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RESEARCH ARTICLE

Physico-chemical and Phytochemical Standardization of Chitrak Haritaki avaleha

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ABSTRACT

Background: *Chitrak Haritaki avaleha* is an important Ayurvedic formulation, mentioned in the Ayurvedic Formulary of India (AFI), Part I. It is commonly used in *Pratishyaya* (chronic rhinitis), *Kasa* (cough), S*wasa* (dyspnoea), *Agnimadhya* (digestive weakness) and *Krimi* (helminthiasis). **Objective-** Present study deals preparation of Chitrak Haritaki Avaleha and its physico chemical and phytochemical standardization to ensure quality. **Materials and methods:** In the present study, a laboratory sample of *Chitrak Haritaki avaleha* (SL) was prepared by traditional method as per AFI and two marketed sample of *Chitrak Haritaki avaleha* (MK-1 and MK-2) were procured from reputed brands. All the three samples were evaluated for various qualitative screening, physico-chemical and phytoconstituents(percentage reducing sugar, tannic acid, gallic acid, piperine, vitamin C and total polyphenols contents). **Results:-** Results of the physico-chemical, qualitative parameters revealed that all the findings were found within the limit as prescribed in Ayurvedic Pharmacopoeia of India (API). But quantitative estimation like percentage reducing sugar, tannic acid, gallic acid, piperine, vitamin C and total polyphenols contents in all the three samples revealed the remarkable variation in the quality of laboratory sample and marketed products. Phytoconstituent were quantified in per gram sample so to meet global standard and to ensure reproducibility. Heavy metal analysis and microbial content were found within limit as per protocol. This study will be helpful in addition to revalidation of the monograph given in the API.**Conclusion-** Present study reflect standardization and phytochemical validation, and open a new concept to standardize the product on phytoconstituent basis.

Key words: Avaleha, Kasa, Shwasa, Gulma, Agnimadhya.

INTRODUCTION

Ayurveda, the world's most ancient yet unique futuristic system of medicine used for healing and maintaining the good health of mankind's. Several ayurvedic formulations are described in ayurvedic text viz. *avaleha* (semi-solid preparation), *asava-arista* (alcoholic preparation), *churna* (powder), *ghrita* (ghee), *taila* (oil), *vati* (tablet) etc. Among them, *avaleha* is the most potent formulation and widely used in the form of food supplement as well as for great medicinal values. *Chitrak Haritaki avaleha* is one of the most common *avaleha* preparation mentioned in AFI-I. It is commonly used in gulma (intra-abdominal swellings), *pratishyaya* (chronic rhinitis), *kasa* (cough), *shwasa* (dyspnoea), *agnimadhya* (digestive weakness) and *krimi* (helminthiasis) [1]. Major ingredients[2] of *Chitrak Haritaki avaleha* are Chitrak(*Plumbagozeylanica* Linn.), Haritaki (*Terminaliachebula* Retz.), Amalaki (*Emblicaofficinalis*Gaertn.) and Guduchi (*Tinosporacordifolia* (Willd.) Miers ex Hook.f. &Thoms.), while minor ingredients are Twak (*Cinnamomumtamala*Nees&Eberm.), Ela (*ElettariacardamomumMaton.),* Trikatu (mixture containing equal amount of rhizome of *Zingiberofficinale*, fruit of *Piper longum*and *Piper nigrum*) [2].

In order to meet the increasing global demand for Ayurvedic, herbal and herbo-mineral medicines, it is essential to ensure the quality and consistency of drugs to achieve their safety and maximal efficacy. In Ayurveda, whole medicinal action is due to synergistic effect of each agent in spite of single constituent [3]. Now day's analytical techniques like thin layer chromatography (TLC), high performance liquid chromatography (HPLC),

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atomic absorption spectroscopy (AAS), infrared spectroscopy (IR), nuclear magnetic spectroscopy (NMR), UV-visible spectroscopy are available for more recent and advanced analysis [4]. Standardization of *Chitrak Haritaki avaleha* is a challenging task because it is a polyherbal formulation, contains many phytochemical constituents and determination of each constituent is not an easy task. The standardization techniques mentioned in API is not sufficient as it do not describe the standardization on their phytoconstituents. So in present study offer physico chemical and phytochemical standardization to ensure quality of product and thereafter compared in order to know the variation in marketed sample.

