Comparative standardization study of two marketed Ayurvedic formulations of Hingwashtak Churna

Kute Vaishali, M Shruti, Mavoori Praharsha, Machiraju Jagadeeswari and Lakkaraju Mounika

Abstract
Standardization of herbal formulation is essential in order to assess the quality of drugs for therapeutic value as it is a plant-derived material containing raw ingredients. According to an estimate of World Health Organization (W.H.O) most of the developing countries rely on traditional medicines. Hence, it has given a detailed protocol in 1999 for the standardization of herbal drugs. Hingwashtak Churna is an Ayurvedic formulation which is used to treat stomach ailments to maintain proper function of the gastrointestinal tract, maintain eliminative function. It is also used in the treatment of vata imbalance and vata related diseases like bloating joint diseases etc. A scheme was developed for standardization of Hingwashtak churna for two marketed formulations and in-house. Various parameters such as physical, chemical and biological were carried out for quantitative and qualitative analysis of selected formulations and they showed promising results.

Keywords: Standardization, traditional medicine, Hingwashtak Churna, quality control

1. Introduction
The use of herbs as medicine is the oldest form of healthcare known to humanity and has been used in all cultures throughout history. Herbal medicines are herbs or herbal products used for their therapeutic or medicinal value. They may come from any part of the plant but are most commonly made from leaves, roots, bark seeds, and flowers. They are eaten, swallowed, drunk, inhaled, or applied topically to the skin. Herbal products often contain a variety of naturally-occurring biochemical’s from plants, many of which contribute to the plant’s medicinal benefits.

Herbal medicines are coming from variable sources. So the quality of herbal medicine is an important aspect to get the desirable effects. Standardization of herbal medicines is the process of prescribing a set of standards or inherent characteristics, constant parameters, definitive qualitative and quantitative values that carry an assurance of quality, efficacy, safety and reproducibility. Specific standards are worked out by experimentation and observations, which would lead to the process of prescribing a set of characteristics exhibited by the particular herbal medicine. Hence standardization is a tool in the quality control process [1].

1.1 Need for standardization of herbal medicine
There are several problems associated with herbal medicines. 1. Herbal drugs are usually mixtures of many constituents. 2. The active principle(s) is (are), in most cases unknown. 3. Selective analytical methods or reference compounds may not be available commercially. 4. Plant materials are chemically and naturally variable. 5. The source and quality of the raw material are variable. 6. The methods of harvesting, drying, storage, transportation, and processing also affect herbal quality. 7. This is further complicated by the use of combination of herbal ingredients as being used in traditional practice. It is common to have as many as five different herbal ingredients in one product. Thus batch-to-batch variation starts from the collection of raw material itself in the absence of any reference standard for identification. These variations multiply during storage and further processing. Hence for herbal drugs and products, standardization should encompass the entire field of study from cultivation of medicinal plant to its clinical application [1].
1.2 Quality control of herbal medicine
According to WHO, standardization and quality control of
herbs is the process involved in the physicochemical
evaluation of crude drug covering aspects, such as selection
and handling of crude material, safety, efficacy and stability
assessment of finished product, documentation of safety and
risk based on experience, provision of product information to
consumer and product promotion. Attention is normally paid
to such quality indices such as Macro and microscopic
examination, Foreign organic matter, Ash values to judge the
identity and purity of crude drug total ash, sulphated ash,
water soluble ash and acid insoluble ash etc., Moisture
content, extractive values, crude fiber, qualitative chemical
evaluation, toxicological studies.[1]

Hingwasthak Churna is used for digestion and treatment of
Vata ailments. It is prepared from 8 ingredients viz. Piper
nigrum Linn (Black Pepper Powder), Zingiber officinale
Roscoe(Dry Ginger Powder), Cuminum cyminum
Linn(Cuminmin), Nigella sativa (Black Cumin Seed), Piper
longum (Long pepper powder), Trachyspermum ammi Sprague(Ajowan), Saindav lavan (Rock Salt), Ferula foetida
Regel (Asafetida Powder). All these ingredients are collected
from various locations and various sources. So there are
chances of variation in phytoconstituents which may result in
decreased level of therapeutic efficacy. Saindav lavan,
Asafetida being hygroscopic there are chances of increase in
moisture content which will degrade other ingredients. In the
present study two marketed formulations and one in-house
formulation have been tested for physical, chemical and
biological properties to develop comparative data on
standardization and quality control of selected three
formulations of Hingwasthak Churna [2].

