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Article · March 2021

DOI: 10.17605/OSF.IO/8X4PU

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WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 3, 1854-1866. Research Article ISSN 2277– 7105

CHARACTERIZATION AND ANALYTICAL STANDARDIZATION OF *AGASTYA HARITAKI*: A POLYHERBAL AYURVEDIC FORMULATION

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Article Received on
13 January 2021,

Revised on 03 Feb. 2021,
Accepted on 23 Feb. 2021

DOI: https://doi.org/10.17605/OSF.IO/8X4PU

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ABSTRACT

Introduction: Ayurveda the Indian traditional system of medicine is categorized under complementary and alternative medicine (CAM) by National Cancer Institute (NCI) on the basis of non-availability of safety and efficacy data. **Materials and Methods:** In present study an attempt is made to standardize one of the poly herbal Ayurvedic formulation *Agastya Haritaki*(AH). Four samples of AH are prepared using different SOPs and subjected to quality control parameters like Ayurvedic parameters organoleptic characters, physicochemical parameters, nutritional value, heavy metal analysis, microbial analysis, antioxidant study, HPTLC finger printing and anti-microbial study. **Result:** Four values for each analytical parameter were obtained providing the range for the particular parameter. **Discussion and Conclusion:** Values of organoleptic characters was comparable with the values given for various *Avaleha* in The Ayurvedic Pharmacopoeia of India and was well within the limit for Heavy metals and microbial load. Also the antioxidant property is remarkable with 5.75-7.89 mg and the inhibition zone for microbes like Pseudomonas pneumonae, Streptococcus aureus, E. coli and Candida albicans are 11-13 mm, 11-14 mm, 09-15 mm and 0-15 mm correspondingly.

KEYWORDS: Ayurveda, CAM, NCI, *Agastya Haritaki*, Antioxidant.

INTRODUCTION

Ayurveda the ancillary therapeutic system is considered as Complementary and Alternative Medicine (CAM) worldwide. National Cancer Institute (NCI) defines CAM as treatment

modality used in addition to (complementary) or instead of (alternative) standard treatments.[1] Though used since centuries, insignificant documented facts are available about safety and efficacy of Ayurvedic preparation.

Agastya Haritaki (AH) is a semisolid polyherbal Ayurvedic dosage known as *Avaleha* form prepared with decoction, herbal powders, sweetening agent like jaggery and unctuous substances like clarified butter and oil. AH is used vividly to treat numerous types of Respiratory disorders like cough, pthisis, dyspnea, asthma, hiccough, coryza, intermittent fever, wrinkles and greying of hair. It also acts as nutraceutical with adapto-immuno-neuro-endocrino-modulator properties.[2, 3, 4] This formulation in dose of two fruits of *Haritaki* every day cures wrinkling of the skin & graying of hair (process of ageing) and promotes complexion, longevity as well as strength.[5, 6, 7] SOP is clearly mentioned in Ayurvedic texts and substitute to unavailable drugs or procedure is also stated;[8,9] but due to various unavoidable issues like non-availability of fresh *Haritaki* throughout year, ensuing single SOP is not feasible for pharmaceutical preparation. Also diverse quality of raw drugs due to weather and altitude leads to non-uniformity in terms of quality and efficacy. Thus, maintaining the quality standard of a poly herbal formulation is a challenging task. In AH fresh *Haritaki* and new barley is advised to use.[10]

Well prepared avaleha should come up thread like (if taken out in a rod), sink in water, take fingerprints or shapes if rolled between fingers, give pleasant smell, possess good color and taste.[11]

According to Ayurvedic classics the shelf life of *Avaleha* is one year.[12] however, it has been revised to three years.[13]

Primary Objective

To standardize *Agastya Haritaki Rasayana*.

Secondary Objective

To obtain acceptable range for different analytical parameter.

MATERIALS AND METHODS

Four samples of AH are prepared using four different form of *Haritaki* viz. fresh wet *Haritaki* fruits, dry *Haritaki* fruits, *Haritaki* powder and boiled *Haritaki* fruits following SOP to estimate the maximum possible variation in yield and analysis of samples.

