Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(3): 1923-1927
Received: 16-03-2020
Accepted: 18-04-2020

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Preparation and quality evaluation of hingwashtak churna: A polyherbal formulation

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Abstract
Ayurveda is a traditional medicinal system of India, having a unique approach and principle to study and treatment of various disorders. The polyherbal formulation of Hingwashtak Churna is prepared as per Ayurvedic Formulary of India. It is used as ailment for various gastro intestinal diseases like acidity, gastric ulcers, Bloating joint disease, etc. Hingwashtak Churna is also used as a remedy for Primary dysmenorrhea. The present study was carried out to check quality and purity of formulation using various parameter such as Organoleptic characteristics, Microscopic evaluation, Phytochemical testing, Physicochemical analysis, Microbial analysis, UV spectrometry, Thin Layer Chromatography (TLC), Fingerprinting of High Performance Thin Layer Chromatography (HPTLC) was done using Piperine as a standard as it is the active ingredient in the Hingwashtak Churna to check adulterants and all the ingredients are present in the formulaton. This control measures are essential to establish quality of formulation which can be incorporated while developing the pharmacopoeial standards.

Keywords: Hingwashtak Churna, physicochemical, phytochemical, analytical evaluation

Introduction
The Ayurveda is an oldest medicinal system of India used for the treatment of several diseases with fundamental principle and theory-based principle, in Sanskrit the meaning of "Ayu" is life and "Veda" is knowledge or science. The whole meaning of Ayurveda is a science of life. Ayurveda identifies three basic type vatta, pitta and kapha. This principle can be related to basic biology of the body. Vatta is required to create movement so that fluids and nutrients get to the cells. Vatta is energy of movement, pitta is energy of digestion and kapha is energy of lubrication and structure. The current study deals with the polyherbal formulation of Hingwashtak Churna which was prepared according to Ayurvedic formulary of India. The Hingwashtak Churna is used for the treatment of digestive disorder like acidity, gastric ulcers, Bloating joint disease, etc. Hingwashtak Churna is also used as a remedy for Primary dysmenorrhea. The Hingwashtak Churnna was prepared using Sendha namak, Ginger, Long pepper, Black pepper, Hing, Black cumin, Jeera, Ajwain.
Rock salt (Sendha namak) in traditional Ayurvedic practices, rock salt is used as a home remedy for various digestive ailments, including stomach worms, heartburn, Bloating, constipation, stomach pain, and vomiting. Ginger powder (*Zingiber officinale)* is used as a remedy to cure indigestion. Black pepper (*Piper nigrum)* and Long pepper (*Piper longum)* it is used to prevent the formation of gases in the gastro-intestinal tract or eases its passing. The alkaloid piperine present in both serves many medicinal purposes. It is digestive, appetizer and also acts as a tonic. Hing (*Ferula foetida*) and Black cumin (*Nigella sativa)* are used for treatment of several diseases of stomach. It is one of the best alternates available for the problem of flatulence and is an important part of most of the digestive formulations. Jeera (*Cuminum cyminum)* Cumin stimulates saliva production, secretion of digestive fluids and excretion of bile, and additionally, it provides increased movement of the intestines, which generally improves digestion. Ajwain (*Trachyspermum Ammi)* is one of the best herbal wonder drug for gas, flatulence and indigestion also used in treatment of hyperacidity.
The Hingwashtak Churna was prepared by using all the specified ingredients as per Ayurvedic formulary of India. And to authenticate the quality and purity of the Hingwashtak Churna formulation was done using various parameters such as organoleptic character, microscopic evaluation, phytochemical test, physicochemical test, Thin Layer Chromatography (TLC), High Performance Thin Layer Chromatography (HPTLC) fingerprinting for identification and quantification of Piperine in Hingwashtak Churna formulation. Control measures are essential to establish safety of formulation.

Hingwashtak Churna has been authenticated using various parameters and can be incorporated while developing the pharmacopoeial standards. **(**Rashmi Saxena Pal, Volume: 5, 2018) [10].

