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Research Article

PHARMACEUTICAL EVALUATION OF *CHATURTHAMALAKA RASAYANA*-AN AYURVEDA COMPOUND

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ABSTRACT

In *Ayurveda*, *Rasayana* is mentioned as a unique preventive as well as curative therapy to achieve healthy long life. The modern medical world is seeking for an alternative class of immunomodulatory drugs, which is nothing but the category of *Rasayana*. Among this *Rasayana* formulation, *Chaturthamalaka Rasayana* (CR) is the one that formulation, mentioned just after *Chyavanprasha Avaleha* in classical literature of *Charaka Samhita*. CR consists of four formulations. It is nothing but the simple permutation and combination of *Amalaki* (*Emblica officinalis* Gaertn.), *Bibhitaki* (*Terminalia bellerica* Roxb.) and *Haritaki* (*Terminalia chebula* Retz.) collectively called as *Triphala*. In present study, it was prepared according to the classical standard operative procedure (SOP) so as to evaluate a physicochemical and phytochemical profile of CR as per standard lab protocols, for all the four formulations under CR, along with the main three raw drugs which ensure the quality. This pharmaceutical analysis differentiates one compound from another by developing its identification markers. As the CR has not been yet studied before, it will be more helpful to evaluate the pharmaceutical screening of this four formulations as an initial step towards standardization. In the future, this study will be helpful to prepare the monograph of CR in the *Ayurvedic formulary of India* (AFI).

KEYWORDS: *Chaturthamalaka, Rasayana, Charaka, Ayurveda, Triphala,* Pharmaceutical.

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 to 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 *Chaturthamalaka Rasayana* (CR) mentioned in *Charaka Samhita*, may be one of the suitable option for this *Chyavanprasha Avaleha.*[13] Before evaluating the pharmacological and clinical aspect of CR, it is necessary to conduct the pharmaceutical study for quality assurance. As the CR has not been studied yet before, it will be more helpful to evaluate the physicochemical and phytochemical screening of these four formulations,

as an initial step towards standardization for the future studies. Standardization starts right from the collection of raw materials up to their clinical applications and efficacy. 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 to standardize the *Ayurveda* formulations.

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 was 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.

Classical method of CR preparation [14]

Authenticated raw drugs were added in a four different combinations I, II, III and IV 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) [15]. (*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 allowed 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*). Equal quantity of *Ghrita*, *Madhu*, *Tila (Sesamum indicum* L.*,* authentication *Pishti*, *Tila Tailam* and *Sharkara* were added in the above prepared four combinations. (*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.

Test Drug Preparation

For the analysis purpose, the final quantity of raw drugs were taken [Table 1] in the proportions mentioned in the classical reference.

Table 1: Formulation composition of CR

S.No.	*Dravya*	Test drug I	Test drug II	Test drug III	Test drug IV
1.	*Amalaki* (*Swinna*)	125gm	125gm	**--**	100gm
2.	*Bibhitaki* (*Swinna*)	**--**	125gm	125gm	100gm
3.	*Haritaki* (*Swinna*)	125gm	**--**	125gm	100gm
Total Pulp (wt.)		**250gm**	**250gm**	**250gm**	**300gm**
4.	*Ghrita*	250gm	250gm	250gm	300gm
5.	*Madhu*	250gm	250gm	250gm	300gm
6.	*Tila*	250gm	250gm	250gm	300gm
7.	*Tila Tailam*	250gm	250gm	250gm	300gm
8.	*Sharkara*	250gm	250gm	250gm	300gm
Test drug (wt.)		1500gm	1500gm	1500gm	1800gm
9.	*Dadhi*	Added in an equal quantity, same as other constituents, at the time of examination.			

Method of Physico-chemical evaluation

CR was analysed by using standard qualitative and quantitative parameters. All the procedures were conducted at pharmacology laboratory, post-graduation department of *Dravyagunavigyana*, National Institute of *Ayurveda* (N.I.A.), Jaipur, Rajasthan. As the preparation contains the fresh raw material, due to which the presence of more moisture content may create preservation problem. Hence the parameters selected for the analysis were moisture content [16], pH value [17], total ash value [18], acid insoluble ash value [18], water insoluble ash value [18], alcohol soluble extractive [19], water soluble extractive [19], total – reducing – non reducing sugar value [20], Rf value of TLC [21] along with phytochemical analysis of the main fresh raw drugs i.e. *Amalaki, Haritaki, Bibhitaki* and all four test drugs combinations under CR. Thin layer chromatography (TLC) studies were carried out after making appropriate solvent system with ethanolic extract of CR. As the CR was not yet studied before. So we were try to figure out that with different solvent combinations and after trials we got the final mobile solvent phase as Toluene:Ethyl acetate: Formaldehyde (6.5:3:0.5) which was found to be more appropriate for the study.

