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## Standardization study of sample of *Haridra* (*Curcuma longa* Linn.)

Soni Manisha, Patil Shilpa and Gupta Laxmi Narayan

**Abstract**

**Background:** Raw material standardization before preparation of any formulation is of prime importance. In this article we will deal with the *Haridra* (*Curcuma longa* Linn.) a well-known versatile drug used traditionally as well as routinely by mankind since long time. There are lots of researches available on its different therapeutic applications and standardization.

**Aim:** To standardize *Haridra* (*Curcuma longa* Linn.) by using parameters laid down by Ayurvedic Pharmacopoeia of India.

**Material and Method:** Dried Rhizome of *Haridra* was studied macroscopically and microscopically. *Haridra* powder was tested for physico-chemical characteristic like loss on drying, total ash, acid insoluble ash, water soluble extractive, alcohol soluble extractive, Phyto- constituent presence, Thin layer chromatography, FTIR, AAS and microbial contamination.

**Result:** The test results are comparable with the standard values given in Ayurvedic Pharmacopoeia of India and found within the prescribed range. FTIR reveals the presence of amide group, AAS confirmed the heavy metal presence below permissible limit and absence of any microbial growth was determined by microbial study.

**Keywords:** *Curcuma longa* Linn, raw material, dried rhizome

**Introduction**

Standardization and quality control of raw drug is very necessary. For maintaining the quality of formulation it is essential that the raw materials which are used should be of desired quality and process involved in its preparation should be followed correctly so that the final product will be a good quality product. The quality of the product is dependent on the quality of the raw material and the quality of the extraction, formulation and manufacturing processes. The safety and benefits of a product are directly related to its quality, just as the quality of the raw material depends on practices in the agricultural supply line.

*Haridra* consists of the dried and cured rhizomes of *Curcuma longa* Linn. (Fam. Zingiberaceae), a perennial herb extensively cultivated in all parts of the country, crop is harvested after 9-10 months when lower leaves turn yellow rhizomes carefully dug up with hand-picks between October-April. It posses *Katu*, *Tikra Rasa*, *Ushna Virya*, *Ruksha Guna*, *Katu Vipaki*. It works as *Krimighna*, *Kushtaghna*, *Varnya*, *Vishaghna* and *Pramehanashak* [1].

**Material and Method**

Raw *Haridra* Rhizome was procured from of Gola Dinanatha market, Varanasi and it was analysed by using physico-chemical tests in the Department of Dravyaguna, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University. (Fig. no.1 and Fig. no.2)

Dried Rhizome of *Haridra* was studied macroscopically and microscopically. *Haridra* powder was tested for physico-chemical characteristic like loss on drying, total ash, acid insoluble ash, water soluble extractive, alcohol soluble extractive, phyto- constituent presence, Thin layer chromatography, FTIR, AAS and microbial contamination.

**A. Macroscopic characteristic of powder of *Haridra***

Morphological characters of *Haridra* powder were observed and tabulated as table no 1. (fig. no.1 and 2)

**B. Microscopic characteristic of powder of *Haridra***

The transverse section of the rhizome shows cork as an outer layer followed by epidermis, cortex, endodermis and ground tissue. Cork composed of thin walled brown cells which is large and polygonal in shape.

Epidermis consists of thin walled cubical cells of various dimension. The cortex consists of thin walled rounded parenchymatous cells and having oil cells. These cells filled with gelatinized starch grains and yellow colouring matter. The ground tissue were parenchymatous and filled with gelatinized starch grains and yellow pigment. Fibrovascular bundle and oil cells scattered throughout ground tissue (Fig no 3).

### C. Identification

- On the addition of Concentrated Sulphuric acid or a mixture of Concentrated Sulphuric acid and alcohol to the powdered drug, a deep crimson colour was produced.
- A piece of filter paper is impregnated with an alcoholic extract of the powder, dried, and then moistened with a solution of Boric acid slightly acidified with Hydrochloric acid, dried again, the filter paper assumes a pink or brownish red colour which becomes deep blue or greenish-black on the addition of alkali <sup>[1]</sup>.

### D. Physico-chemical analysis of *Haridra*

The Physico-chemical analysis of raw drug was done with the help of following mentioned tests.

#### i) Determination of Foreign Matter <sup>[2]</sup>

##### Method

100 g of the drug sample spread out in a thin layer. The foreign matter should be detected by inspection with the unaided eye, Separated and weighted it and percentage was calculated.

