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Detailed Pharmacognostical and Pharmaceutical Evaluation of Combine Formulation of Triphala Yavakuta (1:2:4)

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ABSTRACT

Triphala is an important Ayurvedic formulation containing, *Haritaki* (*Terminalia chebula* Retz),Vibhitaki (*Terminalia bellarica Roxb*),*Amalaki* (*Emblica officinalis L.*) as a ingredients. All the constituents are available and prepared according to the reference present in *Yoga Ratnakara Madhyama Khanda. Triphala* (*Haritaki, Vibhitaki and Amalaki*) classified as an important medicine of the *Rasayana* and *Cakshusya* group which is mainly indicated in *Prameha, Sthoulya* and *Kustha* and also it is believed to promote health, immunity and longevity. Though they are individually very potent drug and have their own specific mode of action on different disease conditions. *Triphala* the sense of its *Rasa, Vipaka* and to some extent its *Prabhava* is similar to its three contents but its Virya, *Doshagnata* and *Guna* are not exactly similar to the composing three drugs. This is due to *Samyoga Samskara* by which the clinical efficacy of particular drug changes several methods are adopted to prepare Triphala some uses equal proportions (1:1:1) some in different proportions (1:2:4). Till date there is only one scientific work has been carried out and less publication of 1:2:4 combination forms of *Triphala*. In the present study the *Yavakuta* formulation of *Triphala* was subjected to Pharmacognostical (microscopic), HPTLC, and pharmaceutical (evaluation of various physiochemical parameters) evaluation in order to prepare a preliminary profile of the formulation. The sample was subjected for various phytochemical parameters like water soluble extractive (55.8%w/w), alcohol soluble extractive (42.2% w/w), ash value (0.89% w/w), loss on drying (11.39% w/w), and the pH (5.0), HPTLC. The HPTLC, solvent system was Toluene:ethyl acetate (9:1), showed the presence of 5 spots at 254 nm and 2 spots at 366 nm. Thus the physiochemical and microscopic characters achieved may provide guidelines for standardization of formulation, *Triphala Yavakuta* combine formulation (1:2:4).

Keywords: *Triphala Yavakuta*, HPTLC, Pharmacognostical, Physiochemical Evaluation

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INTRODUCTION

Triphala is a drug widely used in many disorders due to its various pharmacological activities. *Triphala* is composed of the three myrobalans[1] *Terminalia chebula* Retz. (*Haritaki*), *Terminalia bellerica* Roxb. (*Vibhitaki*) and *Emblica officinalis* Gaertn. (*Amalaki*) and is one of the most commonly used Ayurveda preparations[2]. It contributes to the balance of all the three *Doshas Vata*, *Pitta* and *Kapha*. *Amalaki* for *Pitta*, *Vibhitaki* for *Kapha* and *Haritaki* for *Vata*[3]. *Ama*, the accumulation of unwanted products or toxins in the body is the major reason for many diseases and physical conditions. The main advantages associated with *Triphala* are promotes good digestion, colon cleaning, blood purification, mental alertness, better eyesight etc[4]. *Triphala* is usually a combination of equal parts of all the three fruits in powdered form. But in classics depending upon the *Doshic* combination and disease condition proportion of these three drugs varies[5]. Triphala is known for its medicinal properties such as anti-aging, antianaemic, antibacterial, anticancerous, antidiabetic, antidiarrhoeal, antimutagenic, antioxidant, antiparasitic, antiviral, cardio protective, hepatoprotective, hypocholesterolaemic, radio protective and colon cleanser. All of the three constituents of Triphala are active and shows slight difference in activities under different sets of environmental conditions but the combination all three showed a significant and efficient effect as compared to individual components. Triphla is rich in active ingredients like tannins, carbohydrates, saponins, ellagic acid, sorbitol and ascorbic acid[6].

Till date there is only one scientific work has been carried out and less publication of 1:2:4 combination forms of *Triphala*. In the present study the *Yavakuta* formulation of *Triphala* was subjected to Pharmacognostical (microscopic), HPTLC, and pharmaceutical (evaluation of various physiochemical parameters) evaluation in order to prepare a preliminary profile of the formulation.

MATERIALS AND METHOD:

Collection of the drug:

Mature fruits of *Haritaki*, *Vibhitaki* and *Aamalaki* as Unequal quantities like 1:2:4 were procured from the Pharmacy of IPGT & RA, Jamnagar, India. Their characteristics were confirmed in the Pharmacognosy of IPGT & RA, Jamnagar, by correlating their morphological and microscopical features with relevant literature.