Phyto-chemical analysis [22]

Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. A plant cell produces two types of metabolites: primary metabolites involved directly in growth and metabolism (carbohydrates, lipids and proteins), and secondary metabolites not involved in metabolic activity (alkaloids, phenolics, sterols etc) but act as defense chemicals. Freshly prepared extracts of all four test drugs along with samples of *Amalaki* (*Emblica officinalis* Gaertn.), *Bibhitaki* (*Terminalia bellerica* Roxb.) and *Haritaki* (*Terminalia chebula* Retz. were tested for the presence of various active phytocompounds like carbohydrates, triterpenoid alkaloid, amino acids, protein, saponin, glycosides, phenols, flavonoid, steroids, tannins etc.

Chromatography [23]

Thin layer chromatography (TLC) is a tool for separation and identification of chemical constituent. It is a technique in which a solute undergoes distribution between two phases, a stationary phase acting through adsorption and a mobile phase in the form of a liquid. The adsorbent is a relatively thin, uniform layer of dry finely powdered material applied to a glass, plastic or metal sheet or plate. Glass plates are most commonly used. Separation may also be achieved on the basis of partition or a combination of partition and adsorption, depending on the particular type of support, its preparation and its use with different solvent. Identification can be effected by observation of spots of identical Rf value and about equal magnitude obtained, respectively, with an unknown and a reference sample chromatographed on the same plate. A visual comparison of the size and intensity of the spots usually serves for semi-quantitative estimation.

TLC plates used were coated with 0.25 mm layer of silica gel G60F254 with fluorescent indicator (Mercks). Each plate dimension is 10 cm long and 2 cm width. Activation of pre-coated silica gel G60F254 was done by drying in hot oven at 105^0 C for one to two hour.

Mobile solution[24]

For *Amalaki* - Acetone:Chloroform:Formic acid: Toluene:Methanol (2:2:5:0.5:3:2)

For *Bibhitaki* - Chloroform:Ethyl acetate:Formic acid (5:4:1)

For *Haritaki* - Toluene:Ethyl acetate:Formic acid (5:4:1)

For Test drug I, II, III and IV - Toluene:Ethyl acetate: Formaldehyde (6.5:3:0.5)

Sample was applied with the help of capillary 1 cm above the base of T.L.C. plate. Then it was dipped in mobile solution. T.L.C. plate was removed from the mobile solution immediately after the spot reached the 1 cm below the top of the T.L.C. plate. For Visualization, 1% aq. Ferric Chloride solution for *Amalaki, Haritaki and Bibhitaki* Sample. Anisaldehyde Sulphuric Acid for Test drug I, II, III and IV. Measured and recorded the distance of each spot from the point of its application and calculated Rf. value by dividing the distance travelled by the spots by the distance travelled by the front of the mobile phase.

OBSERVATIONS AND RESULTS

The observations of physicochemical and phytochemical analysis of raw drugs is mentioned in [Table 2 and 4], where as that of test drug I, II, III and IV is in [Table 3 and 5] respectively.

Physio-chemical analysis

Table 2: Physiochemical analysis of raw drugs

S.No.	Physicochemical standards	*Amalaki* (Fresh Fruit)		*Bibhitaki*		*Haritaki*	
		Results % w/w	API standard value	Results % w/w	API standard value	Results % w/w	API standard value
1.	Moisture content	88.882%	NLT 80%	7.98%	Not mention	8.65%	Not mention
2	PH Value	2.67	Not mention	4.07	Not mention	4.62	Not mention
3.	Water soluble extractive value	63.5%	NLT 50%	62.1%	NLT 35%	65.314%	NLT 60%
4.	Alcohol soluble extractive value	43.94%	NLT 40%	32.952%	NLT 8%	51.526%	NLT 40%
5.	Total ash	4.13%	NMT 7%	5.568%	NMT 7%	2.508%	NMT 5%
6.	Acid insoluble ash	0.764%	NMT 2%	0.466%	NMT 1%	0.50%	NMT 5%
7.	Water soluble ash	2.34%	Not mention	2.942%	Not mention	3.186%	Not mention