#### ii) Determination of Loss on Drying

##### Method

1-2 g powder of sample was taken in a clean and dried silica crucible. The sample was kept in an oven at 105<sup>0</sup>C for 5 hrs. After 5 hrs crucible was picked out from oven and weighed it and calculated the percentage w/w of loss on drying with respect to the dried drug.

#### iii) Determination of Total Ash

##### Method

2-3 g weighed sample was taken in silica crucible and incinerated at temperature not exceeding 450<sup>0</sup>C, until free from carbon. The sample was cooled and weighed. Then the percentage of ash with respect to air dried sample was calculated

#### iv) Determination of Acid Insoluble Ash

##### Method

The ash obtained from above method (total ash determination) was transferred to flask and added 25 ml of 6 N HCl and boiled for 5 min. Cooled it and insoluble matter was filtered by ash less filter paper, washed with hot water and dried. Then it was taken in Gooch crucible and ignited to constant weight. Then weighed it and calculated the percentage of insoluble ash with respect to the air-dried drug.

#### v) Determination of Alcohol Soluble Extractive Value

##### Method

The accurately weighed powdered drug was taken in conical flask and added with 50 ml of Methanol and shaken for 6 hrs continuously then allowed to stand for 18 hrs. Next day the extract was filtered very carefully without loss of solvent. The filtrate was evaporated to dryness in oven 105<sup>0</sup> C to a

constant weight. The percentage of Methanol soluble extractive was calculated with respect to air-dried drug.

#### vi) Determination of Water Soluble Extractive

##### Method

The accurately weighed powdered drug was taken in conical flask and added with 50 ml of distilled water and shaken continuously for 6 hrs. Then allowed to stand for 18 hrs. Next day the extract was filtered very carefully without loss of solvent. The filtrate was evaporated to dryness in oven at 105<sup>0</sup>C to a constant weight.

The percentage of water- soluble extractive was calculated with respect to air-dried drug.

The test results are depicted in table no 2.

### E. Phytoconstituent analysis of *Haridra*

The Phytoconstituent analysis of raw drug was done with the help of following mentioned tests. <sup>[3]</sup>

#### i) Test for alkaloids (Mayer's test)

##### Method

1 ml of sample was diluted to 10 ml with acidic ethanol, boiled and filtered. To 5 ml of the filtrate, 2 ml of dilute ammonia, 5 ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid and Mayer's reagent was added. (Mayer's reagent: Dissolve 1.358 g of HgCl<sub>2</sub> in 60ml of water and pour into a solution of 5 g of KI in 10 ml of water, add sufficient water to make 100 ml). The formation of reddish brown precipitate (with Mayer's reagent) confirms the presence of alkaloids.

#### ii) Test for Flavonoids (Shinoda test)

##### Method

1 ml of sample treated with few drops of concentrated HCl and magnesium turnings (0.5 g). The presence of flavonoids is indicated by formation of magenta colour within 3 minutes,

#### iii) Test for Tannins / Phenolics

##### Method

1 ml of sample stirred with 10 ml of distilled water, filtered and a few milliliters of 5 % ferric chloride was added to the filtrate. A deep blue colouration confirmed the presence of tannins,

#### iv) Test for Saponins

##### Method

1 ml of sample diluted with distilled water to 20 ml and shaken in graduated cylinder for 15 minutes. One cm layer of foam indicated the presence of saponins,

#### v) Test for Steroids (Salkowski test)

##### Method

1 ml of sample was dissolved in 10 ml of chloroform and an equal volume of concentrated sulphuric acid was added by the sides of the test tube. The upper layer turning red and sulphuric acid layer turning yellow green fluorescence indicates the presence of steroids,

#### vi) Test for glycosides (Keller-Killiani test)

##### Method

1 ml of sample diluted to 5 ml using water 2 ml of glacial acetic acid containing one drop of ferric chloride solution was added. To this 1 ml of concentrated sulphuric acid was added.

A brown ring at the interface indicated the presence of glycosides.

#### vii) Test for Amino acids and Proteins

##### Method

1 ml sample dissolved in 2 ml of 1% ethanolic Ninhydrin solution. The formation of violet colour showed the presence of amino acids and proteins. The test results are depicted in table no 3.