Preparation of the drug:

Collected dried fruits of *Triphala* (*Haritaki, Vibhitaki, Amalaki*) as Unequal quantities like 1:2:4 made into coarse powder with the help of manual grinder, and mixed together to get till well mixture.

Table 1: Ingredients of *Triphala Yavakuta* (*Yo.Ra, Madyama Khanda* 1)

Sr No.	Sanskrit Name	Botanical Name	Proportion
1	*Haritaki*	*Terminalia chebula Retz.*	1 Part
2	*Vibhitaki*	*Terminalia bellarica Roxb.*	2 Part
3	*Amalaki*	*Emblica officinalis L.*	4 Part

Pharmacognostical evaluation:

Various characters like colour, odour, taste and touch are recorded by using sensory organs.[7] Powder microscopy of the finished product was done without stain and after staining with Phloroglucinol+HCl micro photographs were taken under Carl- Zeiss Trinocular microscope attached with camera[8] By Powder microscopy observed the characters, determined the chemical nature of the cell wall along with the form and chemical nature of the content of the cells.

Pharmaceutical evaluation*:*

Triphala Yavakuta was subjected to physicochemical study in order to develop analytical profiles. In this phase following parameter were carried out -Loss on drying at 1100C, pH value, ash value, water soluble extractive, alcohol soluble extractive[9].

High Performance Thin Layer Chromatography.

In HPTLC study of *Triphala Yavauta*, methanol extract of *Triphala yavakuta* was spotted on pre-coated silica gel GF 60254 Aluminium plate by mean of Camag Linomate V sample applicator fitted with a 100µl Hamilton syringe. The mobile phase consisted of Toluene: Ethyl acetate a ratio of 9:1 v/v. After development, densitometric scan was performed with a Camag TLC scanner III in reflectance in absorbance mode at 254 and 366 nm under control of Win CATS Software (V1.2.1.Camag). Then, the plate was sprayed with Vanillin Sulphuric acid followed by heating and then visualized in daylight. Densitogram curve of HPTLC of *Triphala Yavakuta* is given in **plate no** . The colour and Rf values of resolved spots of HPTLC were noted. (Table 4)[10,11].

RESULTS AND DISCUSSION

PHARMACOGNOSTICAL STUDY

Organoleptic Characters:

Color, Odor, Taste, Touch and Texture and powder nature of *Haritaki, Vibhitaki,* and *Amalaki* were recorded by sensory characters and results were as follows,[12]

Table 2: Organoleptic characters of *Triphala Yavakuta*: Individual

Characters	*Haritaki*	*Vibhitaki*	*Amalaki*
Colour	Yellowish Brown	Yellowish Brown	Dark Brown
Odour	Characteristic	Characteristic	Characteristic
Taste	Astringent & Bitter	Bitter & Astringent	Sour & Astringent

Touch	Fine	Fine	Rough
Texture	Fine	Fine	Rough

Organoleptic characters of *Triphala* - Combine formulation:

Colour, Odour, Taste, Touch and Texture and powder nature of equal and un equal proportion of *Triphala* were recorded by sensory characters and results were as follows,

Table 3: Organoleptic characters of *Triphala*: Combine formulation

Characters	*Triphala* (1:1:1)	*Triphala* (1:2:4)
Colour	Yellowish Brown	Dark yellowish Brown
Odour	Characteristic	Characteristic
Taste	Astringent	Astringent
Touch	Fine	Fine
Texture	Fine	Fine

Plate 1: Triphala Yavakuta (1:2:4)

