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HARIDRA (*CURCUMA LONGA* LINN.) – A PHYTO - PHARMACOGNOSTICAL ANALYSIS

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ABSTRACT:

INTRODUCTION: Turmeric is a spice which is derived from rhizome of *Curcuma longa* (Linn.), from Zingiberaceae (ginger) family. *Haridra* is being used traditionally in spices as well as therapeutically. *Haridra* contains curcuminoids, desmethoxycurcumin, bidesmethoxycurcumin, dihydrocurcumin, phytosterols, fatty acids and polysaccharides. In Ayurveda (Indian traditional medicine), turmeric has been used for its medicinal properties for various indications and through different routes of administration, including topically, orally and by inhalation. **AIM:** - To analyse pharmacognostical, physicochemical and phytochemical parameters of *Haridra* collected from market. **METHODS:** In present analytical study raw drug *Haridra* was collected from market and authenticated. After this pharmacognostical, physicochemical and phytochemical parameters were tested according to prescribed format for sample collected from market. **OBSERVATIONS AND RESULTS:** Pharmacognostical, physicochemical, phytochemical and TLC, the collected sample of *Curcuma longa* (Linn.). **CONCLUSION:** All results received from pharmacognostical, physicochemical and

phytochemical studies were analysed. Results of this study would be helpful in identification and authentication of raw drug *Curcuma longa* (Linn.) along with knowledge of safety and efficacy of drug.

KEY WORDS: Pharmacognostical, *Haridra*, *Curcuma longa* (Linn.), Curcuminoid.

INTRODUCTION:

Curcuma longa (Linn.) belongs from Zingiberaceae family is cultivated extensively all over India as a commercial crop. It is a tall herb, root stock large ovoid in shape with sessile, cylindrical tubers having orange coloured inside it. It is classified in all *samhita* along with *nighantu* of Ayurveda.

*Acharya Charak*ⁱ has classified *Haridra* in *Kusthaghna*, *Visaghna*, *Lekhaniya Mahakashaya* and *Sushruta*ⁱⁱ described in *Haridradi* and *Mustadi Gana*. In *Bhavprakash nighantu*ⁱⁱⁱ it is mentioned in *Haritakyadi Varga*. The historical evidence of *Harida* is traced from *Vedic* period, *Samhita* period and ancient *Nighantu* period to current modern texts. *Harida* is a very famous and common using spices, herb, as well as drug beneficial in many disorders like *prameha*, *dadru kustha*, *vatarakta*, *kaphaja trisna*, *panduroga*. It is also used as *pitta shamaka*, due to *tikta rasa* and *kapha shamaka* due to *tikta - katu rasa*, *ushana virya* and *katu vipaka*. *Haridra* is attributed *Varnya*, *Twachya*, *Ruchikara*, *sheetapittahara*, *kusthaghana* properties^{iv}. *Bhavprakash Nighantu*^v has mentioned four types of *Haridra* as *Haridra*, *Amraharidra*, *Vanaharidra*, and *Daruharidra*.

The main aim behind this study is to know about adulteration and substitutes added in market samples of *Haridra*. Hence, authentication of the *Curcuma longa* (Linn.) on macroscopic and microscopic level is the need of hour.

MATERIALS AND METHODS:

All procedures related to phytochemical, physiochemical and microscopic study of collected sample were carried out according to standard procedure mentioned in authentic book.

Collection of sample:

Haridra was purchased from market (Shri ram Herbals) Jaipur district, Rajasthan state. The crude drug was identified and authenticated by CSIR - National Institute of Science Communication and Information Resources, New Delhi - 110012 (NISCAIR), vide reference number NISCAIR/RHMD/Consult/2019/3487-88-2 as *Curcuma longa* (Linn.) and belongs to Zingiberaceae family. After this, raw drug was packed and subjected for organoleptic and other analytic studies.

Procedure:

The present study of the plant *Haridra* (*Curcuma longa* Linn.) rhizome is performed in some sections namely: Analysis of pharmacognostical, physicochemical, phytochemical parameters and Thin Layer Chromatography (TLC).

Pharmacognostical study:

Pharmacognostical study of test sample was carried on the basis of morphological characters such as taste, colour, odour etc.