त्रिकटुकमजमोदा सैन्धवं जीरकं द्वे समधरणधृतानामष्टमो हिङ्गुभागः ।
प्रथमकवलभुक्तं सर्पिषा चूर्णमेतज्जनयति जठराग्निं वातरोगांश्च हन्ति ।।३७।।
(भैषज्यरत्नावली, अग्निमांद्यादिरोगाधिकार; ३७)

Fig 1: Sanskrit Shlok

Materials and Methods

Collection of the Powder Drug

Hingwashtak Churna consists of the eight main ingredients in the dry powder form, the powder of Rock salt, *Piper nigrum (seed), Piper longum (fruit), Zingiber officinale (rhizome), Cuminum cyminum* (fruit), *Ferula foetida, Nigella sativa (seed), Trachyspermum ammi*. All these ingredients were brought from the local market (Ayurvedic shop) and were stored in a dry, moisture free space.

Formulation composition

Hingwashtak Churna was prepared as per the procedures mention in the Ayuvedic formulatory of India. All the ingredients were taken in the equal parts and were mixed together and stored in a dry, air tight container. (Table 1, Figure 2), (Rashmi Saxena Pal, 2018) [10].

Fig 2: Ingredients of Hingwashtak Churna

Table 1: Composition of *Hingwashtak Churna*

Sr.no	Constituents	Common name	Parts
1	Piper nigrum	Black	50 gms
2	Piper longum	Indian long pepper	50 gms
3	Zingiber officinale	Ginger	50 gms
4	Nigella sativa	Black cumin	50 gms
5	Cuminum cyminum	Jeera	50 gms
6	Trachyspermum ammi	Ajwain	50 gms
7	Ferula foetida	Hing	50 gms
8	Rock salt	Sendha namak	50 gms

Evaluation of Hingwastak churna

Organoleptic evaluation

Organoleptic evaluation were studied with their colour, taste, appearance, texture, odour etc, (Siddiqui, 1995) [11].

Preliminary phytochemical analysis

Samples of Hingwashtak churna were subjected to test separately to check the presence of saponins, tannins, alkaloids, glycosides, steroids, carbohydrates, Phenolic compounds, Flavonoids, Starch, Sterols, etc by the standard procedure, (Rashmi Saxena Pal, 2018) [10].

Physico Chemical Investigation

Determination of foreign matter

About one to two grams of the sample is weighed, the matter which appears different in colour and texture is considered to be the foreign matter. The matter with different colour and texture were separated and weighed, from this method the percentage of foreign matter was calculated, (Mehak Sharma, 2019).

Determination of Alcohol Soluble Extraction

Take about 1.5 ml of the chloroform in 600 ml of the distilled water. In 5 gm of the sample powder add 100 ml of the prepared solution of chloroform in a conical flask, shaking frequently and then allow to stand for 18 hrs. mix the filter rapidly taking care not to lose any solvent. 25 ml of the filterate is transferred to a tared flat bottom dish and evaporated to dryness on a water bath. The residue is dreid at 105 °C for 6 hrs. then cool in a desicator for 30 min and weighed immediately, (Divya N, 2017) [5].

Determination of Water Soluble Extract

About 5 gm of the sample is weighed and transfer it to the conical flask of capacity 100 ml. Add about 25 ml of the distilled water to it and place it on rotatory shaker at 140 rpm for 24 hrs. Then it was filtered and kept for drying in an hot air oven at 80 degree for 24 hrs. Weigh the sample again and the difference in the weight was recorded and water soluble extract was calculated, (Divya N, 2017) [5].

Determination of total Ash Content

Take 2 gm of feed sample prepared in a tarred crucible. Char at low temp. First and then incinerate the material in a muffle furnace for 4 hrs or more until free from all carbonaceous materials and ash is white or greyish white. Cool the crucible and ash partly on asbestos sheet and then in a desiccators and weight. Repeat the process of ignition in the muffle furnace, cooling and weighing at half an hour interval until the difference between two successive weighing is less than 1mg. Note the lowest weight, (Divya N, 2017) [5].

Microscopic Evaluation

Place the powdered material on the slide. Add 1-3 drops of dilute Safranin. In case of Starch identification add 1-3 drops of Iodine solution. Add cover slip. Remove any excess liquid that may exude from under the cover slip by blotting around its edges gently with filter paper, (Indian Pharmacoepeia 1996) [8].

Total Viable Count

Weigh 1gm of formulation (sample) and transfer it into sterile test tube and add 5ml of distilled water. Vortex it and perform serial dilution. Make serial 10 fold dilution up to 10^{-5} for total viable count. Transfer 0.1 ml formulation sample 10^{-3} to 10^{-5} dilution on NA and 10^{-3} to 10^{-5} for Saboraud dextrose agar. Spread the sample suspension uniformly on media. Incubate at 30-35 ^{0}C for 24 hours. Count cfu (colony forming unit) for bacteria and fungi, (Arun Sarjoshi, 2014) [2].