2. Materials and Methods
2.1 Materials
2.1.1 Plant Materials: (Ingredients)
Piper nigrum Linn (Black Pepper Powder), Zingiber officinale
Roscoe (Dry Ginger Powder), Cuminum cyminum
Linn (Cuminmin), Nigella sativa (Black Cumin Seed), Piper
longum (Long pepper powder), Trachyspermum ammi Sprague(Ajowan), Saindav lavan (Rock Salt), Ferula foetida
Regel (Asafetida Powder). All these ingredients are collected
from local market, authenticated by morphological and
physico chemical study. Then all crude drugs were powdered to 40
mesh size and stored in tightly closed container for further
study [2].

2.1.2 Marketed formulations of Hingwasthak Churna
Formulation -1: Baidyanath
Formulation -2: Zandu
Formulation-3: In-house formulation: Prepared by mixing all
8 ingredients in equal quantity [2].

2.2. Methods
2.2.1 Physical methods
a) Determination of Ash Values
1. Total Ash Value: 1 gms of churna was weighed accurately
in a previously ignited and tared silica crucible. The material
was then ignited by gradually increasing the heat to 500-
600°C until; it appeared white indicating absence of carbon. It
is then cooled in a desiccator and total ash in mg per gm of
air-dried material is calculated[3, 4, 5, 6, 7].
2. Acid Insoluble Ash Value: To the crucible containing total
ash, 25 ml of dil. HCL was added and boiled gently for
5minutes, and then about 5 ml of hot water was added and
transferred into crucible. The insoluble matter was collected
on an ash less filter paper. This was then washed with hot
water until filtrate is neutral and the filter paper along with the
insoluble matter was transferred into crucible and ignited to
constant weight. The residue was then allowed to cool and
then weighed[8, 4, 5, 6, 7].

b) Determination of Extractive Values
This method determines the amount of active constituents
extracted with solvents from a given amount of medicinal
plant material. It is employed for materials for which as yet no
suitable chemical or biological assay exists.
1. Water Soluble Extractive Value: 5 gms of churna was
accurately weighed and placed inside a glass stoppered
conical flask. It is then macerated with 100 ml of
chloroform water for 18 hours. It was then filtered and
about 25 ml of filtrate was transferred into a china dish
and was evaporated to dryness on a water bath. It was
then dried to 105°C for 6 hours, cooled and finally
weighed[5, 4, 5, 6, 7].
2. Alcohol Soluble Extractive Values: Ethanol was used as
solvent in place of chloroform water and remaining
procedure was the same as that of water-soluble
extractive value[3, 4, 5, 6, 7].

c) Determination of moisture content
A clean china dish was taken and dried in a hot air oven at
105°C for 30 min. Then 1.0 gm of powder sample was placed
into it. The sample was then dried in an oven drying was
continued till a constant weight of sample was obtained (6
hours). After drying, the dish was allowed to cool to room
temperature in a desiccator before weighing and then the
weight of dried sample was recorded. The percentage loss on
drying was calculated with reference to powder sample taken
initially [3].

2.2.2. Chemical methods
a) Semi Quantitative method
1. Limit test for Iron
Procedure: Limit test was performed in Nessler’s cylinder. 2
ml of test and standard solutions (20ppm) were taken in
separate cylinders and then 2ml of 20% solution of citric acid
and 0.1 ml thioglycollic acid were added. The solution was
then mixed and made alkaline with iron free ammonia, diluted
to 50ml with distilled water. It was then allowed to stand for
5minutes and colour obtained in sample was compared with
that of standard colour. If the colour produced in test is more
when compared to that of standard solution then the sample
was said to fail the limit test and said to pass the test if vice
versa occurs [8, 9].

b) Qualitative method
2. Thin Layer Chromatography
Preparation of extract: 0.5 gm of the powdered plant material
extracted with 10 ml of methanol by heating on water bath for
30 min and filtered, filtrate was used for TLC.
Precoated silica gel plates were used for developing TLC.
Samples were applied to the plates from the edges of the plate,
with capillary. The plates were developed by the
ascending technique, to a distance of 80 mm, at 25±5°C,
relative humidity 50–60%, in a glass chamber with a stainless
steel lid, using the mobile phase Hexane: Ethyl acetate (9.5:0.5). The chamber was saturated with solvent system vapors for 20 minutes. After development, plates were dried using a hot-air dryer, and observed under UV cabinet. Rf values are calculated [5].