#### F. Thin Layer Chromatography

Chromatography was performed on 10x 10cm silica gel 60 F254 HPTLC plate. 10 mg of each sample was moisten with few drop of water and taken in 1 ml of AR methanol and 10 mg/ml samples was prepared 10µl of samples were spotted on TLC plate. The TLC tank Saturated with solvent system of Ethyl acetate: methanol: Hydrochloric acid 8:1.5:0.5 for about 30 minutes the plate was run in this tank for about 10cm and spots were clearly visible at Rf about 0.12, 0.2 and 0.30 were observed.

#### G. Determination of heavy metals i.e. Lead, Cadmium, Arsenic and Mercury by Atomic Absorption Spectrophotometry<sup>[4]</sup>

##### Methods

Preparation of a test solution: 3 g of the test substance was transfer to a clean, dry, 300- ml Kjeldahl flask. Clamp the flask at an angle of 45° and added a sufficient quantity of a concentrated nitric acid to moisten the substance thoroughly. It was warm gently until the reaction commences, allowed the reaction to subside and added portions of the same acid mixture, heating after each addition, until a total of 18 ml of the acid has been added. Increased the amount of heat, and boiled gently until the solution darkens. After cooling 2 ml of nitric acid added and heated again until the solution darkens. Continued the heating, followed by addition of nitric acid until no further darkening occurs, then heated it strongly to the production of dense, white fumes. Cooled cautiously and added with 5 ml of water, boiled gently to produce dense, white fumes, and continued heating until the volume is reduced to a few ml. cautiously cooled and added 5 ml of water, and examine the colour of the solution. If the colour is yellow, cautiously added 1 ml of 30 per cent hydrogen peroxide, and again evaporated to the production of dense, white fumes and a volume of 2 to 3 ml. If the solution is still yellow, addition of 5 ml of more water and the peroxide. It was cooled, diluted cautiously with a few ml of water, and rinse into a 50-ml colour-comparison tube, taking care that the combined volume does not exceed 25 ml. blank solution was prepared following the same procedure omitting the sample.

3 standard solutions were prepared of the element being examined of different concentrations, covering the 25 to 200 percentage of the range that may be present in the sample solution. Separately added the corresponding reagents as that for the test solution and prepared the blank reference solution with the corresponding reagents. Calibrated, operated the instruments as per manufacturer's recommendations and analytical condition was set which was suitable for the analysis of lead, cadmium, arsenic, and mercury. Absorbance was measured of the blank reference solution and each reference solution of different concentrations separately, readings were recorded and calibration curve was drawn with the average value of 3 readings of each concentration on the ordinate and the corresponding concentration on the abscissa. (Fig.no. 4.10)

Interpolated the mean value of the readings obtained with the test solution on the calibration curve to determine the concentration of each heavy metal.

#### H. Total Aerobic count (Bacteria, Fungi Yeast and Moulds)

##### Method

10 gm or 10 ml of the sample homogenized with 5 g of polysorbate 20, heat to not more than 40° C. It was mixed carefully while maintaining the temperature in the water-bath or in an oven. 85 ml of buffered sodium chloride-peptone solution pH 7.0 was added or any other suitable medium shown to have no antimicrobial activity under the conditions of the test, heated to not more than 40° C if necessary. This temperature maintained for the shortest time necessary for formation of an emulsion and in any case for not more than 30 min. If necessary, adjusted the pH to about 7.

#### Membrane filtration

Membrane filters having 50 mm in diameter and having a nominal pore size not greater than 0.45 µm the effectiveness of which in retaining bacteria has been established for the type of preparation being examined was used. 10 ml or 1 gm of the sample transfer to each of two membrane filters and filter immediately. It was diluted and each membrane was washed by filtering through it three or more successive quantities, each of about 100 ml, of a suitable liquid such as buffered sodium chloride-peptone solution pH 7.0. For fatty substances add to the liquid polysorbate 20. One of the membrane filters transferred which was intended for the enumeration of bacteria, to the surface of a plate of casein soyabean digest agar and the other, intended for the enumeration of fungi, to the surface of a plate of Sabouraud dextrose agar with antibiotics. Plates incubated for 5 days, unless a more reliable count obtained in shorter time, at 30°C to 35°C in the test for bacteria and 20°C to 25°C in the test for fungi. Number of colonies counted that is formed. per gm or per ml for bacteria and fungi separately along with yeast and mould.