UV Spectrophotometry

The methanolic extract of formulation was analysed under UV Spectrometer ranging on various wavelength from 200nm

up to 700nm. The UV was analysed in order to get exact maximum absorbance. (Gupta Vishvnath, 2011) [7].

Chromatographic Evaluation Preparation of Standard

Piperine standard was prepared in methanol with initial concentration of 1000 ppm. Further dilution of 10 ppm was prepared using mobile phases. (Alok K. Hazar, 2017) [1].

Preparation of Sample

All the raw materials and prepared formulation powders were dissolved in Methanol and kept overnight. Next day all the solutions were filtered through whattman filter paper to obtain clear extracts. (Alok K. Hazar, 2017) [1].

High Performance Thin Layer Chromatography (HPTLC)

10µl of the filtered solution of formulation extract and standard was applied on the HPTLC plate as per conditions mentioned Table [2]. (Alok K. Hazar, 2017) [1].

Table 2: Conditions for HPTLC of Hingwahtak Churna

Stationary Phase	HPTLC plates silica gel
Plate Size	10.0 x 10.0 cm
Mobile Phase	(Hexane : Ethyl acetate : Glacial acetic acid) (3 : 1 : 0.1)
Saturation Time	20 mins
Standard Used	10 ppm Piperine
Spot Volume	10 µl
Band Length	8.0 mm
Solvent Front	80mm
Wavelenth and Lamp	Ultra Violet Lamp at 336nm
Sample Applicator	CAMAG Linomat 5
Sample Detection	CAMAG Visualizer : 200480
Number of Tracks	6

Results and Discussion

The In-house preparation of Hingwashtak Churna was done by following principles and directives given by Ayurvedic Formulary of India. The various parameters were done and was compared with the limits mentioned in the research paper. The Physicochemical and sensory features are mentioned below in Table [3] and Table [4]. Organoleptic evaluation and the determination of foreign matter was done to check the presence of foreign matter, taste, texture and odour etc. The formulation was analysed by microscopic evaluation under 10X as well 1000X, in order to see any contaminations are present in raw matierals as well as in finished formulation. The results of Microscopic evaluation of formulation showed presence of Epidermis, Starch granules, Vessels, Parenchyma figure [3-6]. The Phytochemical testing was done according to the protocol. The results of Phytochemical parameters showed presence of various secondary metabolities and is mentioned in Table [5]. Along with Physicochemical test, heavy metal determination test was seperately carried out and it was observed that the formulation shows absence of crude and heavy metals in raw matierals as well as formulation. Heavy metal Determination results are mentioned in Table [6].

Further the methanolic extract of formulation was scanned in UV spectrometer ranging from 200-700nm. It was observed that the maximum absorbance was 1.653 at 300nm. The UV spectra is mentioned in Table [7], Figure [7]. Microbiological studies were done for checking the growth of bacteria, fungi by using NA plates and Sabourauds plates. The results are mentioned in table [8]. In this result growth of any microorganism was not observed therefore we can say that the churn was of good quality. The HPTLC fingerprinting was performed to check the presence of standard in the formulation. The standard used in HPTLC is Piperine as it's the active ingredient in Hingwashtak churna. The fingerprinting was done of the raw matierals as well as the formulation against standard Piperine. The results are mentioned in Figure [8].

Table 3: Organoleptic Characteristics of *Hingwashtak Churna*

Sr. No	Parameters	Formulation
1.	Colour	Dark brown
2.	Odour	Characteristic
3.	Taste	Pungent
4.	Appearance	Powder

Table 4 Physicochemical Parameters of *Hingwashtak Churna*

Sr.no	Test	Results
1	Loss on Drying	7.7 %
2	Total Ash value	16.85 %
3	Alcohol Soluble Extraction	29.42 %
4	Water Soluble Extraction	22.012 %
5	Bulk Density	0.431 g/ml
6	Tapped Density	0.5250 g/ml
7	Hausner ratio	1.218
8	Powder Compressibility	17.90 %

