sample II can be differentiated from other reaming 3 samples on the basis of colour, odour and taste However, it is very difficult to identify the rest three samples based on organoleptical examination.

Under powder microscopy, the test drug I, containing *Amalaki* and *Haritaki*, show violet coloured starch, pale yellow coloured cellulose, red coloured cell nuclei, pink coloured protein, red coloured cutin, fibers, tracheids, and trichomes. The test drug II, containing *Amalaki* and *Bibhitaki* shows violet coloured starch grains, pale yellow coloured cellulose, red coloured cell nuclei, pink coloured protein elements, red coloured cutin, fibers, vessels, trichomes & green coloured tannin contains. The test drug III, containing *Haritaki* and *Bibhitaki* shows red coloured cutin, violet coloured starch grains, pale yellow coloured cellulose, red coloured cell nuclei, fibers, tracheids, trichomes, pink coloured protein elements, green coloured tannin contains & vessels. And the test drug IV, containing all three, *Amalaki*, *Haritaki* and *Bibhitaki* shows red coloured cell nuclei, violet coloured starch grains, pale yellow coloured cellulose, red coloured cutin, fibers, tracheids, trichomes, vessels, green coloured tannin contains & vessels in the identification. It is a complex process to known the wholesome of test drug only on the basis of their pharmacognostical study as it is amalgamation of drugs. That's why further lab as well as clinical investigations is needed to confirm its action.

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CONCLUSION

Any plant which is used medicinally requires detail study prior to its use because the therapeutic efficacy is absolutely depends on the quality of the plant used. It is also the first step to standardize a drug which is the need of the day. If the raw drugs are adulterated, then the quality of preparation cannot give the desirable results. So before using a drug it's very much essential to carry out its detailed pharmacognostical study. The morphological examination which includes macroscopic and microscopic characterization is preliminary study to decide authenticity of drugs. In above study, CR was prepared according to the classical standard operative procedure (SOP) mentioned in *Charaka Samhita*, as it has not been prepared or studied yet before. On the basis of the observations recorded and analysis, it suggests that, the results of this work is very encouraging & indicate that *Triphala* should be studied more extensively to confirm & reveal its potential therapeutic effects because it is easily available in the fresh form without any adulteration. On the other hand, CR might be used as a cost effective and adulteration free *Rasayana* overcome to the use of *Chyavanprasha Avaleha,* which is more

costly, having large number of ingredients with issues of unavailability, if found, mostly with adulteration in the market. Advanced screening of the CR may be the area of interest for researchers and will be more helpful towards further in vivo and in vitro studies.

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