Hartaki	Vibhitaki	Amalaki	Triphaa Yavakuta

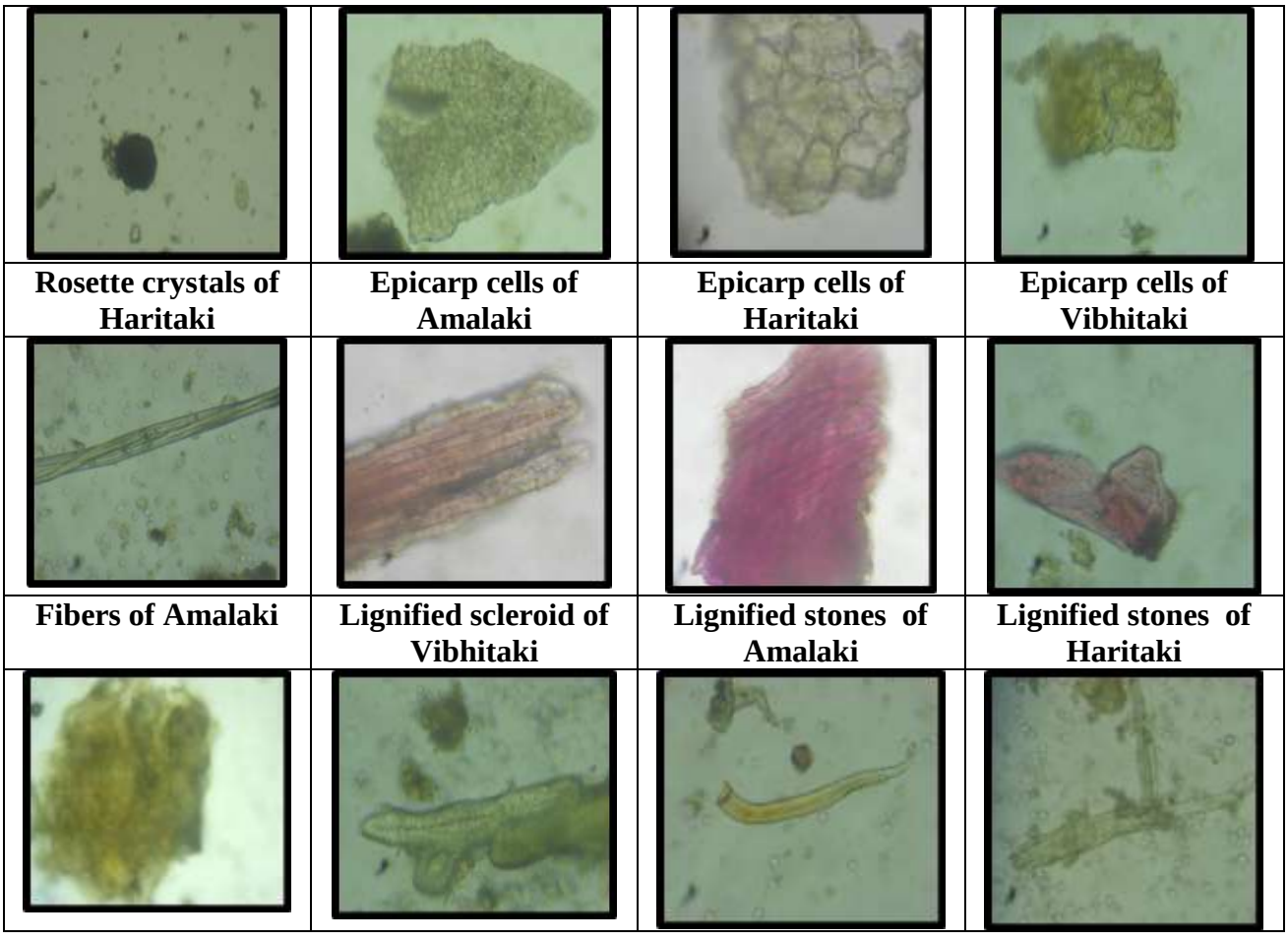
Microscopic characters:

Microscopic characters of individual raw drugs as such shows definite variation, the compound formulation in different proportion also varies microscopically this may be due to the effect of Panchamahabuta constitution. The main interaction of Panchamahabhuta are Pitted stone cells with wide lumen, fibers with wide lumen indicates that characters may be influenced by Vayu and Akash. The specific cells and characters were loosely arranged with the influence may be Jala. The clumping and dissolving nature of rosette crystals because of excess addition of Amalaki indicating influence of Agni ultimately integrate the potency of the formulation. The 1:1:1 shows less number of simple fibers, silica crystals and sclerides with lumen, trichome with tannin and clear rosette crystals. Whereas 1:2:4 results shows large number of simple fibers, silica crystals and sclerides with lumen, trichome without tannin and clumped rosette crystals. The characters which are less concentration with lumen, clumping nature of crystals and silica crystals mainly may due to variant ingredient proportion.