2.3.3. Biological methods

**Estimation of Microbial Content**

Preparation of agar media: Peptone (0.5 g), Beef extract (0.3 g), NaCl (0.5 g) was weighed and transferred into 250 ml conical flask, then 10 ml distilled water was slowly added to it. The flask was swirled gently to dissolve the ingredients. The final solution was clear. The pH was adjusted with 0.1N NaOH solution (if acidic) or 0.1N HCl (if alkaline) using pH meter technique. Then 2 g of agar was added to above prepared Nutrient Broth (100ml). The mixture was heated to above 100°C to dissolve agar completely the flask was plugged with cotton and sterilized the medium by autoclave at 121°C, 15 psi Pressure for 15 min [9,10,11]. Preparation of samples, inoculation and incubation: Each formulation (0.1 gm) was weighed and dissolved in 10 ml autoclaved distilled water separately, inoculated on to the petri dish containing 3/4 th agar media and incubated at 28°C for 5 days. Then it was observed growth of microorganism colonies. The colonies were counted using digital colony counter [9,10,11].

3. Results and Discussions

3.2. Physical methods

**3.2.1. Ash Value:** The ash remaining following ignition of medicinal plant materials is determined by different methods which measure total ash, acid insoluble ash. The total ash method is designed to measure the total amount of material remained after ignition. This includes both "Physiological ash" which is derived from the plant tissue itself, and "non physiological ash", which is the residue of the extraneous matter (e.g. sand and soil) adhering to the plant surface. Acid – insoluble ash is the residue obtained after boiling the total ash with dilute hydrochloric acid, and igniting the remaining insoluble matter. This measures the amount of silica present, especially as sand and siliceous earth. Ash values for three formulations and results are described in table 1.

**Table 1: Ash value**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Total Ash Value (w/w)</th>
<th>Acid insoluble ash (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formulation 1</td>
<td>15 ± 1</td>
<td>11.167 ± 1.6</td>
</tr>
<tr>
<td>Formulation 2</td>
<td>26.333 ± 1.52</td>
<td>22.333 ± 1.53</td>
</tr>
<tr>
<td>Formulation 3</td>
<td>26 ± 1.3</td>
<td>23.333 ± 1.53</td>
</tr>
</tbody>
</table>

* Mean ± SD (n=3)

**3.2.2. Extractive value:** This method determines the amount of active constituents extracted with solvents from a given amount of medicinal plant material. Extractive value is indicative of nature of chemical constituents present in crude drug i.e. polar, medium polar and non-polar. It is carried out for all three formulations using polar and medium polar solvent and results are described in table 2. It was found that constituents in formulations are medium polar.

3.2.3. Determination of moisture content: Moisture content is useful to determine stability of constituents and susceptibility of crude drugs to undergo microbial attack. Moisture content for all three formulations was determined and results are tabulated in table 3.

**Table 2: Extractive value**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Water Soluble Extractive value (w/w)</th>
<th>Alcohol soluble extractive value (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formulation 1</td>
<td>26.3333 ± 3.21</td>
<td>30.5 ± 0.5</td>
</tr>
<tr>
<td>Formulation 2</td>
<td>24.8667 ± 2.01</td>
<td>43.83 ± 0.76</td>
</tr>
<tr>
<td>Formulation 3</td>
<td>40.3333 ± 0.57</td>
<td>40.5 ± 0.5</td>
</tr>
</tbody>
</table>

* Mean ± SD (n=3)

**Table 3: Moisture Content Determination**

<table>
<thead>
<tr>
<th>Formulation</th>
<th>% Loss on drying (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formulation 1</td>
<td>0.09 ± 0.87</td>
</tr>
<tr>
<td>Formulation 2</td>
<td>0.09 ± 0.78</td>
</tr>
<tr>
<td>Formulation 3</td>
<td>0.12± 0.66</td>
</tr>
</tbody>
</table>

* Mean ± SD (n=3)

3.3. Chemical methods

3.3.1. a). Semi Quantitative Methods

Limit test for Iron: Ingested iron can have an extremely corrosive effect on the gastrointestinal (GI) mucosa, which can manifest as nausea, vomiting, abdominal pain, hematemesis, and diarrhea. Therefore it is necessary to determine whether iron content in acceptable limits or not. All the three samples were found to be less turbid compared to the standard solution. Hence, it passes the limit test for Iron as described in below figure 1.

