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Research Article

PHARMACOGNOSTICAL AND PHYTOCHEMICAL EVALUATION OF HARIDRA (CURCUMA LONGA LINN.) Prachisha.P.C^{1*}, Sumit Nathani²

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KEYWORDS: Haridra, Curcuma longa, Avurveda, Pharmacognosy, Phytochemicals.

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ABSTRACT

Curcuma longa Linn. is a very commonly used herb in the day-to-day life for therapeutic and culinary purposes. Haridra commonly known as 'Golden Spice' belongs to Zingiberaceae family and is very well known for its pharmacological actions like hepatoprotective, anti-allergic, immunestimulant, anti-cancer, anti-tumor, anti-hyperlipidemic, anti-oxidant, antimicrobial, anti-diabetic etc. According to the growing regions there are so many varieties of *Haridra* like Kerala, Tamil Nadu, Maharashtra, Andhra Pradesh, Orissa and North Eastern varieties. All medicinal plants contain phytochemicals or bioactive compounds which plays the key role in its therapeutic action. Most of the drugs show variations in these phytochemicals due to its growing regions which results in severe variations of its quality and efficacy. Also the globalisation and increasing demand for Ayurveda drugs resulted in unavailability of authentic and quality drugs in market which meets the quality standards. **Objective**: Kerala variety of Turmeric is very well known for its high Curcumin, which is up to 6.5%. Present study is to evaluate the quality of Kerala variety of sample. Material and methods: This study involves Haridra pharmacognostical and phytochemical analysis of Kerala variety of Haridra samples to ensure its purity, safety and quality. **Observations** and Results: All the findings of the phytochemical and pharmacognostical study were within the standards of quality as per API. **Conclusion:** The present sample of Haridra, the Kerala variety was found to be rich in quality and was safe, pure and authentic.

INTRODUCTION

Email:

Herbs and plants have been used by the mankind for its therapeutic value since the beginning of the human civilization. Many herbal spices are used to enhance the flavour, colour and aroma of food and also as medicines to relive pain and treat many different disorders. One of the most valuable spice herbal plants is Haridra commonly known as 'Golden Spice'. The taxonomical classification of Kingdom-Plantae, Phylum-Haridra is Spermatophyta, Sub-phylum- Angiospermae, Class-Monocotyledonae, Order- Zingiberales, Family-Zingiberaceae, Genus- Curcuma, Species- longa. Linn., Latin Name - Curcuma longa Linn.^[1]

As per Ayurveda, Acharya Charaka classified it under Shirovirechana, Bahiparimarjanopayogi, Kusthaghna, Vishaghna and Lekhaneya mahakasaya.^[2] Acharya Sushruta classified it under Haridradi Gana,

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Mustadi Gana, Lakshadi Gana, Vatasamshamana Kaphasamshamana dravya, Tikta Rasa dravya, dravya.^[3] Acharya Vagbhata classified it under Vachaharidradi Gana, Mustadi Gana and Tikta Rasa dravya.^[4] The commonly used synonyms of Haridra Krimiahni. Kaanchani. Nisaakhva. are Peeta. Yoshitpriya, Hattavilasini, Mehaghni, Ranjani, Lomasamulika, Vishaghni, Haldi and Pindaharidra.^[5] The vernacular names of Haridra are English-Turmeric, Telugu- Pasubu, Hindi- Haldi, Hardee, Kannada- Arishina, Malayalam- Manjal, Mannal, Tamil-Manjal.^[6]

Curcuma longa Linn. is an aromatic herb. It has a large underground perennial rhizome of one ovoid tuberous root-stock called 'Mother' and several narrower sessile cylindrical accessory tubers called 'Fingers'. The stem less leafy growing arises from these root stock and it reaches up to one meter height. The usual time of flowering is from September to November. Useful part of *Haridra* is *Kanda* (Rhizome).^[7]

Due to increasing demand of Ayurveda medicine for the rapidly growing population, lack of proper knowledge of drug identification or collection, to increase quantity and sales there occur a complementary loss of quality and adulteration of drugs. Pharmacognostical studies helps to provide the correct identification of the samples and there by authentify the purity, safety and efficacy of the drug. Phytochemicals are the naturally occurring chemical compounds in plants which contribute to its colour, taste, smell, actions and other properties. The phytochemical study helps to discover the bioactive profile of the plants of therapeutic importance. The organic and inorganic substances present in a plant like alkaloids, tannins, saponins, phenols, flavinoids etc are tested to understand its complete pharmacodynamics. The main objective of this study is to do the pharmacognostical, physiochemical, phytochemical and chromatography study of the sample of Haridra collected from Thrissur district of Kerala.

