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Standardization of a Polyherbal Formulation

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ABSTRACT

Standardisation of herbal formulations is essential in order to assess the quality of drugs, based on the concentration of their active principles. The present paper reports on standardisation of Hingwashtak churna, a polyherbal ayurvedic medicine used as a carminative, digestive, astringent and as an antacid. Hingwashtak churna was prepared as per the Ayurvedic Formulary of India. In-house preparation and three marketed samples have been standardised on the basis of organoleptic characters, physical characteristics and physico-chemical properties. The set parameters were found to be sufficient to evaluate the churna and can be used as reference standards for the quality control/quality assurance laboratory of a Pharmaceutical house.

KEYWORDS: Hingwashtak churna, polyherbal formulation, physicochemical parameters, standardization.

INTRODUCTION

Standardization is an essential factor for polyherbal formulation in order to assess the quality of the drugs based on the concentration of their active principle. It is very important to establish a system of standardization for every plant medicine in the market, since the scope for variation in different batches of medicine is enormous. Plant material when used in bulk quantity may vary in its chemical content and therefore, in its therapeutic effect according to different batches of collection e.g. collection in different seasons and/or collection from sites with different environmental surroundings or geographical location. The increasing demand of the population and the chronic shortage of authentic raw materials have made it incumbent, so there should be some sort of uniformity in the manufacture of Ayurvedic medicines so as to ensure quality control and quality assurance (5). The World Health Organization (WHO) has appreciated the importance of medicinal plants for public health care in developing nations and has evolved guidelines to support the member states in their efforts to formulate national policies on traditional medicine and to study their potential usefulness including evaluation, safety and efficacy (5). Hingwashtak churna is a polyherbal Ayurvedic medicine used as a carminative, digestive, astringent and as an antacid (2). The present paper reports on the standardisation of Hingwashtak churna based on organoleptic characters, physical characteristics and physico-chemical properties.

MATERIALS AND METHODS

Plant material

Hingwashtak churna consists of eight ingredients viz., *Piper nigrum*, *Piper longum*, *Zingiber officinale*, *Carum carvi*, *Cuminum cyminum*, *Apium graveolens*, *Ferula foetida*, and Rock salt (Saindhava Lavana) (5). All these ingredients were procured from the local market of Udupi, Karnataka, India and were authenticated by botanist Dr. K. Gopal Krishna Bhat, Professor of the Department of Botany, Poorna Prajna College, Udupi, Karnataka. A voucher specimen (PP 1-7) of the same has been deposited in the museum of the Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal for future reference.

Preparation of Hingwashtak churna

The churna was prepared as per the procedure given in Ayurvedic Formulary of India. All the ingredients (100g each) viz. *Piper nigrum*, *Piper longum*, *Zingiber officinale*, *Carum carvi*, *Cuminum cyminum* and *Apium graveolens* were fried in equal quantities. The fried ingredients were then powdered separately, passed through 80 # sieve and then mixed together in an equal proportion with *Ferula foetida* (12.5 gm) fried in ghee and rock salt (12.5gm) to get uniformly blended churna.

Marketed samples: The marketed sample of various brands of Hingwashtak churna i.e. Baidyanath (B), Dabur (D), Zandu (Z) and the In-house preparation (I) were standardised based on their organoleptic characters, physical characteristics and physico-chemical properties.

Organoleptic evaluation

Organoleptic evaluation refers to evaluation of formulation by colour, odour, taste, texture etc. The

organoleptic characters (6) of the samples were carried out based on the method as described by Siddiqui et. al.

Physico-chemical investigations

Physico chemical investigations of the formulations carried out including determination of extractive values and ash values (4,5).