**b). Qualitative methods**

TLC Finger print profile: As the focus of the study was to identify formulations from its adulterants using physical, chemical and biological methods all the collected formulations were subjected to TLC fingerprint profiling to compare the difference in chemical constituents present. The fraction of methanolic extracts of all formulations were spotted on a TLC plate and developed in Hexane: Ethyl acetate (9.5:0.5). The plate was observed under UV 254 nm and UV 366 nm (Figure 2; Table 4).

*Fig 1: Limit Test for Iron 1, Formulation 1; 2, Formulation 2; 3, Formulation 3; 4, Standard for iron (20ppm)*
Fig 2: TLC fingerprint profile of methanolic extract of Formulations. Tracks: 1: Formulation 1; 2: Formulation 3; 3: Formulation 2

Formulations 1 and 2 are lacking in few spots than Formulation 3. This means that some of the constituents were absent in formulations 1 and 2.

Table 4: Rf values of Formulations

<table>
<thead>
<tr>
<th>Spot number</th>
<th>Rf values (Under UV 254 nm)</th>
<th>Rf values (Under UV 366 nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Track 1</td>
<td>Track 2</td>
</tr>
<tr>
<td>1</td>
<td>0.055</td>
<td>0.018</td>
</tr>
<tr>
<td>2</td>
<td>0.1296</td>
<td>0.055</td>
</tr>
<tr>
<td>3</td>
<td>0.425</td>
<td>0.166</td>
</tr>
<tr>
<td>4</td>
<td>0.55</td>
<td>0.425</td>
</tr>
<tr>
<td>5</td>
<td>0.648</td>
<td>0.55</td>
</tr>
<tr>
<td>6</td>
<td>0.77</td>
<td>0.722</td>
</tr>
<tr>
<td>7</td>
<td>0.833</td>
<td>0.833</td>
</tr>
<tr>
<td>8</td>
<td>-</td>
<td>0.944</td>
</tr>
</tbody>
</table>

3.4. Biological methods
3.4.1. Estimation of microbial load
The microbial content for all the three media was performed and colonies are counted using digital colony meter and was found within the limits. This indicates that the microbial load in the all three formulations was permissible. Number of colonies found in each formulation is given below.

- **Formulation-1**: 101
- **Formulation-2**: 269
- **Formulation-3**: 156

4. Conclusion
Collected plant materials were authenticated by Morphology and Microscopy. All the plant materials were found to be genuine by comparison with literature. In standardization of marketed formulation physical evaluation it was found that Hingwastak churna has ash value within limits as well as it has polar and medium polar constituents 25-30 % w/w respectively. Moisture content is in permissible limits. There is less chance for hydrolysis of constituents and microbial attack. In chemical evaluation it was found that iron content is within limit i.e. less than 20 ppm. From reports of TLC it was observed that some of the constituents were absent formulation 1 and 2 which were found in In-house formulation. Identification and quantitative determination of variation in the constituents present in all three formulations can be done in future by using modern analytical techniques like HPLC, HPTLC. Total microbial count was done to evaluate the microbial load in the formulations. It was observed number of colony forming units in all formulations were below 300. Therefore the microbial load in all formulations is within limits. The study shows that the contents of formulation presents within the permissible limits as per WHO, all these investigations are not specified in the standard literature such as in pharmacopoeia, which could helpful in authentication of *Hingwastak* churna. The result of present study will also serve as reference monograph in the preparation of drug formulation.

5. Reference
5. World Health Organization (Geneva). Quality Control Methods for Medicinal Plant Materials, A.I.T.B.S. Publishers and Distributors, 10, 22, 28, 46, 61, 64.