Microscopic Evaluation of Hingwashtak Churna

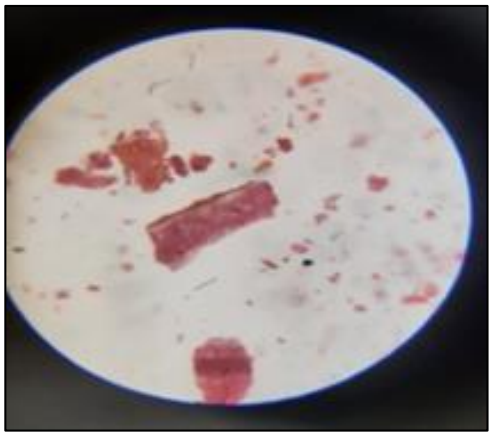


Fig 1: Epidermis

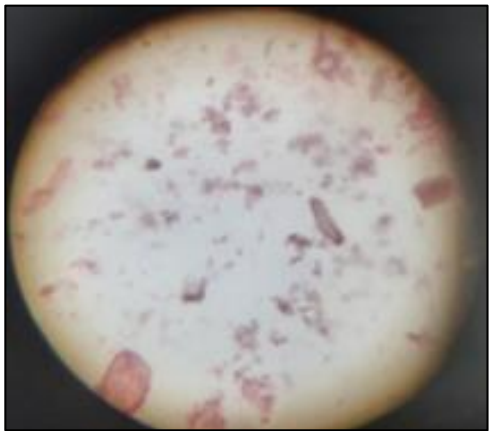


Fig 2: Starch granules

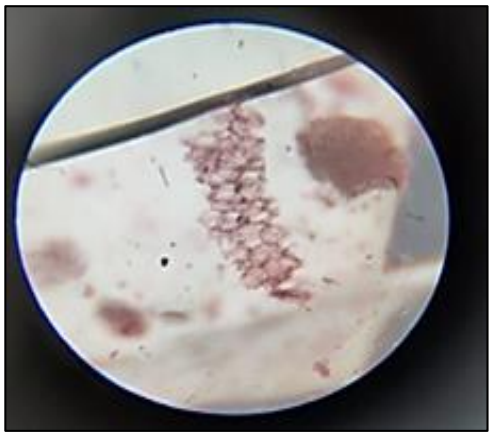


Fig 3: Parenchyma

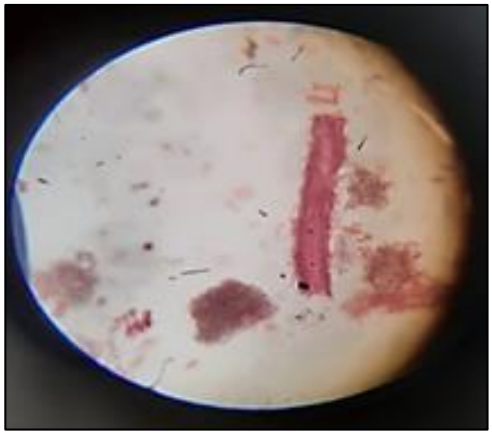


Fig 4: Vessels

Table 5: Phytochemical Evaluation of *Hingwashtak Churna*

Sr No.	Test	PN	PL	ZO	NS	CC	TA	FF	F
1	Tannins	+	-	+	-	+	+	-	+
2	Alkaloid	+	+	+	+	+	+	-	+
3	Glycoside	+	+	+	-	+	+	-	+
4	Flavonoids	+	-	-	+	+	-	-	+
5	Steroids	-	-	+	-	-	+	+	-
6	Saponin	+	+	+	+	+	+	+	+
7	Phenolic compound	-	-	-	-	-	-	-	-
8	Sterols	-	-	-	-	+	+	+	-
9	Anthocyanin	-	-	-	-	-	-	-	-
10	Terpenoid	-	-	-	-	-	+	-	+
11	Starch	-	-	-	-	-	-	-	-
12	Carbohydrate	-	-	+	-	-	-	+	-

Keys: + Present, - Absent

Keywords: PN- *Piper nigrum*, PL- *Piper longum*, ZO- *Zingiber officinale*, NS- *Nigella sativa*, CC- *Cuminum cyminum*, TA- *Trachyspermum ammi*, FF- *Ferula foetida*, F- Formulation.