Fluorescence analysis (6)

The powdered samples were exposed to Ultraviolet light at wavelength of 254nm and 366nm. Fluorescence analysis was carried out in accordance with the procedure reported by Kokoshi et. al. One mg of powdered drug was placed on a micro slide and observed under UV 366, UV 254 and in daylight to observe the fluorescent characteristics of the powder, if any. One mg of the powdered drug was placed on a micro slide and treated with one ml 1N HCl and observed under UV 366, UV 254 and in daylight while wet. One mg of the powdered drug was placed on a micro slide and treated with one ml 1N NaOH and the slide observed after a few minutes in daylight, under UV 254 and UV 366. One mg of the powdered drug was placed on a micro slide and treated with one ml 1 N NaOH in one ml methanol and observed under UV 366 and UV 254 and in daylight while still wet. One mg of the powdered drug was placed on a micro slide and treated with one ml 50% KOH and observed under UV 366 and UV 254 and in daylight while still wet. One mg of the powdered drug was placed on a micro slide and treated with one ml 50% H₂SO₄ and observed under UV 366 and UV 254 and in daylight while still wet. One mg of the powdered drug was placed on a micro slide and treated with one ml Conc. H₂SO₄ and observed under UV 366 and UV 254 and in daylight while still wet. One mg of the powdered drug was placed on a micro slide and treated with one ml 50% HNO₃ and observed under UV 366 and UV 254 and in daylight while still wet. One mg of the powdered drug was placed on a micro slide and treated with one ml conc. HNO₃ and observed under UV 366 and UV 254 and in daylight while still wet. One mg of the powdered drug was placed on a micro slide and treated with one ml acetic acid and observed under UV 366 and UV 254 and in daylight while still wet. One mg of the powdered drug was placed on a micro slide and treated with one ml iodine water and observed under UV 366 and UV 254 and in daylight while still wet.

Determination of pH

The pH of different formulations in 1% w/v and 10% w/v of water soluble portions were determined using

pH paper (Range 3.5-6) and (6.5-14) with standard glass electrode at 24°C.

Estimation of sodium content (7, 8)

Sodium content was estimated by flame photometry by using a flame photometer.

This was done as follows-

A stock solution of NaCl 10% was prepared in distilled water & further dilutions were made to get 0%, 2%, 4%, 6%, 8%, 10% respectively for preparing the standard graph shown in table 6. Sodium content of the formulations was estimated by flame photometric method based on the measurement of emission intensity in nano meters. The method was validated for linearity, precision and accuracy. The method obeyed Beer's law in the concentration range 1-10 µg/ml. When a standard drug solution was assayed repeatedly (n = 6), the mean error (accuracy) and relative standard deviation (precision).

Determination of physical characteristics of powder formulation (9, 10)

Physical characteristics like bulk density, tap density, angle of repose, Hausner ratio and Carr's index were determined for different formulations. The term bulk density refers to method used to indicate a packing of particles or granules. The equation for determining bulk density (Db) is $Db = M/V_b$ where M is the mass of the particles and V_b is the total volume of the packing. The volume of the packing can be determined in an apparatus consisting of a graduated cylinder mounted on a mechanical tapping device (Jolting Volumeter) that has a specially cut rotating can. Hundred gm of weighed formulation powder was taken and carefully added to the cylinder with the aid of a funnel. The initial volume was noted and the sample was then tapped until no further reduction in volume was noted. The initial volume gave the bulk density value and after tapping the volume reduced, giving the value of tapped density.

Angle of repose has been used as an indirect method of quantifying powder flowability, because of its relationship with interparticle cohesion. The fixed funnel and the free standing cone method employs a funnel that is secured with its tip at a given height (H), above the graph paper that is placed on a flat horizontal surface. Powder or granules was carefully poured through the funnel until the apex of the conical pile just touched the tip of the funnel. Thus, with R being the radius of the conical pile. $\tan \alpha = H/R$ or $\alpha = \arctan H/R$, where α is the angle of repose.

Hausner ratio is related to interparticle friction and as such can be used to predict the powder flow

properties. The equation for measuring the Hausner ratio is D_f / D_o

Where, D_f = Tapped density and D_o = Bulk density.