In the test sample of *Haridra* no growth of any microbe observes after 72 hrs. It proves the anti microbial property of *Haridra*. (fig no. 5)

#### I. FTIR

Infrared spectroscopy probes the molecular vibrations. Functional groups can be associated with characteristic infrared absorption bands, which correspond to the fundamental vibrations of the functional group.<sup>[5]</sup>

A 50 mg ethanolic extract of *C. longa* and *C. xanthorrhiza* was added with 950 mg KBr IR grade and grinded in mortar until homogenous. The mixture was placed on Horizontal Attenuated Total Reflectance (HATR) accessory at controlled ambient temperature (20°C). The FTIR spectra of all samples were scanned using a FTIR spectrophotometer ABB MB 3000 FTIR spectra were scanned in the mid infrared region of 4000-650 cm<sup>-1</sup> with resolution of 4 cm<sup>-1</sup> and number of scanning of 32. The samples were placed in good contact with HATR accessory. All spectra were rationed against a background of air spectrum. After every scan, a new reference air background spectrum was taken. These spectra were recorded as absorbance values at each data point in triplicate.<sup>[6]</sup>

The first step for analysis of *Haridra* using FTIR spectroscopy is the selection of wave numbers region. The use of FTIR spectra at selected regions can increase the accuracy

of analytical results (Vazquez *et al.*, 2000). The selection of wave numbers region is based on its capability to provide the high coefficient of determination (R<sup>2</sup>) and low values of errors, either in calibration model known as Root Mean Square Error of Calibration (RMSEC) or in prediction model called with Root Mean Square Error of Prediction (RMSEP) (Rohman, 2012b). After optimization step, finally, wave numbers region of 2000-950 cm<sup>-1</sup> was selected for prediction.

Figure 4 revealed the correlation between actual values determined by FTIR at wavenumbers of 2000-950 cm<sup>-1</sup>. The high value of R<sup>2</sup> and low value of RMSEC indicated the high accuracy and precision of analytical method.

In the raw test sample, the broad band at 3428 cm<sup>-1</sup> in the IR spectrum belongs to the O-H hydrogen bonding stretching frequency. The peak at 2925 cm<sup>-1</sup> indicates C-H stretching frequency. The strong peak and weak peak at 1644 cm<sup>-1</sup> and 1544 cm<sup>-1</sup> corresponds to carbonyl amide(-C=O) and N-C stretching of amide frequencies, respectively. The weak bands at 1455 cm<sup>-1</sup> corresponds to -COO- bending frequency. The strong peak 1042 cm<sup>-1</sup> indicates the stretching frequency of ether (CO) linkage. The test result are tabulated as table no 5.

## Result

The observed test result can be depicted as:

**Table 1:** Macroscopic characteristic of powder of *Haridra*

S. No	Parameters	Observation of seeds
1.	Nature	Coarse powder
2.	Colour	Bright yellow
3.	Odour	Aromatic
4.	Taste	Characteristics
5.	Texture	Smooth & fibrous
6.	Size	Sieve with mesh aperture of 1.70 mm

**Table 2:** Physico-chemical Characteristic of *Haridra*

S.N.	Analytical test	Findings (%)	Reference values (API; part 1, Vol. I)
1.	Purity	Absent	Foreign material <2%
2.	Loss on drying at 105 °C	4.05±1.74	-----
3.	Total ash value	8.7±0.53	<9%
4.	Acid insoluble ash	0.8±1.23	<1%
5.	Hydro-alcoholic extract	22.5±2.17	-----
6.	Water soluble extract	23.1±4.39	>12%
7.	Alcohol soluble extract	11.8±2.49	>8%

**Table 3:** Phyto-constituent analysis of *Haridra*

S.N.	Phyto-constituent	Findings
1.	Alkaloids	-ve
2.	Flavonoids	-ve
3.	Proteins	+ve
4.	Saponins	+ve
5.	Tannins	-ve
6.	Glycosides	-ve
7.	Free amino acids	-ve

**Table 4:** Heavy metal analysis of *Haridra* by AAS

S.N.	Heavy Metal	Values (ppm)
1.	Arsenic	0.0194
2.	Lead	0.0383
3.	Mercury	0.0036
4.	Cadmium	0.0028

**Table 5:** FTIR analysis of *Haridra*

S.N.	Absorption Band peak	Possible interaction
1.	3428	N-H st; O-H st
2.	2925	C-H st
3.	1644	C=O st (amide-I)
4.	1544	N-C=O stsym. (amide-II)
5.	1455	(COO)- stsym
6.	1042	C-O-C st of ethe