Table 6: Heavy Metal Evaluation of *Hingwashtak Churna*

Tests	Results
Bismuth	-
Chromium	-
Copper	-
Cadmium	-
Nickel	-
Zinc	-
Cobalt	-
Lead	-

Table 7: UV spectra

Wavelength	Absorbance
200	0.051
250	1.586
300	1.653
350	1.579
400	0.556
450	0.115
500	0.08
550	0.016
600	0.048
650	0.091
700	0.03

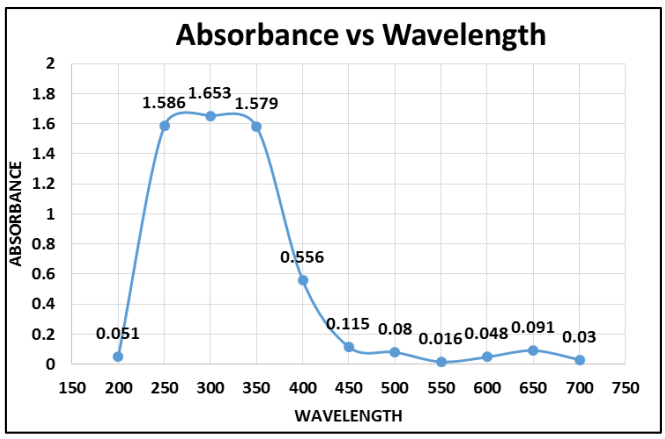
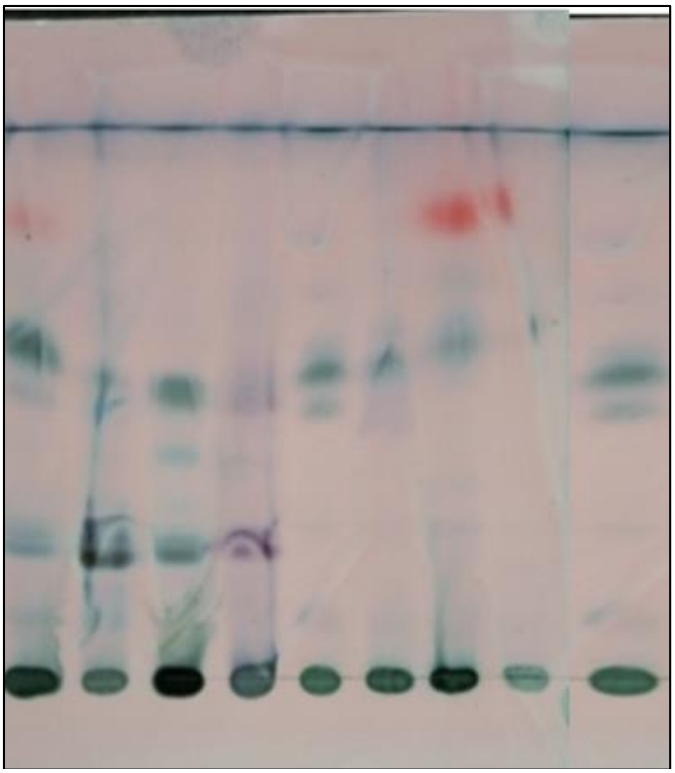


Fig 7: (200-700 nm)

Table 8: Microbiological Assay of Hingwashtak Churna

Media	Organism	Dilution	Cfu/0.1ml	Cfu/1ml	Average
Nutrient AGAR	Aerobic	10-3	232 x 10^3	23.2 x 10^4	7 x 10^5
		10-4	34 x 10^4	34 x 10^4	
		10-5	4 x 10^5	40 x 10^4	
Nutrient AGAR	Anaerobic	10-3	-	-	-
		10-4	-	-	
		10-5	-	-	
Sabourauds AGAR	Fungi	10-3	-	-	-
		10-4	-	-	
		10-5	-	-	



Key: Track 1: Formulation, Track 2: *Piper nigrum*, Track 3*: Piper longum*, Track 4*: Zingiber officinale*, Track 5*: Nigella sativa*, Track 6*: Cuminum cyminum*, Track 7*: Trachyspermum ammi*, Track 8*: Ferula foetida*, 9: Standard.

Fig 8: HPTLC Fingerprinting of Hingwashtak Churna

Conclusion

Authentication is one of the crucial and important steps that helps in maintaining a proper standard and quality of drugs. The formulations was prepared as per Ayurvedic Formulary of India, and it was authenticated by organoleptic characterization, Phytochemical testing, microscopic evaluation, physicochemical testing, and using modern analytical instruments like UV spectroscopy, High performance thin layer chromatography profiling. The results obtained shows the good quality of formulation and can be used as reference while setting the pharmacopoeial standards to ensure the quality of formulation.

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