Carr's index is another indirect method of measuring the powder flow from bulk density. The equation for measuring Carr's index is

$$\% \text{ Compressibility} = \frac{D_f - D_o \times 100}{D_f}$$

Where, D_f = Tapped density, D_o = Bulk density

RESULTS AND DISCUSSION

In-house standard formulation was prepared according to the Ayurvedic Formulary of India (2). Water soluble and alcohol-soluble extractive values are given in Table 2 and ash values (total ash, acid insoluble ash and water soluble ash) in Table 1. The ash values (5) of the samples were carried out based on the method as described by World Health Organization (WHO) guidelines for medicinal plant materials. The physico-chemical comparisons between in-house formulation and marketed formulations are given in Table 3. The results obtained with the market formulations and the

in-house formulations were found to be comparable and variation was insignificant. Acid -insoluble ash value for in-house formulation was found to be 1.98 ± 0.92 , in case of the marketed formulation this was found to be 3.33 ± 1.72 (average value along with standard of deviation) ($n = 3$). pH values from 1% W/V and 10% W/V solution revealed that while the in-house formulation is nearly neutral, the market formulation was slightly acidic. Estimation of sodium content shows that in-house formulation contains less sodium than of other marketed formulations (Table 7). In Tables 8 and 9 the fluorescent analyses have been presented. The physical characteristics of the in-house formulation and three market formulations (average values along with standard deviation) ($n = 3$) are shown in Table 5. The results of the market formulations and in-house formulation were found to be comparable. The flowability of the formulation was found to be poor in both market formulations and in-house formulation, which was further confirmed by high values of Hausner ratio and Carr's index.

Table 1: % Ash values of individual ingredients present in Hingwashtak churna (w/w)

Samples	Total ash	Acid insoluble ash	Water soluble ash
	Mean (n=6) $\pm$ SD	Mean (n=6) $\pm$ SD	Mean (n=6) $\pm$ SD
<i>Piper nigrum</i>	5.5491 $\pm$ 0.8679	0.7037 $\pm$ 0.0525	2.1187 $\pm$ 0.0606
<i>Piper longum</i>	4.8925 $\pm$ 0.0568	0.6325 $\pm$ 0.1081	2.5825 $\pm$ 0.1337
<i>Z.officinale</i>	10.68 $\pm$ 1.3356	0.35 $\pm$ 0.0976	1.4225 $\pm$ 0.3335
<i>Carum carvi</i>	4.475 $\pm$ 0.3849	0.4525 $\pm$ 0.0561	3.1085 $\pm$ 0.2909
<i>Cuminum cyminum</i>	9.985 $\pm$ 0.5603	1.3742 $\pm$ 0.2773	6.7562 $\pm$ 0.2337
<i>Apium graveolens</i>	7.9316 $\pm$ 0.2872	0.5625 $\pm$ 0.2036	5.58 $\pm$ 0.0697
<i>Ferula foetida</i>	1.17 $\pm$ 0.1101	0.335 $\pm$ 0.1161	0.8425 $\pm$ 0.0125

Table 2: Extractive values of individual ingredients present in Hingwashtak churna

Samples	Alcohol soluble (%)	Water soluble (%)
	Mean (n=6) $\pm$ SD	Mean (n=6) $\pm$ SD
<i>Piper nigrum</i>	8.98 $\pm$ 1.1814	8.4325 $\pm$ 0.1228
<i>Piper longum</i>	10.055 $\pm$ 1.2318	10.705 $\pm$ 1.007
<i>Z.officinale</i>	7.83 $\pm$ 1.6563	15.8025 $\pm$ 0.9656
<i>Carum carvi</i>	38.0325 $\pm$ 1.0741	18.9275 $\pm$ 1.5226
<i>Cuminum cyminum</i>	29.7125 $\pm$ 0.3364	20.285 $\pm$ 1.949
<i>Apium graveolens</i>	20.045 $\pm$ 1.6018	23.3525 $\pm$ 1.7162
<i>Ferula foetida</i>	60.2825 $\pm$ 0.7791	70.4532 $\pm$ 0.9213

Table 3: Physico-chemical characteristics of Hingwashtak churna formulations.