**Fig no 1 and Fig no 2:** Macroscopic structures of *Haridra*

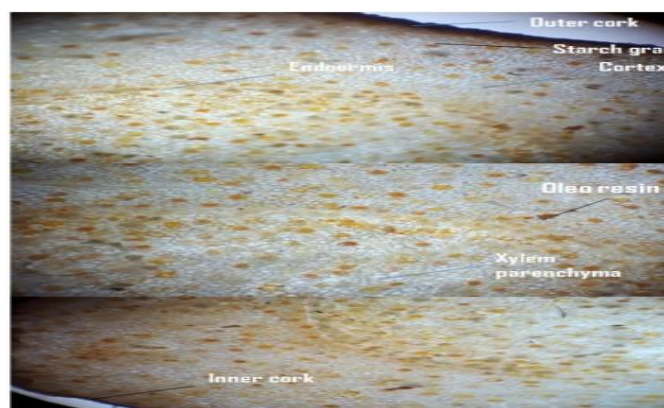


**Fig 1:** *Haridra* dried rhizome



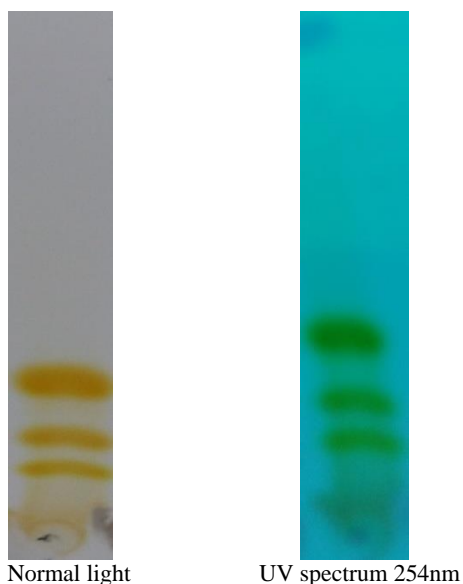
**Fig 2:** *Haridra* Powder

**Fig. no 3.** Microscopic structure of *Haridra*



**Fig 3:** The transverse section of the rhizome

**Fig no 4: Thin layer chromatography of *Haridra***

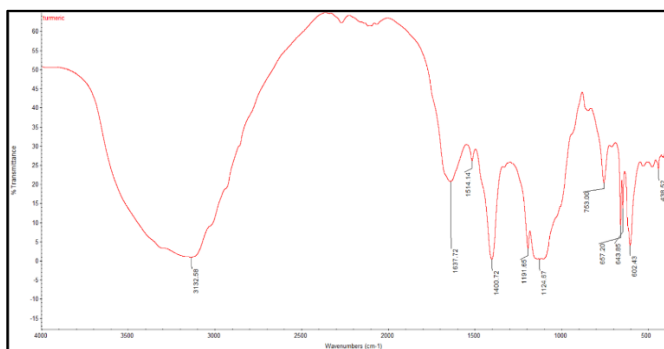


**Fig 4: TLC of *Haridra***

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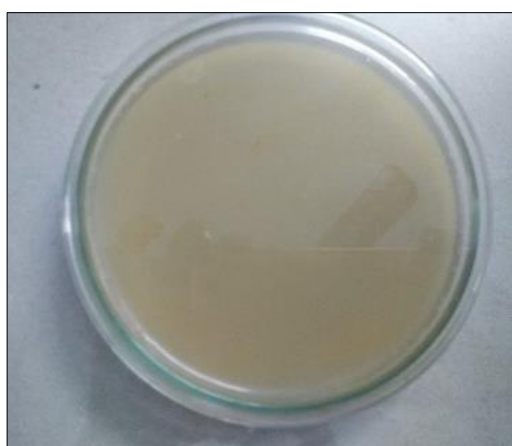
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**Fig no. 5: FTIR Spectra of *Haridra***



**Fig 5: FTIR Spectra of *Haridra***

**Fig 6: Microbial contamination Analysis**



**Fig 6: Microbial Assay of *Haridra***

**Conclusion**

The test results of physico-chemical and phyto-constituent analysis were comparable with the standard values given in Ayurvedic pharmacopoeia of India and found within the prescribed range. FTIR reveals the presence of amide group, AAS confirmed the heavy metal presence below permissible limit and absence of any microbial growth was determined by microbial study.