Physicochemical study:

Moisture Content^{vi}: Moisture content was determined by placing weighed sample of 5 g of drug in oven at 105° for 5 hours, and calculated weight of sample for every 30 minute, until the weight of the sample came out to be constant, no variation of weight was recorded. This sample was allowed to cool at room temperature in a desiccator for 1 hour before weighing.

pH^{vii}: The pH value of an aqueous liquid may be defined as the common reciprocal of the hydrogen ion concentration expressed in gram per liter. It practically means the quantitative indication of the acidic or basic nature of a solution.

The pH of a given solution is measured by using digital pH meter. First the pH meter was standardized. Tablets of different pH were taken and each tablet was dissolved in 100 ml of distilled water to prepare solutions of different pH. The instrument was switched on and left for some time until required different pH solutions appeared. Buffer solution was taken in the beaker and the electrode was dipped in it. Same procedure was repeated for the other buffer solution 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.

Extractive values^{viii}:

Alcohol Soluble Extractive: - 5 g coarsely powdered air dried drug was macerated with 100 ml of alcohol of the specified strength in a closed flask for twenty-four hours. It was then continuously shaken for six hours using rotary shaker and allowed to stand for eighteen hours. The content was filtered using filter paper. The filtrate was transferred to a pre-weighed flat bottomed dish and evaporated to dryness on a water bath. Then, the dish was kept in oven at 105°, to constant weight and weigh. The percentage of alcohol-soluble extractive was calculated with reference to the air-dried drug.

Water Soluble Extractive: -5 g coarsely powdered air dried drug was macerated with 100 ml of water of the specified strength in a closed flask for twenty-four hours. It was then continuously shaken for six hours using rotary shaker and allowed to stand for eighteen hours. The content was filtered using filter paper. The filtrate was transferred to a pre-weighed flat bottom dish and evaporated to dryness on a water bath. Then, the dish was kept in oven at 105°, to constant weight and weigh. The percentage of water-soluble extractive was calculated with reference to the air-dried drug.

Ash value^{ix}:

Total Ash: - Weighed accurately 2 g of the air-dried drug in a silica dish and incinerated at a temperature not exceeding 450° C until free from carbon. Then, cooled and weighed. Percentage of ash value was calculated on the basis of air - dried drug.

Acid Insoluble Ash: - Boiled the total ash with 25 ml of 2M hydrochloric acid for 5 minutes, collected the insoluble matter in a Gooch crucible or on an ash less filter paper, washed with hot water, ignite, cool in a desiccator and weighed. Calculate the percentage of acid insoluble ash with reference to the air - dried drug.

Water Soluble Ash: - Boiled the total ash for 5 minutes with 25 ml of water; collected the insoluble matter in a Gooch's Crucible or on an ash less filter paper, washed with hot water and ignite for 15 minutes at a temperature not exceeding 450°C. Subtract the weight of the insoluble matter from the weight of the ash; the difference in weight represented the water soluble ash. Calculate the percentage of water soluble ash with reference to the air - dried drug.

Phytochemical Analysis:

Phytochemical examinations were carried out for all the extracts as per the standard methods.

Carbohydrates:

Molisch's test: - 2 ml of test solution was taken in a test tube and 2 ml 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. A purple colour ring at the junction of the two layers, if formed, indicated the presence of carbohydrate.

Benedict's test: - It is used for reducing sugars and composed of mainly copper sulphate and sodium hydroxide. To the 4 ml of aqueous solution of drug, 1 ml of Benedict's solution was added and heated almost to boiling. Solution appears green, yellow, orange, red or brown colour in order of increasing concentrations of simple sugar in the test solution.

Fehling solution test: - It is generally used for reducing sugars and composed of two solutions, which are mixed in situ. Fehling solution A composed of 0.5% of copper sulphate whereas Fehling solution B composed of Sodium Potassium Tartarate. Equal volumes of Fehling A and Fehling B solutions were mixed (1 ml each) and 2 ml of aqueous solution of drug was added followed by boiling for 5-10 minutes on water bath. Formation of reddish brown coloured precipitate due to formation of cuprous oxide indicates presence of reducing sugar.