Table 4: Powder microscopic characters of *Triphala* (individual and combine) drug

Sl.no.	*Haritaki*	*Vibhitaki*	*Amalaki*	*Triphala Yavakuta* (1:2:4)
1	Epicarp cells	Fibers	Fibers	Large no of simple fibers
2	Epidermal cells	Trichome base filled with tannin	Starch grains	Mesocarp cells
3	Mesocarp cells	Mesocarp cells	Mesocarp cells	Pitted stone cells
4	Lignified fibres	Rosette crystals	Stone cells	Pitted vessels
5	Tannin	Sclerides	Stone cell in group	Rosette crystals clumped
	Pitted stone	Compound starch grains	Silica crystals	Large quantity of Sclerides , lumen
7	Pitted vessels	Stone cells	Tannin	Stone cells + pitted pharanchymatous
8	Starch grains simple and compound	Tannin	-	Trichomes without Tannin
9	Sclerides	Stones cells+Trichome	-	Large no of silica crystals

Plate 2- Powder microscopic characters of *Triphala Yavakuta*



Rosette crystals of Haritaki | **Epicarp cells of Amalaki** | **Epicarp cells of Haritaki** | **Epicarp cells of Vibhitaki**

Fibers of Amalaki | **Lignified scleroid of Vibhitaki** | **Lignified stones of Amalaki** | **Lignified stones of Haritaki**

Mesocarp cells of Amalaki	Scleroid of Amalaki	Trachoma of Vibhitaki	Scleroid of Vibhitaki

Physicochemical analysis:

Results of physicochemical analysis i.e. loss of drying, ash value, water soluble extract, alcohol soluble extract and pH valve are shown in table 5.

Table 5: Physico – chemical parameters.

Sl No	Analytical parameter	Results
1.	Loss on Drying	11.35%w/w
2.	Ash value	0.89%w/w
3.	Water soluble extractive	55.8%w/w
4.	Alcohol soluble extractives	42.2%w/w
5.	pH value	5.0

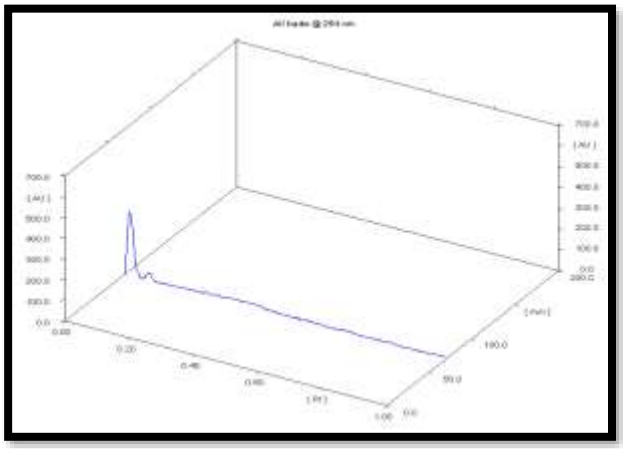
High performance thin layer chromatography (HPTLC):

The color and Rf values of resolved spots of HPTLC were noted. (Table 6)

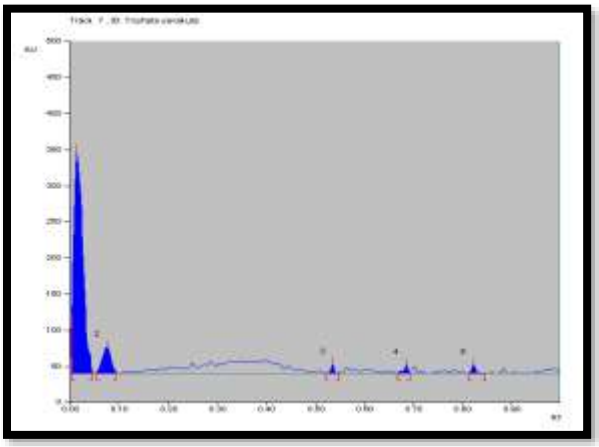
Table 6: R_f values of Methanolic extract of *Triphala Yavakut*a obtained by HPTLC

Sample Name	Visualize under short UV (254 nm)		Visualize under short UV (366 nm)	
	No. of spots	Rf value	No. of spots	Rf value
Yavakut* of *Triphala	5	0.01, 0.08, 0.54, 0.69, 0.82	2	0.01, 0.54