SOP for preparation of AH1: The ingredients of the formulation (Table 1) were procured. The ingredients were identified and authenticated. Foreign matters were thoroughly removed. Ingredients 2-22 were coarsely powdered with the help of disintegrator machine and sieved through sieve number- 44 N. Ingredient 27 was finely powdered with the help of pounding machine and electric grinder and sieved through mesh - sieve number 85N.

Table 1: Ingredients of Agastya Haritaki.

S.No.	Ingredients	Latin name	Part Used	Quantity(gm)
Main Ingredients				
1.	Haritaki	*Terminalia chebula*	Fruit (Pulp)	As per sample
2.	Bilva	*Aegle marmelos*	Stem Bark	19.2
3.	Agnimantha	*Clerodandrum phlomidis*	Stem Bark	19.2
4.	Shyonaka	*Oroxylum indicum*	Stem Bark	19.2
5	Kashmari	*Gmelina arborea*	Stem Bark	19.2
6.	Patala	*Stereo suaveolens*	Stem Bark	19.2
7.	Brhati	*Solanum indicum*	Whole Plant	19.2
8.	Shalaparni	*Desmodium gangeticum*	Whole Plant	19.2
9.	Prishniparni	*Uraria picta*	Whole Plant	19.2
10.	Kantakari	*Solanum virginianum*	Whole Plant	19.2
11.	Goksura	*Tribulus terrestris*	Whole Plant	19.2
12.	Bala	*Sida cordifolia*	Root	19.2
13.	Shankhpushpi	*Convolvulus pluricaulis*	Whole Plant	19.2
14.	Kapikachhu	*Mucuna pruriens*	Seed	19.2
15.	Sathi	*Hedychium spicatum*	Rhizome	19.2
16.	Hastipippali	*Scindapsus officinalis*	Dry Fruit	19.2
17.	Apamarg	*Achyranthes aspera*	Root	19.2
18.	Pippalimula	*Piper longum*	Root	19.2
19.	Chitraka	*Plumbago zeylanica*	Root	19.2
20.	Bharngi	*Clerodandrum serratum*	Root	19.2
21.	Puskarmula	*Inula racemosa*	Root	19.2
22.	Barley	*Hordeum vulgare*	Seed	614
23	Water	-	3.072 lt.	23
Sweetening Agent				
24.	Guda	Jaggery	-	960
Oleaginous substances				
25.	Go Ghirta	Cow ghee		38.4
26.	Tila Taila	Tila oil	Oil	38.4
Adjuvants				
27.	Pippali	*Piper longum*	Fruit	38.4
28.	Madhu	Honey		38.4

Preparation of sample AH1: The prepared coarse powder of ingredients (2 – 21) was kept in container. Water was added and kept over-night for making decoction. Next day fresh *Haritaki* fruits were tied in a clean piece of cotton cloth in heap of barley and bundle was

made. This bundle was suspended in container containing the ingredients of decoction for soaking. This whole set up (*Dola-yantra*) was kept over mild flame. Bundle was removed and *Haritaki* was deseeded. The fruit was made into pulp by gently pressing over sieve (No. 40). Rest of the liquid was reduced to 1/4th to obtain decoction. The decoction was strained through muslin cloth. The pulp was fried in mixture of Oil & Cow Ghee till the fumes continues to appear, the color of the pulp changes to golden brown, the pulp leaves oil and pulp becomes stick like while rubbing between fingers.

The jaggery was mixed in decoction and cooked to obtain syrup. The fried *Haritaki* pulp was added and cooked on mild flame till the mixture is soft to touch, takes shape of finger crevices on pressing, doesn't spread while dropping in water, is stable and can be easily picked with finger.

Appearance of specific odor, color and taste confirmed desired consistency. The fine powder of adjuvant (*Pippali*) mixed vigorously to form a homogenous mixture. Honey was added on cooling.

Preparation of sample AH2: Same no of Dry *Haritaki* was taken; seeds were removed and made into fine powder. And mixed with water to make paste. Rest of the procedure remains same.

Preparation of sample AH3: *Haritaki* powder was taken half the quantity of wet *Haritaki*, made in to paste by mixing water. Rest of the procedure remains same.

Preparation of sample AH4: The seeds of the boiled *Haritaki* were removed. Whole fruit of *Haritaki* was fried till the fumes continues to appear, golden brown color. The jaggery syrup was prepared. The fried *Haritaki* fruit was added to syrup and subjected to mild flame till the appearance of desired consistency.