Table 3: Physiochemical analysis of Test drug I, II, III and IV

Sr.No.	Physicochemical standards	Test drug I	Test drug II	Test drug III	Test drug IV	API standard value
1.	Moisture content	49.198%	46.87%	51.32%	47.292%	Not mention
2	PH Value	5.65	5.17	4.72	4.85	Not mention
3.	Water soluble extractive value	30.41%	23.552%	24.504%	28.866%	Not mention
4.	Alcohol soluble extractive value	32.486%	21.886%	29.068%	26.466%	Not mention
5.	Total ash	1.058%	1.222%	1.394%	1.058%	Not mention
6.	Acid insoluble ash	0.086%	0.086%	0.124%	0.05%	Not mention
7.	Water soluble ash	0.344%	0.316%	0.464%	0.332%	Not mention
8.	Total sugar	13.496%	12.797%	23.9857%	13.8075%	Not mention
9.	Reducing sugar	5.253%	6.848%	6.256%	6.338%	Not mention
10.	Non reducing sugar	8.243%	5.949%	17.7297%	7.4695%	Not mention

Phytochemical Analysis

Table 4: phytochemical analysis of raw drugs

Name of Test	***Emblica officinalis* Gaertn**		***Terminalia bellerica* Roxb**		***Terminalia chebula* Retz**	
	Aq. Ext.	**Alco. Ext.**	**Aq. Ext.**	**Alco. Ext.**	**Aq. Ext.**	**Alco. Ext.**
Carbohydrate						
Molish test	-ve	-ve	-ve	+ve	-ve	-ve
Benedict test	+ve	+ve	+ve	+ve	+ve	+ve
Fehling test	-ve	+ve	+ve	-ve	-ve	-ve
Barfoed test	-ve	+ve	+ve	+ve	+ve	-ve
Alkaloids						
Dragendorff test	+ve	+ve	+ve	+ve	-ve	-ve
Wagner's test	-ve	+ve	+ve	+ve	-ve	-ve
Hager's test	+ve	-ve	+ve	-ve	+ve	-ve

Amino acids						
Ninhydrine	+ve	-ve	-ve	-ve	-ve	-ve
Protein						
Biuret test	+ve	-ve	-ve	-ve	+ve	-ve
Xanthoproteic test	-ve	+ve	-ve	-ve	-ve	-ve
Millon test	+ve	-ve	-ve	-ve	-ve	-ve
Saponin						
Foam test	+ve	-ve	+ve	+ve	+ve	+ve
Glycosides						
Borntrager's test	+ve	-ve	-ve	+ve	-ve	+ve
Phenolic compound						
	+ve	+ve	+ve	+ve	+ve	+ve
Flavonoids						
Shinoda test	+ve	-ve	-ve	+ve	-ve	+ve
Steroids						
Salkowski test	-ve	-ve	+ve	+ve	-ve	-ve
Tannins						
$FeCl_3$ test	+ve	+ve	+ve	+ve	+ve	+ve
Lead acetate test	+ve	+ve	+ve	+ve	+ve	+ve
Pot. Dichromate test	+ve	-ve	+ve	-ve	+ve	-ve

Table 5: Phytochemical analysis of Test drugs

Name of Test	Test drug I		Test drug II		Test drug III		Test drug IV	
	Aq. Ext.	Alco. Ext.	Aq. Ext.	Alco. Ext.	Aq. Ext.	Alco. Ext.	Aq. Ext.	Alco. Ext.
Carbohydrate								
Molish test	-ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve
Benedict test	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Fehling test	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve
Barfoed test	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Alkaloids								
Dragendorff test	-ve	+ve	+ve	+ve	-ve	+ve	-ve	+ve
Wagner's test	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve
Hager's test	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve
Amino acids								
Ninhydrine	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve
Protein								
Biuret test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Xanthoproteic test	-ve	+ve	-ve	+ve	+ve	+ve	+ve	+ve
Millon test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
Saponin								
Foam test	+ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve
Glycosides								

Borntrager's test	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve
Phenolic compound								
	+ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve
Flavonoids								
Shinoda test	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve
Steroids								
Salkowski test	-ve	-ve	+ve	+ve	-ve	-ve	+ve	+ve
Tannins								
$Fecl_3$ test	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Lead acetate test	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve
Pot. Dichromate test	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve

Chromatography

The Rf value of raw drugs is mentioned in [Table 6] along with [Fig 1] and The Rf value of all test drugs I,II,III and IV is mentioned in [Table 7] along with [Fig 2].