Alkaloids: -

Dragendorff's reagent test: - 2 ml of test solution was taken in a test tube in which 2 ml of the Dragendorff's reagent (Mixture of Potassium Iodide and Bismuth sub nitrate solution) was added. An orange precipitate if formed indicated presence of alkaloids.

Wagner's Test: - Drug solution was taken in a test tube in which few drops of Wagner's reagent (dilute Iodine solution), formulation of reddish-brown precipitate.

Hager's Test: - A saturated aqueous solution of picric acid was employed for this test. When the test filtrate was treated with this reagent, an orange yellow precipitate was obtained which indicates the presence of alkaloids.

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 gives characteristic deep blue or pale yellow colour due to the formation of complex between two ninhydrin molecule and nitrogen of free amino acid.

Proteins: -

Biuret test: - A few mg of the residue was taken in water and 1 ml 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.

Xanthoproteic test: - A small quantity of test sample was taken with 2 ml of water and 0.5 ml of concentrated nitric acid was added to it. Development of yellow colour indicates the presence of proteins.

Millon's test: - A small quantity of test sample was taken and 2 to 3 ml of millon's reagent was added. The white precipitate slowly turning to pink, indicate the presence of proteins.

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 saponin.

Glycosides: -

Borntrager's test: - 1 ml of Benzene and 0.5 ml of dilute ammonia solution was added to the extract and was observed for the formation of reddish pink colour.

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

Steroids: -

Salkowski reaction: - Few mg of extract was taken in 2 ml of chloroform and 2 ml 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.

Tannins: -

Ferric chloride solution: - A 5% solution of ferric chloride in 90 % alcohol was prepared. Few drops of this solution were added to a little of the above filtrate. 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.

Thin Layer Chromatography (TLC): -

Thin layer chromatography is a tool for separation and identification of chemical constituents. Thin layer chromatography is a technique in which a solute undergoes distribution between two phases, a stationary phase acting through adsorption and a mobile phase in the form of a liquid. The adsorbent is a relatively thin, uniform layer of dry finely powdered material applied to a glass, plastic or metal sheet or plate. Glass plates are most

commonly used. Separation may also be achieved on the basis of partition or a combination of partition and adsorption, depending on the particular type of support, its preparation and its use with different solvent. Identification can be effected by observation of spots of identical R_f value and about equal magnitude obtained, respectively, with an unknown and a reference sample chromatographed on the same plate. A visual comparison of the size and intensity of the spots usually serves for semi-quantitative estimation.

Chromatography plates: - T.L.C. plate coated with 0.25 mm layer of silica gel 60 F₂₅₄ with fluorescent indicator was used. (Each plate dimension is 10 cm long and 2 cm width)

Activation of pre-coated Silica gel 60 F₂₅₄: -Plates were dried in hot oven at 105⁰ C for one and half hour.

Preparation of mobile solution: - Chloroform: Ethanol: Glacial acetic acid (9.5:0.5:0.1)

Preparation of test solution: - 4 g powdered drugs were extracted with 100 ml of ethanol (90%) in a Soxhlet's apparatus consecutively three times. Extract was filtered and concentrated to 10 ml.

Sample application: - Samples were applied with the help of capillary 1 cm above the base of T.L.C. plate. Then it was dipped in mobile solution. T.L.C. plate was removed from the mobile solution immediately after the spot reached the 1 cm below the top of the T.L.C. plate.

Visualization: - Anisaldehyde sulphuric acid spray.

R_f Value: - Measured and recorded the distance of each spot from the point of its application and calculated R_f value by dividing the distance travelled by the spots by the distance travelled by the front of the mobile phase.

OBSERVATION AND RESULTS

The different pharmacognosy parameters were studied and evaluated in order to standardize the drug. The results of pharmacognosy parameters i.e. microscopic study, physicochemical parameters, phytochemical analysis and TLC have been cited below.