The test for the preparation of avaleha is done at two step viz. a) for syrup preparation (*asannapakalakshana*) i.e. *tantumatva* (thread like appearance), *apsumajjana* (sinks in water) and *sthiratva* (firmness due to proper consistency); and b) for avaleha preparation (*Sidhalakshana*) i.e. *peedithomudra* (fingerprints on pressing), *gandha-varna-rasodbhava* (specific odor, color, taste) and *sukhamarda* (soft to roll).[14] The *Lehya* should be kept in glass, porcelain jars and nonreactive metal container.[15]

Analytical Testing: The prepared samples *Agastya Haritaki* (AH1, AH2, AH3 and AH4) were subjected to analytical testing parameters like organoleptic characters, physicochemical parameters, nutritional value, heavy metal analysis, microbial analysis, antioxidant study, HPTLC and anti-microbial study. For reference purpose the range of standards given for various avaleha formulation in API Part II; Vol I, II and III are used.[16,17,18]

Organoleptic characters

- Description
- Colour
- Odour
- Taste
- Consistency

Physico -Chemical analysis

- Loss on drying
- pH (10% aqueous extract)
- Total ash
- Acid- insoluble ash
- Water soluble ash
- Water -soluble extractive
- Alcohol- soluble extractive
- Total sugars
- Reducing sugar
- Non Reducing Sugar
- Total Tannins

Nutritional value

- Total Fat
- Total Energy

Test for heavy metals

- Cadmium
- Lead
- Arsenic
- Mercury

Microbial analysis

- Total Aerobic Microbial count
- Total Yeast and Mould count

Antioxidant study

HPTLC finger printing

Anti-microbial Study

- Pneumococcal pneumonia
- Streptococcus aureus
- E Coli
- Candida Albicans

RESULTS AND DISCUSSION

Organoleptic characters

The *Sparsha* (Texture), *Rupa* (Color), *Rasa* (Taste), *Gandha* (Odor) and Consistency was uniformly found as soft, dark reddish brown, sweetish astringent, characteristic sweet and semisolid respectively for all the samples (Table 2).

Table 2: Organoleptic characters of Agastya Haritaki samples.

S. No.	Parameters	AH1	AH2	AH3	AH4
1.	*Sparsha (*Texture)	Soft	Soft	Soft	Soft
2.	*Rupa* (Color)	Dark reddish brown	Dark reddish brown	Dark reddish brown	Dark reddish brown
3.	*Rasa* (Taste)	Sweetish astringent	Sweetish astringent	Sweetish astringent	Sweetish astringent
4.	*Gandha* (Odor)	Characteristic Sweet	Characteristic Sweet	Characteristic Sweet	Characteristic Sweet
5	Consistency	Semisolid	Semisolid	Semisolid	Semisolid

Physico-Chemical analysis

Loss on drying: Loss on drying is non-specific analytical technique designed to measure the water and volatile content when the sample is dried at 105^0 C for three hours. Moisture is leading cause for the deterioration of the drugs and formulations. Lower the moisture content better will be the stability of drugs (Table 3). Loss on drying for AH is not more than 10.52% - 11.86% (API - Not more than 6%-32%).

pH value: The pH value is measured at 10% dilution and is one of the main factors influencing the quality of medicine. It controls chemical and microbiological reactions. In

present study pH value is almost uniform i.e. 4.5-4.6 (API-3.3-6.6) and samples are suitable for oral intake and drug absorption.

Ash value: Ash value is the residue after incineration helpful in determination of quality and purity of powdered drugs. It removes all traces of organic matter, leaving ash consisting of carbonates, phosphates and silicates of sodium, potassium, calcium and magnesium.[19]

A high ash value is indicative of contamination, substitution, adulteration with inorganic matter, or carelessness in preparing the formulation. In present study ash value is not more than 1.9%-2.2% (API Not more than 1.4%-12). These values were found to be reasonably low indicating very low contamination.

Acid Insoluble Ash: The acid insoluble ash is the part of the total ash which is insoluble in diluted hydrochloric acid. The test was carried out to detect adulteration with exhausted drugs and to ensure percentage of silica and oxalates which are introduced accidentally or purposely at the time of preparing the formulation. In present study Acid insoluble ash value is not more than 0.38%-0.4% (API not more than 0.13%-2%).