Parameter	In-house formulation Mean (n=3) $\pm$ SD	Market formulation Mean (n=3) $\pm$ SD
Water-soluble extractive	27.45 $\pm$ 1.09	34.67 $\pm$ 1.97
Alcohol-soluble extractive	18.35 $\pm$ 1.32	17.66 $\pm$ 0.92
Total ash values	22.93 $\pm$ 1.28	23.43 $\pm$ 0.79
Acid-insoluble ash	1.98 $\pm$ 0.92	3.33 $\pm$ 1.72
Water soluble ash	12.65 $\pm$ 0.76	7.38 $\pm$ 1.87
pH of 1% W/V formulation solution	7.23 $\pm$ 0.67	6.59 $\pm$ 0.68
pH of 10% W/V formulation solution	7.35 $\pm$ 0.97	6.80 $\pm$ 0.47

Table 4: Organoleptic properties of different Hingwashtak churna formulations

Different Formulations	Appearance	Colour	Taste	Odour
In- House	Powder	Light brown	Salty	Characteristic odour
Baidyanath	Powder	Brown	Salty	Characteristic odour
Dabur	Powder	Light brown	Salty	Characteristic odour
Zandu	Fine Powder	Yellow	Sour	Characteristic Odour

Table 5: Physical characteristics of different Hingwashtak churna formulations

Parameters	In-house formulation Mean (n=3) ± SD	Market formulation Mean (n=3) ± SD
Tap density	0.5416 ± 0.07	0.3446 ± 0.02
Bulk density	0.4387 ± 0.03	0.5503 ± 0.01
Angle of repose	42.6 ± 0.27	41.03 ± 1.26
Hausner ratio	1.21 ± 0.08	1.1 ± 0.01
Carr's Index	15.03 ± 0.79	11.62 ± 1.97

Table 6: Standard graph of sodium by flame photometry method.

Concentration (%)	Emission intensity (λ)
10	80
8	65
6	49
4	31
2	15
0	0

Table 7: Sodium content in different Hingwashtak churna formulations

Different formulations	Sodium content (%)
Baidyanath	7.46
Dabur	7.71
Zandu	9.55
In house	6.43

Table 8: Powder fluorescence test of Ferula foetida present in Hingwashtak churna and in-house Hingwashtak churna formulation

Material	Ferula foetida			In-house Formulation		
	Day light	UV 254 nm	UV 366 nm	Day light	UV 254 nm	UV 366 nm
Powder	P.Y.	Y.	L.Y.	P.Y.	BR.	F.Y.
In NaOH(1N) in water	P.Y.	BR.	L.Y.	Y.	G.Y.	Y.
P + In HCl (1N)	C.	C.	W.	Y.	BR.	Y.
P + In NaOH (1N) in methanol	Y.	BR.	W.	Y.	G.Y.	Y.
P + 50% KOH	Y.	BR.	Y.	Y.	G.Y.	Y.
P + 50% H ₂ SO ₄	PI.	PI.	BL.	Y.BR.	Y.	F.B.
P + 50% HNO ₃	Y.	G.Y.	BL.	O.	Y.	Y.
P + Conc. HNO ₃	Y.	G.Y.	G.Y.	Y.BR.	Y.	Y.
P + CH ₃ COOH	Y.	G.Y.	W.	Y.	BR.	Y.
P+ Conc. H ₂ SO ₄	PI.	PI.	BL.	Y.BR.	Y.	F.B.
P + Iodine in water	Y.	Y.	BR.	BR.	BL.	BR.

BL: Bluish light, BR: brown, PY: Pale yellow, Y: yellow, G: Green, LG: Light green, LY: Light yellow, GY: Grayish yellow, LB: Light blue, FY: Fluorescent yellow, Y.BR. Yellowish brown