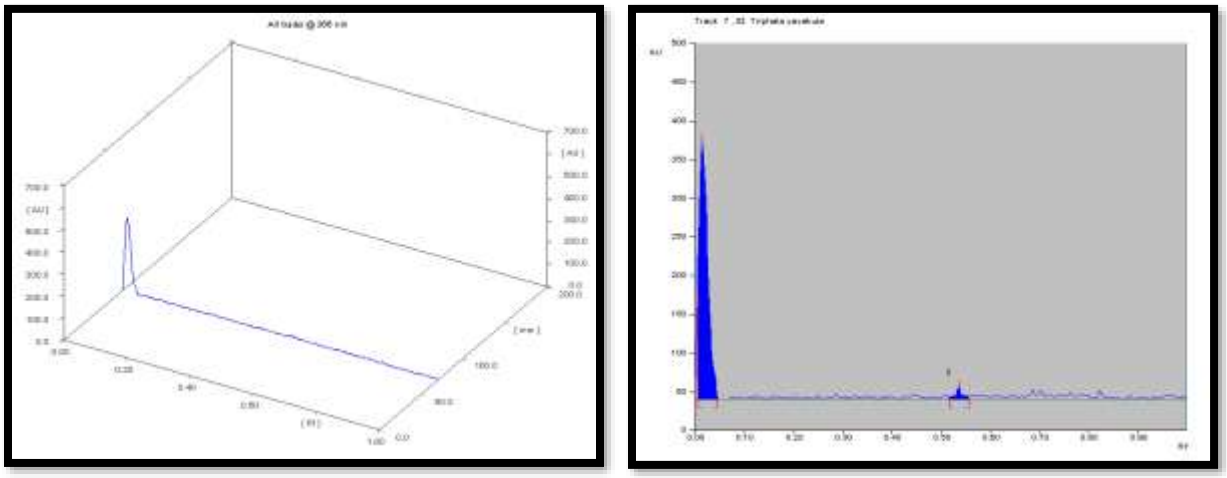
Plate 3. HPTLC in *Triphala Yavakuta*



254nm – 3D View

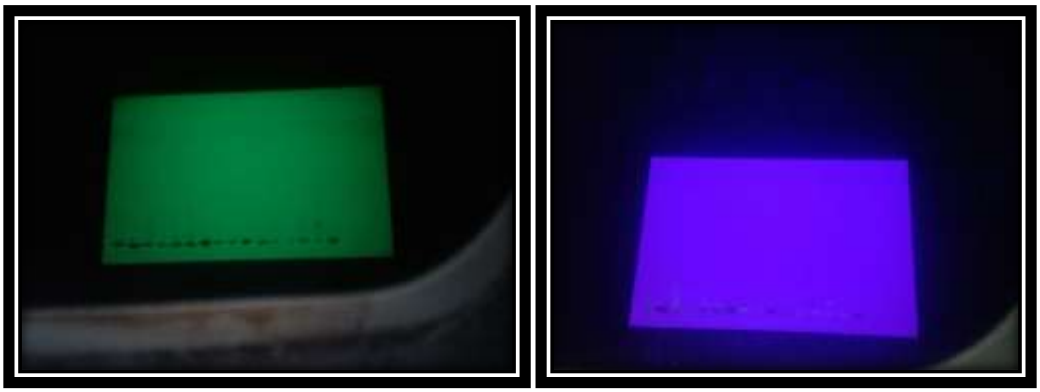


254 nm - Peak View



366 nm – 3D View **366 nm - Peak**

Plate 4. HPTLC plates of *Triphala Yavakuta*



At 254 nm **At 366 nm**

DISCUSSION:

Organoleptic characters:

Organoleptic characters of individual raw drug vary in color, odor and taste. When they are combined together, quantitatively also there is a definite change in the characters. Here 1:2:4 mixture shows strong astringent, dark yellowish brown and characteristic taste, color and odor respectively.

Microscopic characters:

The 1:2:4 shows large number of simple fibers, silica crystals and sclerides with lumen, trichome without tannin and clumped rosette crystals. These changes may be due to variant ingredient proportion and also influence of *Panchamahabhuta* constitution. The efficacy of the drug too changes in both the combinations.Due to Acidic pH the crystals of *Amalaki* dissolved the crystals of *Haritaki* and *Vibhitaki*. So, *Amalaki* has *Chakshushya* action more than *Haritaki* and *Vibhitaki*.

On the basis of above special findings we selected *Triphala Yavakuta* ratio as 1:2:4. The HPTLC showed that 2 spots were found at 254nm and 5 spots at 366nm.

CONCLUSION:

So on the basis of above pharmacognostical, physico-chemical analyzing details, we can prove fact that individual and different proportions of *Triphala* act differently in many clinical conditions.This study scientifically reveals that the combines formulation (1:2:4 *Triphala Yavakuta*) shows genuinely of the finished product (representing *Haritaki, Vibhitaki* and *Amalaki*) but some of the characters are changed due to the combination, quantity and influence of Panchamahabuta constitution; this preliminary information can be used for reference in future for further research development.

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