MATERIALS AND METHODS

Preparation of *Chitrak Haritaki avaleha*

*Chitrak Haritaki avaleha*was prepared in laboratory as per the classical method prescribed in API by using authentic ingredients [5].Ingredients were procured from the local market in Varanasi and authenticated by Prof. A. K. Singh, Department of Dravyaguna, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi. Two marketed samples (MK-1 & MK-2) of reputed brands were purchased from local market in Varanasi.

Standatdization of *Chitrak Haritaki avaleha*

All the three samplesof *Chitrak Haritaki avaleha*(SL, MK-1 and MK-2) were analyzed for organoleptic properties, physicochemical parameters (loss on drying, total ash value, acid insoluble ash value, alcohol soluble extractive value, water soluble extractive value, pH value, acid value and fiber content), qualitative analysis of various category of phytochemical constituents and quantitative estimation of % reducing sugar, total polyphenols, tannins, gallic acid, vitamin C and piperine content. Laboratory sample (SL) was also analyzed for heavy metal content.

Organoleptic characterization

All the three sample of *Chitrak Haritaki avaleha*were analyzed for appearance, colour, taste, odour and touch.

Qualitative study

*Chitrak Haritaki avaleha*was extracted with methanol, chloroform and water separately for their preliminary phytochemical screening to evaluate the presence of phytoconstituents like alkaloid, carbohydrate, triterpenoid, saponins, phenol content and flavonoids [7].

Physico-chemical characterization

All the physico-chemical parameter (Determination of loss on drying, total ash content, acid insoluble ash content, alcohol soluble extractive, water soluble extractive, pH, Acid value and fiber content) were evaluated following API[8].

Quantitative estimation of phyto-constituent

1. Reducing sugar content

Approximately 25 g of sample was taken and transferred to 250 ml flask. 10 ml of neutral lead acetate solution was added and dilute up to volume with distilled water and filter. An aliquot of 25 ml of clarified filtrate was transferred to 500 ml volumetric flask containing about 100 ml of water. Ammonium oxalate was added in small quantity until there was no further precipitation. Volume was made up to the mark and filtered. 50 ml filtrate was transferred to a 50 ml burette. 5ml each of Fehling A and Fehling B were taken into 25 ml conical flask. 10 ml water and 2 drops of Methylene blue indicator were added to it. Boil it over heat and titrate with filtrate filled in burette.

$$\% \text{ Reducing sugar} = \frac{\text{Dilution x factor of fehling x 100}}{\text{Wt of sample x titre value}}$$

2. Total tannin content [9]

Tannic acid was dissolved in water to make a standard solution of 20µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml, and 100 µg/ml concentrations. 1 ml of tannic acid standard solution was taken and added by 1 ml of Folin-Ciocalteau reagent and 4ml 20% sodium carbonate solution and volume was made up to 10 ml. The absorbance of tannic acid was measured at 725nm and calibration curve was plotted between absorbance and concentration. Now sample was extracted in water and filtered, filtrate was diluted and added by 1ml of FC reagent, 4ml sodium carbonate and then made up the volume to 10 ml. Sample dilution was leftfor 40 minute and then absorbance was measured at 725 nm in UV spectroscopy and compared with calibration curve. The entire samples were analyzed in triplicate. Result was found as total tannin equivalent to tannic acid in per gm of sample.