Table 6: Chromatography of raw drug samples

Sample	*Amalaki*	*Haritaki*	*Bibhitaki*
Rf Value	0.13, 0.32, 0.37, 0.61	0.16, 0.23, 0.27, 0.53	0.14, 0.26, 0.41, 0.68,
Mobile Solution	Acetone:Chloroform:Formic acid:Toluene:Methanol	Toluene:Ethyl acetate:Formic acid	Chloroform:Ethyl acetate:Formic acid
Visualization	1% aq. Ferric Chloride	1% aq. Ferric Chloride	1% aq. Ferric Chloride

Table 7: Chromatography of Test drugs

Test Drug	I	II	III	IV
Rf Value	0.16, 0.27, 0.52, 0.59, 0.65, 0.75	0.16, 0.27, 0.51, 0.60, 0.66, 0.75	0.17, 0.28, 0.53, 0.60, 0.67, 0.75	0.17, 0.29, 0.54, 0.64, 0.68, 0.75
Mobile Solution	Toluene:Ethyl acetate:Formaldehyde			
Visualization	Anisaldehyde Sulphuric Acid			

FIGURES

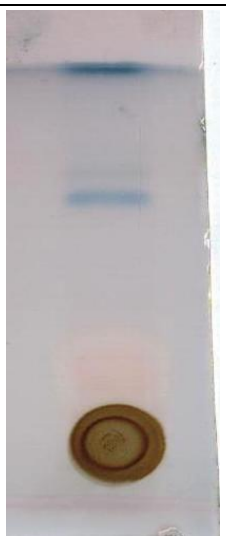
Sample	*Amalaki*	*Haritaki*	*Bibhitaki*
TLC Plates			

Fig 1: Chromatography of raw drug samples

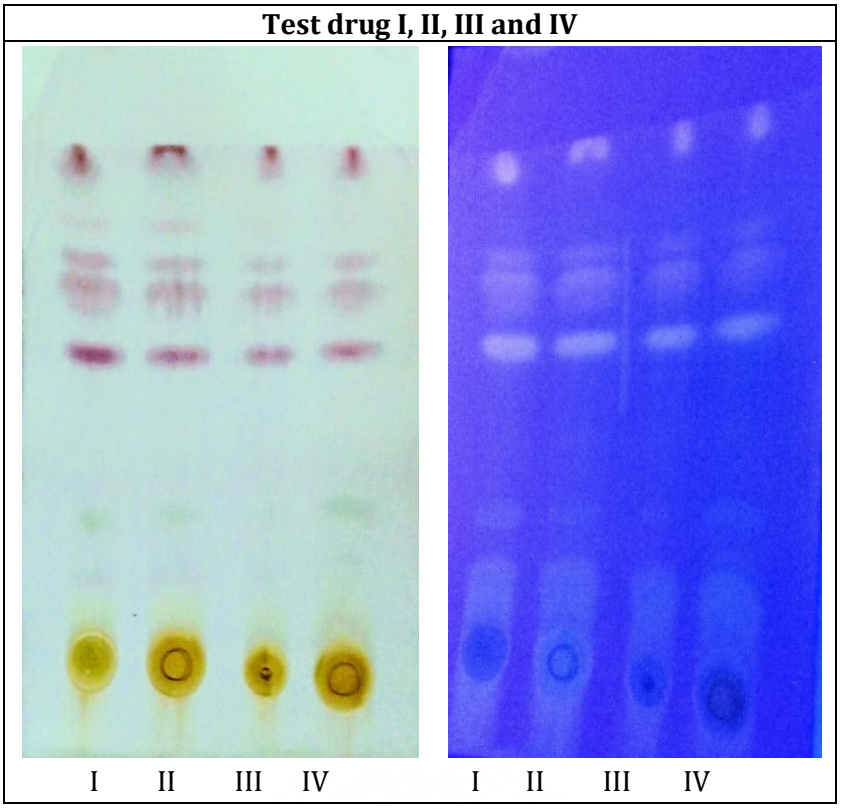


Fig 2: Chromatography of test drug I, II, III and IV.