Water Soluble Ash: Water soluble ash is the part of the total ash content, which is soluble in water. Thus it is the difference in weight between the total ash and the residue obtained after treatment of total ash with water It is a good indicator of previous extraction of water soluble salts in the drug or incorrect preparation.[20] In present study Water soluble ash value is not more than 1.34%-1.65%.

Water soluble extractive: Water soluble extractive value plays an important role in evaluation of crude drugs. Useful for the estimation of nature of constituents extracted with the solvent used for extraction. Less extractive value indicates presence of exhausted material, contamination or improper processing during drying or storage or preparation. In present study Water soluble extractive is not less than 70.1%-71.1% (API not less than 11%-90%).[21]

Alcohol soluble extractive: The alcohol soluble extractive value was also indicative for the same purpose as the water soluble extractive value. Less extractive value indicates addition of exhausted material, adulteration or incorrect processing during drying or storage or formulating. In present study Alcohol soluble extractive is not less than 43.04%-45.1% (API

not less than 6.8%-74%) The water soluble extractive value was higher than alcohol soluble extractive value, this indicates presence of more amount of water soluble content in the plant.

Total sugars: In present study Total sugar are 51.51% - 69.22%.

Reducing Sugar: It is source of Carbohydrate & indicates reduction nature of sugar. If reducing sugar is more in the sample, it will help in oxidation of other ingredients which will lead in degradation of the product. In present study reducing sugar are 15.16%- 29.38% (API Not more than 11%-85%).

Non Reducing Sugar: The non-reducing sugars is indicative of stability or non-degradation of samples. In present study non-reducing sugar are 22.13%-50.17% (API not more than 4.7%-72%).

Total Tannins: Astringent herbs are rich in tannins. Tannins help in protection against infections and pests. Tannins are divided into two classes: hydrolysable tannins (more astringent) and condensed tannins. This expedites wound healing and has anti-inflammatory, hemostatic, antioxidant and antidiarrheal property. In present study total tannins are 1.71% - 4.91% (API not less than 0.4%-5%).

Table 3: Values of physicochemical parameters.

Parameter	AH1	AH2	AH3	AH4
Loss on drying	11.86	10.52	11.06	11.52
pH (10% aqueous extract)	4.5	4.5	4.5	4.6
Ash value (% w/w)	2.2	2.15	2.12	1.9
Acid insoluble Ash(% w/w)	0.38	0.4	0.4	0.39
Water soluble Ash(% w/w)	1.56	1.65	1.50	1.34
Water soluble extractive(% w/w)	71.01	70.06	70.06	70.01
Alcohol soluble extractive(% w/w)	44.93	45.01	44.76	43.04
Total sugars(%)	64.5	66.39	69.22	51.51
Reducing Sugar(%)	15.16	17.77	16.40	29.38
Non Reducing Sugar	49.34	48.62	50.17	22.13
Total Tannins (%)	3.43	4.91	4.35	1.71

Nutritional value

Total fat content of the samples is 1.14%- 3.02% and total energy are 3659.77 Cal/gm - 4281.71 Cal/gm (Table 4).

Table 4: Nutritional Values of *Agastya Haritaki* samples.

Sr. no.	Parameter	Unit	AH1	AH2	AH3	AH4
1.	Total Fat	%	1.14	1.73	1.36	3.02
2.	Total Energy	Cal/gm	3760.42	3923.32	3659.77	4281.71

Test for heavy metals: Tests for heavy metals showed the presence of very small quantity of Lead and Arsenic in the samples of AH i.e. 0.125ppm-0.192 ppm and 0.005 ppm-0.008ppm respectively (Table 5). Although, the values of Lead and Arsenic are within the normal limit and Cadmium and Mercury are absent in all the samples of AH.[22]

Table 5: Heavy Metal Analysis in *Agastya Haritaki* samples.

Sr. no.	Parameter	Limit(ppm)	AH1	AH2	AH3	AH4
1.	Lead	NMT 10	0.189	0.142	0.125	0.192
2.	Cadmium	NMT 0.3	ND	ND	ND	ND
3.	Mercury	NMT 1.0	ND	ND	ND	ND
4.	Arsenic	NMT 3.0	0.005	0.008	0.006	0.006

*ND- Not Detected

†NMT- Not More Than.