Vitamin C estimation [10]

Reagent and chemical was of analytical grade and solutions were prepared by distilled water. Standard solution of ascorbic acid was prepared of 20, 40, 60, 80 and 100 µg/ml. 3 ml of 1mM ferric chloride, 3ml of 5mM potassium Ferrocyanide

were added to 1 ml of sample and volume was made up to 10 ml. Dilution were mixed properly and allowed to stand for 10 min. Absorbance of standard solutions was measured at 709 nm by UV spectroscopy against blank and calibration curve was obtained between absorbance and concentration. The sample was analyzed by similar method and concentration was obtained by calibration curve.

Piperine content [11]

Stock solution of piperine was prepared by dissolving 10 mg of piperine in 100 ml of methanol. Standard solutions of piperine were prepared from stock solution in the concentration range of 2-20 µg/ml in 100 ml volumetric flask using methanol as solvent. The absorbance of piperine standard solutions was measured at 342 nm (λmaxfor piperine) against methanol as blank. Calibration curve was plotted between absorbance and concentration. Sample 5 gm was taken and extracted with methanol for 1 hour. The extract was filtered and re-refluxes the marc left with 50 ml of ethanol for another 1 hours. Filtrates were combined and subjected to concentration in rotary evaporator. Residues obtained were dissolved in methanol and volume made up to 1000 ml with methanol. The absorbance of sample solutions was measured at 342 nm against methanol as blank. Same procedure was repeated for two different days.

Total polyphenol content [12]

Here total polyphenol content was determined with help of standard gallic acid and express as total polyphenol content equivalent to gallic acid. Gallic acid was dissolved in water to make a standard solution of 20µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml, and 100 µg/ml concentrations. 1 ml of gallic acid standard solution was taken and added by 1 ml of Folin-Ciocalteau reagent and 4ml 20% sodium carbonate solution and volume was made up to 10 ml. The absorbance of gallic acid was measured at 760 nm and calibration curve was plotted between absorbance and concentration. Now sample was extracted in water and filtered, filtrate was diluted and added by 1ml of FC reagent; 4ml sodium carbonate and then made up the volume to 10 ml. Sample dilution was leftfor 40 minut and then absorbance was measured at 760 nm in UV spectroscopy and compare to calibration curve. The entire samples were analyzed in triplicate.

Tannic acid content [13]

Tannic acid was dissolved in methanol to make a standard solution of 20µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml, and 100 µg/ml concentrations. 1 ml of tannic acid standard solution was taken and volume was made up to 10 ml. Absorbance of tannic acid was measured at275 nm of wavelength with the help of UV Spectroscopy and calibration curve was plotted between absorbance and concentration. Now sample was extracted in water and filtered, filtrate was diluted and made up the volume up to 10 ml. Sample was scanned at 275 nm in UV spectroscopy and absorbance was measured. The entire sample was analyzed in triplicate. Calculate the result as tannic acid in per gm of sample.

Heavy metal Analysis [14]

Heavy metal is analysed by Atomic absorption spectroscopy. Sample ware dissolved in 50 % HNO_3 for the digestion that was further diluted with distilled water.

Table 1: Ingredients of *Chitrak Haritaki avaleha*

Ingredient	Botanical name	Part used
Chitrak	*Plumbagozeylanica* Linn.	Root bark
Amalaki	*Emblicaofficinalis*Gaertn.	Fruit
Guduchi	*Tinosporacordifolia* (Willd.) Miers ex Hook.f. &Thoms.	Stem
Haritaki	*Terminaliachebula* Retz.	Fruit
Dashamool [6] Bilva root Agnimantha Shyonaka Patala Gambhari Brihati Kantakari Shalaparni Prushniparni Goksharu	 *Aeglemarmelos*Correa *Premnaintegrifolia*Linn. *Oroxylumindicum*Vent. *Stereospermumsuaveolens*DC. *Gmelinaarborea*Roxb. *Solanumindicum L*inn. *Solanumxanthocarpum*Linn. *Desmodiumgangeticum*DC. *Urariapicta*Desv. *Tribulusterrestris*Linn.	Root bark
Tvak	*Cinnamomumzeylanicum*Blume.	Stem Bark
Ela	*ElettariacardamomumMaton.*	Fruit
Patra	*Cinnamomumtamala*Nees&Eberm.	Leaf
Yavakshara	Potassium carbonate	Kshar
Trikatu	(Mixture containing equal amount of rhizome of *Zingiberofficinale*Roscoe, fruit of *Piper longum*Linn. and *Piper nigrum* Linn.)	-