DISCUSSION

All the values for phytochemical analysis of *Amalaki*, *Haritaki* and *Bibhitaki* were found as per standard reference values mentioned in API [23]. Moisture content is the tool to assess stability of the drug and its therapeutic efficacy. Moisture content denotes the hygroscopic nature of the drug. If the water content of the drug is maximum it will cause oxidation of the drug and encourage the bacterial growth. Moisture content of test drug I, II, III and IV was found 49.198%, 46.87%, 51.32% and 47.292% respectively. This means that the test drug 3 is more hygroscopic in nature, which means there are chances of oxidation and bacterial growth in the drug. P^H plays an important role for quality control and enhances the absorption and distribution of drug. PH is a method of quantity analysis of acidic and basic nature of drug. An appropriate PH is useful for quality control of the drug and controls the bacterial growth and increases the local absorption of the drug. PH of test drug I, II, III and IV was found 5.65,5.17,4.72 and 4.85 respectively. They all are acidic in nature. Extractive value is the tool to assess the solubility of drug in particular solvent. Test drug I and III shows more solubility in water as compared to alcohol while test drug II and IV shows more solubility in alcohol as compared to water. Ash is the quantity assessment to determine the inorganic and silica matter present in the plant material. Ash content of root is more in comparison to other parts of the plant. Higher ash value is suggestive of thermo non labile / heat stable or inorganic constituent. Among the four test drug, the total ash value of test drug III was more as 1.394% and that of test drug I and IV found same and lowest which was 1.058%. The acid insoluble ash value of test drug III was more as 0.124% and that of test drug IV shows lowest value which was 0.05%. The water soluble ash value of test drug III was more as 0.464% and that of test drug II shows lowest value which was 0.316%. Non-reducing sugars do not have an OH group attached to the anomeric carbon so they cannot reduce other compounds. All monosaccharides such as glucose are reducing sugars. This process is important in maintaining human life by creating an energy source for the body. This process requires a catalyst, called a reducing agent or oxidizing agent. That's why body need reducing sugar to gain energy for day to day work. Among the four, test drugs II has highest reducing sugar value and test drug I has lowest value while test drug III has more non reducing value and that of test drug II has the lowest one.

Phyto-chemical analysis of *Amalaki*, *Haritaki* and *Bibhitaki* was done for the evaluation of carbohydrates, reducing sugars, ketone functional groups, alkaloids, proteins with aromatic amino acids, protein with secondary and primary amines, tannins, glycosides, phenolic compounds, steroids, and flavonoids. The analysis shows that all the samples contain almost all primary & secondary metabolites which are required for plant's growth &

are present in a plant when plant is fully grown. It means samples were collected at the perfect time with its full growth & are authentic one. Same evaluation was done with four test drugs and it was found that, lab test shows presence of almost all primary and secondary metabolites in all four test drugs except saponin in test drug III and steroids in test drug I and III.

Thin Layer Chromatography (TLC) is a tool for separation and identification of chemical constituent present in the herbs or chemical mixtures. With mobile solution of Acetone: Chloroform: Formic acid:Toluene:Methanol, Alcoholic extract of *Amalaki (Emblica officinalis)* sample shows 4 spots after derivatization with 1% aq. Ferric Chloride. With mobile solution of Toluene:Ethyl acetate: Formic acid, Ethanolic extract of *Haritaki* (*Terminalia chebula*) sample shows 4 spots after derivatization with 1% aq. Ferric Chloride. With mobile solution of Chloroform:Ethyl acetate:Formic acid, Ethanolic extract of *Bibhitaki* (*Terminalia Bellerica*) sample also shows 4 spots after derivatization with 1% aq. Ferric Chloride. Same way TLC was done for all the four test drugs with mobile solution Toluene:Ethyl acetate:Formaldehyde, after derivatization with Anisaldehyde Sulphuric Acid shows 6 spots each, with Rf value 0.16, 0.27, 0.52, 0.59, 0.65, 0.75 for test drug I, with Rf value 0.16, 0.27, 0.51, 0.60, 0.66, 0.75 for test drug II, with Rf value 0.17, 0.28, 0.53, 0.60, 0.67, 0.75 for test drug III and with Rf value 0.17, 0.29, 0.54, 0.64, 0.68, 0.75 for test drug IV. Most of the Rf value were similar in all the four test drugs suggestive of some common chemical constituents as common raw ingredients were used in the drug preparation.

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CONCLUSION

Any plant or formulation, 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**.** The results obtained in this pharmaceutical evaluation are very encouraging & indicate that *Triphala* should be studied more extensively to confirm & reveal other potential therapeutic effects also. In above study, CR was prepared according to the classical textual standard operative procedure (SOP) mentioned in *Charaka Samhita*. The phytochemical analysis had assessed for CR but still need validation through repeated experiment on different batches along with additional important analysis and investigations for the identification of all the active chemical constituents of the test drug to substantiate the clinical efficacy quantity of ingredients. The groundwork requisites for the standardization of CR were try to covered in the above work. As CR was not yet studied before, its advanced screening might be the area of interest for researchers and will be more helpful towards further clinical studies. In the future, this study will be helpful for the preparation of the monograph of CR in the Ayurvedic formulary of India (AFI).

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