Table 9: Powder fluorescence test of different Hingwashtak churna formulations

Material	Baidyanath			Dabur			Zandu		
	Day light	UV 254 nm	UV 366 nm	Day light	UV 254 nm	UV 366 nm	Day light	UV 254 nm	UV 366 nm
Powder	L.Y.	BR.	F.Y.	P.Y.	BR.	P.Y.	P.Y.	BR.	P.Y.
In NaOH(1N) in H ₂ O	Y.	G.Y.	Y.	Y.	G.Y.	Y.	O.	L.Y.	Y.
P + In HCl (1N)	Y.	G.Y.	BR.	L.Y.	Y.	L.Y.	Y.	G.Y.	F.Y.
P + In NaOH (1N) in MeOH	BR.	BR.	F.Y.	Y.	G.Y.	Y.	Y.	G.Y.	F.Y.
P + 50% KOH	Y.	G.Y.	BR.	Y.	G.Y.	Y.	O.	Y.	Y.
P + 50% H ₂ SO ₄	BR.	Y.	F.Y.	Y.	G.Y.	F.B.	BR.	Y.	F.B.
P + 50% HNO ₃	O.	Y.	BR.	Y.	G.Y.	G.Y.	Y.	G.Y.	BR.
P + Conc. HNO ₃	Y.	Y.	L.Y.	Y.	G.Y.	Y.	L.Y.	G.Y.	BR.
P + CH ₃ COOH	BR.	BR.	Y.	L.Y.	G.Y.	L.Y.	Y.	L.Y.	Y.
P+ Conc. H ₂ SO ₄	Y.	G.Y.	F.Y.	Y.	G.Y.	F.B.	BR.	G.Y.	F.B.
P + Iodine in water	BR.	BR.	F.Y.	BR.	BR.	BL.	BR.	BL.	BR.

BL: Bluish light, BR: brown, PY: Pale yellow, Y: yellow, G: Green, LG: Light green, LY: Light yellow, GY: Grayish yellow, LB: Light blue, FY: Fluorescent yellow. F.B.: Fluorescent blue, O: orange

Organoleptic properties of different formulations were found to be similar and are given in Table 4.

Pharmacognostic characters established for the raw materials of Ayurvedic formulation Hingwashtak churna could be employed as Q.C. standards for evaluating its identity and can be used for routine analysis.

Purity and potency of the raw materials and formulations following the procedure given could be performed in QC/QA laboratory of pharmaceutical house.

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REFERENCES

1. Kaviraj Sri Ambidatasastri Ayurvedacharya, *Bhaisjya Ratnawali* (Churbabha sanskurrata Saasthan, publisher of Indian Sanskrit Literature and Distributors, Varanashi, 1951) pp. 242-5.
2. Ayurvedic Formulary of India, part-I, 1st eds., (Government of India, Ministry of Health and Family Planning, Department of Health. New Delhi, 1978) pp. 95-7.
3. Ansel H C, Allen L V JR, Popovich N G. "Powder and granules," in: *Pharmaceutical Dosage Forms And Drug Delivery System*, 17th eds. Lippincott Williams and Wilkins,

227, East Washington Square, Philadelphia, PA 19106 – 3780, 164-8 (2000).

4. *Indian Pharmacopoeia*, (Ministry of Health and Family Welfare, Government of India, New Delhi), Vol II, 1996.
5. Organisation Mondiale De La Sante, Quality control methods for medicinal plant materials, (World Health Organisation, 559, rev.1, Original English, 1992) pp. 159.
6. Siddiqui, Hakim M A. Format for the pharmacopoeial analytical standards of compound formulation, workshop on standardization of Unani drugs, (appendix), 24-25 January, Central Council for Research in Unani Medicine (CCRUM), New Delhi, (1995).
7. Skoog A D, West D M., Holler F J. *Fundamentals of Analytical Chemistry*, 17th eds., (Saunders College Publishing, New York, USA., 1991) pp.613-5.
8. Mendham J, Denney R C, Barnes J D, Thomas M. *Vogel's Text Book of Quantitative Chemical Analysis*, 6th eSds., (Pearson Education Pvt. Ltd., Singapore, 2002) pp. 605-13.
9. Lachman L, Lieberman H A, Kanig J L. *The Theory and Practice of Industrial Pharmacy*, 3rd eds., (Varghese Publishing house, Bombay –14, 1987) pp.183, 316.
10. Aulton M E. *Pharmaceutics*, The science of dosage forms design, 2nd eds., (Churchill Livingstone, New Delhi, 2002) pp. 205-221.