Madhu	Honey	-
Sharkara	Sugar	-

RESULTS

*Chitrak Haritaki avaleha*was evaluated for organoleptic characteristic (**Table 2**), qualitative evaluation (**Table 3**) and quantitative evaluation (**Table 4 & 5**) of ingredient present in it.

Table 2: Organoleptic Character of *Chitrak Haritaki avaleha*

Organoleptic Characters	Laboratory Sample (SL)	Market sample-1 (MK-1)	Market sample -2 (MK-2)
Colour	Blackish Brown	Blackish Brown	Brown
Odour	Spicy, Pleasant odour	Spicy, Pleasant odour	Spicy, Pleasant odour
Taste	Bitter- astringent	Bitter- astringent	Bitter- astringent
Appearance	Thick Semi Solid Mass	Semi Solid	Semi Solid Mass with lesser consistency
Touch	Soft and viscous	Soft and viscous	Soft and viscous

Table 3: Phytochemical evaluation of *Chitrak haritakiavaleha*

		Lab sample (SL)			Marketed sample-1 (MK-1)			Marketed sample -2 (MK-2)		
S. No	Plant Constituents Test / Reagent	Me. Ext.	Chl. Ext.	Aq. Ext.	Me. Ext.	Chl. Ext.	Aq. Ext.	Me. Ext.	Chl. Ext.	Aq. Ext.
1	**Alkaloids**									
	Dragendroff's reagent	+	+	+	+	+	+	+	+	+
2	**Carbohydrates**									
	Molisch's reagent	+	+	+	+	-	+	+	-	+
	Fehling solution	+	-	+	+	-	+	+	-	+
	Reducing sugar test	+	-	+	+	-	+	+	-	+
3	**Triterpenoids**									
	Salkowski test	+	+	+	+	+	-	+	+	-
4	**Saponins**									
	Foam test	+	-	+	+	-	+	+	-	+
5	**Phenolic compounds& tannin**									
	Ferric chloride solution	+	+	+	+	+	+	+	+	+
	Nitric acid test	+	+	+	+	+	+	+	+	+
6	**Proteins & amino acids**									
	Millon's reagent	+	-	-	+	-	+	+	-	+
	Ninhydrin reagent	+	-	-	+	-	-	+	-	+
7	**Flavanoids**									
	Shinoda/Pew test	+	-	+	+	-	+	+	-	+

Table 4: Physico-chemical evaluation data of *Chitrak Haritaki avaleha*

S. No	Parameters	API Standard(4)	SL	MK-1	MK-2
1	Loss on drying 110^0C (% w/w)	Not more than 36%	23. 78	31. 25	37. 59
2	Total Ash Value (% w/w)	Less than 4. 7%	4. 045	3. 362	1. 059
3	Acid insoluble ash (% w/w)	Less than 1%	0. 306	0. 242	0. 433
4	Water soluble extractive (% w/w)	More than 67 %	65. 29	54. 79	77. 92
5	Alcohol soluble extractive (% w/w)	More than 21 %	48. 64	57. 92	32. 60
6	pH value of 1% aqueous solution	6. 4- 6. 6	5. 19	5. 92	6. 02
7	Acid Value	-	10. 36	9. 61	7. 61
8	Fiber content (w/w)	-	13. 087	11. 437	3. 273