Microbial analysis

Total Aerobic Microbial count and Total Yeast and Mould count is given in (Table 6).

Table 6: Total Aerobic Microbial count in *Agastya Haritaki* samples.

Parameter	Unit	AH1	AH2	AH3	AH4
TAMC	Cfu/gm	138	294	58	80
TYMC	Cfu/gm	<10	<10	<10	<10

Antioxidant study: AH3 sample have higher polyphenol content. This may be chebulic acid, a phyto-chemical compound of *Haritaki* and other ingredients. Such active principles have the anti-oxidant properties and therefore, limit the risk of various degenerative diseases associated with oxidative stress (Table 7).

Table 7: Total Antioxidant and Polyphenol in Agastya Haritaki samples.

Parameter	Unit	AH1	AH2	AH3	AH4
Total Antioxidant	mg	6.659	5.749	7.89	6.643
Total polyphenol	%w/w	1.28	1.58	1.68	0.97

HPTLC finger printing

Stationary phase: Pre-coated silica gel 60 F_{254} Aluminium plates

Mobile phase: Toluene: Ethyl acetate: Methanol: Glacial Acetic Acid (6:2.5:1:0.5).

Chamber Saturation Time: 20 mins.

Test Solution: 2.5 gm of drug extracted by cold maceration in methanol and then filter the liquid extract. Make the volume up to 50 ml with methanol.

Derivatising Reagent: Anisaldehyde sulphuric acid

In order to know the presence of plant constituents in the samples, a methonolic extract of all the samples were chromatographed by developing with Toluene, Ethyl acetate, Methanol and Glacial acetic acid (6:2:5:1:0.5) and studied under UV light at 220 (Table 8). For better resolution the chromatograms were sprayed with the Anisaldehyde sulphuric acid and studied under UV light at 540 nm.

Table 8: Rf values of various spots for wavelength 220nm and 540nm.

Sample	Wavelength	No. of spots	R_f value
AH1	220	7	0.29, 0.34, 0.40, 0.47, 0.62, 0.72, 0.87
AH2	220	8	0.34, 0.40, 0.47, 0.59, 0.67, 0.73, 0.81, 0.88
AH3	220	7	0.34, 0.40, 0.47, 0.58, 0.72, 0.82, 0.88
AH4	220	5	0.12, 0.26, 0.44, 0.50, 0.81
AH1	540	9	0.41, 0.50, 0.59, 0.68, 0.71, 0.75, 0.79, 0.82, 0.86
AH2	540	9	0.36, 0.41, 0.60, 0.68, 0.71, 0.76, 0.79, 0.82, 0.86
AH3	540	7	0.35, 0.41, 0.59, 0.67, 0.72, 0.77, 0.86
AH4	540	5	0.44, 0.51, 0.73, 0.80, 0.95

Anti-microbial Study

- Pneumococcal pneumonae
- Streptococcus aureus
- E Coli
- Candida Albicans

The agar well diffusion method is used for the determination antimicrobial activities. The culture medium used in the study was the nutrient agar which is also used for culturing routine pathogens. The inhibition zone for Pseudomonas pneomonae, Streptococcus aureus, E. coli and Candida albicans are 11-13 mm, 11-14 mm, 09-15 mm and 0-15 mm respectively (Table 9).

Table 9: Total Antimicrobial Activity in *Agastya Haritaki* samples.

Sr. no.	Antimicrobial activity	Standard	AH1	AH2	AH3	AH4
1.	Pseudomonas pneomonae	20mm	11mm	13mm	11mm	10mm
2.	Streptococcus aureus	23mm	12mm	14mm	13mm	11mm
3.	E. coli	20mm	12mm	15mm	15mm	09mm
4.	Candida Albicans	37mm	12mm	15mm	15mm	10mm

CONCLUSION

Agastya Haritaki (AH) is a common Ayurvedic formulation in a linctus dosage form with many herbs along with jaggery and honey used judiciously to treat Respiratory ailments. This study shows its nutritional potential, antioxidant property along with therapeutic importance. Formulation is having good amount of moisture content still microbial count is very low and remarkably it bears antimicrobial properties. As the standards for its quality control parameters are absent; the outcome of this study could be used as the reference for standards for the quality control and quality assurance of *Agastya Haritaki*.

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