MATERIAL AND METHODS

Sample collection: The dried and unpolished samples of *Haridra* were collected from Irinjalukkuda in Thrissur district of Kerala. The seedling of this sample was done during June and the harvesting was done during February. After harvesting the samples were washed and boiled for 45 to 50 minutes and this process is known as 'curing'. It was then cut in to splits and dried in concrete drying floor for 10-15 days in sunlight. This dried sample was collected for this study. The dried *Haridra* was grinded into fine Powder using mini pulverizer and was sieved using a vibro sifter. Later alcohol and aqueous extracts of the sample was prepared for the tests. The testing procedures included.

- **1. Pharmacognostical study:** The pharmacognostical study was carried out as organoleptic study and powder microscopy.
- **2. Physiochemical analysis**: The physiochemical parameters of the *Haridra choorna* was analysed for the following:

Determination of foreign matter^[8]

250gm of the *Haridra choorna* was weighed with the electronic balance and a thin layer of sample was spread on a white sheet and examined with lens for any of the foreign matter. Foreign matter was removed and the *Haridra choorna* recollected was weighed again.

Determination of Moisture Content/ Total Soluble Solids ^[9]

Moisture content was determined by placing 5gm of *Haridra choorna* in oven at 105°C for 5 hours. The weight of the sample was calculated every 30 minute, until the weight of the sample came out to be constant or no variation of weight was recorded. This sample was allowed to cool to room temperature in a desiccator before weighing.

Determination of pH^[10]

The pH of aqueous solution of *Haridra choorna* was measured by using digital pH meter. First the pH meter was standardized. Buffer solution was taken in the beaker and the electrode was dipped in it. After washing the electrode thoroughly with distilled water the sample was taken (10% aqueous solution) and electrode was dipped in it and the value of pH was noted.

Determination of Extractive values^[11]

• Determination of Alcohol Soluble Extractive

5gm coarsely powdered air dried *Haridra choorna* was macerated with 100ml of alcohol of the specified strength in a closed flask for twenty-four hours. In a rotary shaker it was continuously shaken for six hours and then allowed to stand for eighteen hours. The content was then filtered using filter paper. The filtrate transferred to a pre-weighed flat bottomed dish was evaporated to dryness on a water bath. Then the dish was kept in an oven at 105°C, to constant weight and weighed.

• Determination of Water Soluble Extractive

Procedure of water soluble extractive is same as that of alcohol soluble extractive value but it was preceded with distilled water instead of alcohol.

Determination of Ash value

• Determination of Total Ash value

5 gm of powdered *Haridra choorna* was put in a silica crucible. The sample was spread evenly into a thin layer. This crucible was placed in a muffle furnace. The temperature of the furnace was set at 450°C for about 6 hrs until the ash was totally free from Carbon. The crucible with the ash was then allowed to cool to the temperature in desiccator and then weighed to constant weight.

• Determination of Acid Insoluble Ash

The total ash obtained was boiled with 25ml of 2M hydrochloric acid for 5 minutes. The insoluble matter was collected in a Gooch crucible, washed with hot water, ignited, cooled in a desiccator and weighed.

• Determination of Water soluble Ash

The total ash was boiled for 5 minutes with 25ml of water. The insoluble matter was collected in

a Gooch's crucible, washed with hot water and ignite for 15 minutes at a temperature not exceeding 450°C. The weight of the insoluble matter was subtracted from the weight of the ash, the difference in weight represented the water – soluble ash.

3. Phytochemical study^[12]**:** Qualitative Phytochemical evaluation tests of both aqueous and alcoholic extracts were conducted for various phytochemicals as follows:

Tests for Carbohydrates

Molisch's Test

To 2ml of test solution taken in a test tube, 2ml of the Molisch's reagent was added and shaken carefully and then about 1ml of conc. H_2SO_4 is poured from side of the test tube and allowed to stand for 1 minute. Formation of purple colour ring at the junction of the two layers will indicate the presence of carbohydrate.

Benedict's Test

It is used for detecting reducing sugars and is mainly composed of Copper Sulphate and Sodium Hydroxide. To the 4ml of aqueous solution of drug, 1ml of Benedict's solution was added and heated almost to boiling.