Table 5: Quantitative evaluation result for *Chitrak Haritaki avaleha*

S. No	Parameter	Lab Sample(SL)	MK-1	MK-2
1	Reducing sugar content (% w/w)	8. 39	13. 25	11. 87
2	Total Tannin content(% w/w)	6. 55	8. 33	4. 65
3	Vitamin C content (% w/w)	2. 92	2. 94	2. 08
4	Piperine content (% w/w)	1. 2	1. 58	0. 86
5	HMF content (% w/w)	0. 23	0. 68	0. 26
6	Total polyphenol content (% w/w)	7. 15	8. 95	3. 6
7	Total tannic acid content (% w/w)	8. 05	11. 91	4. 25

Table 6: Estimation of heavy metals in *Chitrak Haritaki avaleha*

Element	Wavelength	Instrument detection limit	Concentration in ppm	Limit
Cadmium	228.802	0.0027	Not detected	0.3 ppm
Lead	220.353	0.0420	7 ppm	10 ppm
Mercury	253.652	0.0610	0.4 ppm	1 ppm
Arsenic	193.696	0.0530	Not detected	3 ppm

DISCUSSION

Chitrak Haritaki avaleha was evaluated for organoleptic characteristic, qualitative evaluation and quantitative evaluation of ingredient present in it. All sample showed similar organoleptic characters except appearance (Table 2) where laboratory sample was thick semi solid mass while MK-1 was semi solid and MK-2 was having thinner consistency, which could be due to higher moisture content. The taste and appearance was quite similar, except lab sample was more astringent and bitter. MK-2 was sweeter in comparison to other two, reflecting higher content of sweetening agent or less of kasaya dravya

(astringent substance). Phytochemical screening of *Chitrak Haritaki avaleha* shows alkaloids, tannins, flavonoid, saponins, phenolics, carbohydrate, protein and amino acid were present in each sample (Table 3). Since it was prepared with extract of several herbs, it contains most of the phytochemical constituents. Comparative physico-chemical evaluation of various preparations of *Chitrak Haritaki avaleha* was done and finding (Table 4) revealed that physicochemical parameters were within the limits as per the Pharmacopoeial standards (API-2, Vol-1) and some more parameter ware taken for physicochemical standardization. However, there was a significant variation among *Chitrak Haritaki avaleha* samples. Quantitative estimation was done to know reducing sugar, tannin content, gallic acid, vitamin C content etc. (Table 5). Reducing sugar content was found higher in MK-2 (11. 9 %) while lesser in Lab sample (98. 9%) which reflect that MK-1 and MK-2 have higher sugar content. Total tannin content was determined by using UV spectroscopy and it was found more tannin in MK-1 (8. 33 %) while lesser in MK-2 (4. 65 %) which may be due to use of substandard raw materials or substitutes. Total Polyphenol content was present highest in MK-1 which indicates good quality while MK-2 had lower polyphenol content. Vitamin C was quite similar in all the three samples. Piperine content was found highest in MK-1 (1. 58% w/w) and lowest in MK-2 (0. 86 % w/w) that indicate MK-1 may produce good effect in cough disease and produce their effect effectively.Heavy metal estimation were done for cadmium, lead, mercury and arsenic and the result shows that lead was 7 ppm and mercury was 0.4 ppm[Table-6] while other heavy metals were not detected and the value found here complies within limit.Since here phytoconstituent were quantified in per gram sample so to meet global standard and to ensure reproducibility, phytochemical standardization may be used as standard in coming future.

CONCLUSION

The laboratory sample of *Chitrak Haritaki avaleha*was dark brown coloured, pleasant spicy odour, astringent and bitter in taste and thick semi solid mass. The samples were analyzed for their preliminary qualitative testing (Phytochemical screening) which showed that phytochemical constituent like alkaloid, tannins, saponin, glycoside, carbohydrate, amino acids and phenolics compound are present in sample. Physiochemical characterization of *Chitrak Haritaki avaleha*showed that market samples (MK-1) were similar to that of lab sample, while MK-2 exhibiting considerable variation. The data evolved in the present study will be very useful for routine quality control of *Chitrak Haritaki avaleha*and provide new aspect for their standardization and also to control the batch to batch variation (ensure reproducibility).

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