Fehling Solution Test

It is generally used for detecting reducing sugars and composed of two solutions, which are mixed in situ. Fehling solution A (0.5% of copper sulphate) and Fehling solution B (Sodium Potassium Tartarate). Equal volumes of Fehling A and Fehling B solutions were mixed (1ml each) and 2ml of aqueous solution of drug was added and then boiled for 5-10 minutes on water bath.

Tests for Alkaloids

Dragondroff's Reagent Test

2ml of test solution was taken in a test tube in which 2ml of the Dragon Droff's reagent (mixture of Potassium Iodide and Bismuth sub nitrate solution) was added. Formation of orange precipitate indicates presence of alkaloids.

Wagner's Test

Drug solution when added with few drops of Wagner's reagent (dilute Iodine solution) a formation of reddish-brown precipitate indicates presence of alkaloids.

Hager's Test

A saturated aqueous solution of picric acid was used for this test. It was added to the test sample. The formation of an orange yellow precipitate will indicate the presence of alkaloids.

Test for Amino Acids

Ninhydrin Test

The Ninhydrin test is used to detect the presence of alpha-amino acids and proteins containing free amino groups. Protein solution when heated with ninhydrin molecules, it results in formation of complex between two ninhydrin molecule and nitrogen of free amino acid. This gives a characteristic deep blue or pale yellow colour.

Tests for Proteins

Biuret Test

A few mg of the residue was taken in water and 1ml of 4% sodium hydroxide solution was added to it, followed by a drop of 1% solution of copper sulphate. Development of violet or pink colour indicates the presence of proteins.

Millons Test

A small quantity of test sample was taken and 2 to 3ml of millions reagent was added. The white precipitate slowly turning to pink, indicate the presence of proteins.

Test for Saponin

Foam Test

A small quantity of the test sample was taken in a test tube and shaken vigorously with a small amount of sodium bicarbonate and water. A stable, characteristic honeycomb like froth indicates the presence of saponins.

Test for glycosides

Borntragor's Test

1ml of Benzene and 0.5ml of dilute ammonia solution was added to the ethanolic extract and was observed for the formation of reddish pink colour.

Test for Phenolic Compound

The extract was taken in water and warmed; to this 2ml of ferric chloride solution was added and observed for the formation of green and blue colour.

Test for Flavonoids Shinods Test

A small quantity of test sample was dissolved in 5ml ethanol (95%v/v) and reacted with few drops of concentrated hydrochloric acid and 0.5gm of magnesium metal. Appearance of pink, crimson or magenta colour within a minute or two indicates the presence of flavonoids.

Test for Steroids

Salkoweski Reaction

Few mg of extract was taken in 2ml of chloroform and 2ml of concentrated sulphuric acid was added from the side of test tube. The test tube was shaken for few minutes. The development of red colour indicates the presence of steroids.

Test for Tannins Ferric Chloride Solution

A 5 percent solution of ferric chloride in 90% alcohol was prepared. Few drops of this solution was added to the test sample. Appearance of dark green or deep blue colour indicates the presence of tannins.

Lead Acetate

A 10%w/v solution of basic lead acetate in distilled water was added to the test filtrate. Development of precipitate indicates the presence of tannins.

Potassium Dichromate

A solution of potassium dichromate was added to the filtrate. Appearance of dark colour indicates the presence of tannins.

4. Chromatographic Study^[13]

Chromatography is a technique to separate mixture of substances into components on the basis of their molecular structure and molecular composition. Chromatographic separation can be done by using Thin Layer Chromatography (TLC) or Gas Chromatography or Paper Chromatography or Liquid Chromatography. Here TLC is used for separation of mixture and identification of its chemical constituent. TLC is a technique in which a solute undergoes distribution between two phases in which, the stationary phase act through adsorption and a mobile phase acts in the form of a liquid.

RESULTS AND OBSERVATION

The observations and the results of the present study are tabulated below.

1. Pharmacognostical Study

S. No	Parameters	Observations
1	Size	5 to 8cm in length, 1-2cm in diameter.
2	Shape	Mother rhizomes are ovate in shape and shorter in length. Fingers are thin and elongated.
2	Colour	Dark yellow to orange.
3	Odour	Aromatic.
4	Surface	Dry, rough

Table 1: Organoleptic characters of dried Haridra rhizomes

Table 2: Organoleptic characters of Haridra choorna

S. No	Parameters	Observations
1	Color	Yellowish
2	Odor	Aromatic
3	Taste	Bitter, pungent.
4	Texture	Fine powder

Powder microscopy^[14]: For powder microscopy HCl, Iodine, Ferric chloride, Sudan red and saffranin were used for the staining of the *Haridra choorna*. The presence of fibers, starch, crystals, oil glands and parenchyma were observed as shown in Fig no 6.

2. Physiochemical analysis

Table 3: Observations of Physiochemical parameters

S. No	Physiochemical parameters	Observations	API standards ^[15]
1.	Foreign matter	1.326%	Not more than 2.0%
2.	Moisture Content	10.626%	Not more than 12.0%
3.	pH	6.2	5.5 -7.0
4.	Extractive values		
	Alcohol soluble extractive value	9.87%	Not less than 8.0%
	Water soluble extractive value	18.42%	Not less than 12.0%
5.	Ash value	5.8%	Not more than 9.0%
	Acid insoluble ash value	0.61%	Not more than 1.0%
	Water-soluble Ash	4.31%	

3. Phytochemical study

Phytochemicals	Tests	Aq. Ext of Haridra	Al. ext of Haridra
Carbohydrates	1.1	+	+
	1.2	+	+
	1.3	+	+
Alkaloids	2.1	+	+
	2.2	-	+
	2.3	+	-
Amino acids	3.1	+	+
Proteins	4.1	-	+
	4.2	+	+
Saponin	5.1	-	+
Glycosides	6.1	-	+
Phenolic Compound	7.1	-	-
Flavonoids	8.1	+	+
Steroids	9.1	-	+
Tannins	10.1	- man	+
	10.2	+	+
	10.3	+>	

Table 4: Phytochemical analysis of Aqueous & Alcoholic extracts of Haridra choorna

4. Chromatography study

T.L.C. plate coated with 0.25mm layer of silica gel 60 F_{254} with fluorescent indicator was used. Plates were dried in hot oven at 105°C for one and half hour. Mobile solution used was Chloroform, Methanol, Glacial acetic acid (95:5:1).^[16] Test solution used was Alcoholic Extract of *Haridra choorna*. Visualization was done under normal light and Iodine.

44 S 12 1

Table 5: Results of TLC

Distance of Solvent	Rf Value	Visualisation in day light	Visualisation in Iodine Vapour
6.0cm	0.95 0.90	and the second se	
	0.85	-	and the second se
	0.81		
	0.75		
	0.66		
	0.61		
	0.56	and the second se	
	0.50	and the second sec	
	0.45	Contraction of the local division of the loc	
	0.43	and the second se	
	0.35		
	0.30		

DISCUSSION

According to table no:1 and table no:2 we can analyse that the sample of *Haridra* is organoleptically within the limits of API. Picture no: 6 shows the presence of fibers, starch, crystals and oil glands in the sample. According to Table no 3, the presence of foreign matter is 1.326% and the standards as per API are not more than 2%. This indicates the purity of the sample. Moisture content or loss of drying is the water holding capacity of sample. Higher

moisture content in sample denotes that it has a decreased stability. The results within the API standard limits show the stability of the sample. The ash value which is within the standard limits as per API is indicating the authenticity and purity of the present sample. Extractive values within the standards indicate the absence of exhausted or adulterated drugs in the sample.

According to table no:4, the phytochemical screening of the water extract had shown positive results for the presence of carbohydrates, alkaloids, amino acids, proteins, saponins, flavinoids, and tannins. Phytochemical screening of alcohol extract shows presence of carbohydrates, alkaloids, amino acids, proteins, saponins, Glycosides, flavinoids, steroids and tannins.

TLC of the Alcohol extract of Haridra choorna shows bands at $R_{\rm f}$ -0.50 corresponding to that of curcumin and at $R_{\rm f}$ -0.45 corresponding to demothoxycurcumin and at $R_{\rm f}$ -0.30 corresponding to bisdemothoxycurcumin.

CONCLUSION

On the basis of the observations, results and discussions it has been concluded that the present sample of *Haridra Choorna* is within all the standards of quality as per API. All the pharmacognostical, physiochemical, phytochemical and Thin Layer Chromatography study helped in identification and authentication of the sample of *Haridra choorna*.

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Figures

Fig 1: Haridra plant

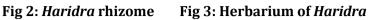




Fig 4: Dried sample of Haridra



Fig 5: Haridra choorna



Fig 6: Powder microscopic features of Haridra choorna



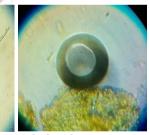
Fibers and starch Stain: HCl



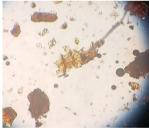
Fibers Stain: Iodine



Crystals Stain: Ferric chloride



Oil glands Stain: Sudan red



Parenchyma Stain: Saffranin