Macroscopic study of *Curcuma longa* (Linn.):

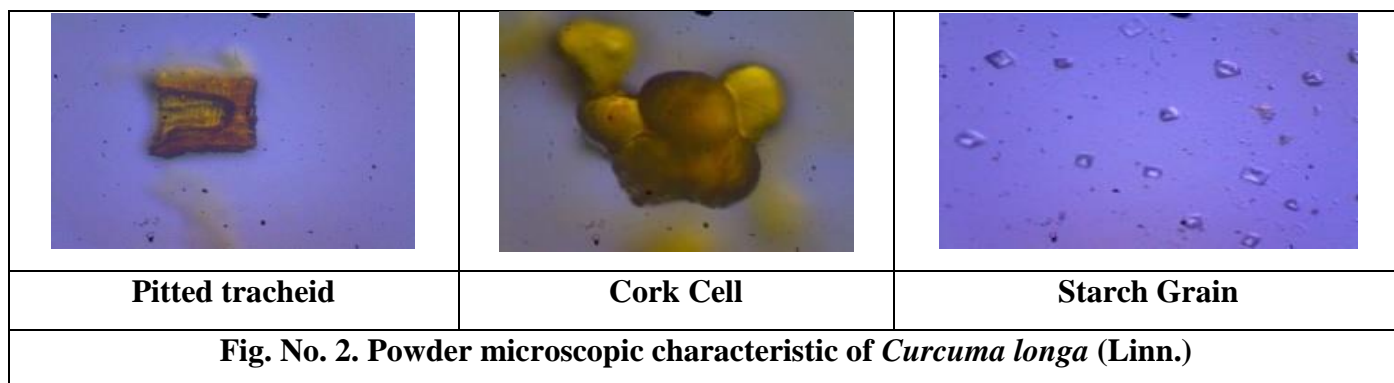


Table no. 1: Macroscopic examination of *Curcuma longa* (Linn.):

S. No.	Sample	Observed		
		Colour	Odour	Taste
1.	<i>Curcuma longa</i> (Linn.)	Yellow	Characteristics	Bitter

Powder microscopic study of *Curcuma longa* (linn.):

In powder microscopy, structure like pitted tracheid, cork cell and starch grains were seen.

**Physicochemical study:**

In this study, moisture content, pH, extractive value (alcohol and water soluble extractive value) and ash values (total ash, acid insoluble ash and water soluble ash) were determined.

Table no. 2: Physicochemical analysis of *Curcuma longa* (Linn.)

S. No.	Physicochemical Standards	Results % w/w	API standard value
1.	Moisture content	10.01%	NMT 12%
2.	pH value	6.4	--
3.	Water soluble extractive value	12.19%	NLT 12%
4.	Alcohol soluble extractive value	9.03%	NLT 8%
5.	Total ash	7.53 %	NMT 9%
6.	Acid insoluble ash	0.53 %	NMT 1%
7.	Water soluble ash	6.74 %	Not mention

Phytochemical analysis:

Phytochemical are nutritive plant chemicals that have protective or disease preventive properties. A plant cell produces two types of metabolites- primary metabolites involved directly in growth and metabolism (carbohydrates, lipids and proteins etc.) and secondary metabolites not involved in metabolic activity (alkaloids, phenols and sterols etc.) but act as defence chemicals. The preliminary phytochemical investigations of aqueous and alcohol extract of *Curcuma longa* (Linn.) were performed which reveals the presence of carbohydrates, alkaloids, amino acids, saponin, glycosides, steroids and tannins.

Table no. 3. Phytochemical analysis of *Curcuma longa* (Linn.):

Name of test	<i>Curcuma longa</i> (Linn.)	
	Aq.	Al.
(+) = Positive and (-) = Negative		
Carbohydrate		
Molish test	+	+
Benedict test	+	+
Fehling test	+	+
Alkaloids		
Dragendorff test	+	+
Wagner's test	-	-
Hager's test	+	+
Amino acids		
Ninhydrin test	+	+
Proteins		
Biuret test	-	+
Xanthoproteic test	+	+
Millon's test	-	+
Saponin		
Foam test	+	-
Glycosides		
Borntrager's test	-	+
Phenolic compound		
Phenolic test	-	-
Steroids		
Salkowski reaction	-	+
Tannins		
FeCl ₃ test	-	-
Lead acetate test	+	+
Potassium Dichromate test	-	+

Table No. 4. Thin Layer Chromatography of *Curcuma longa* (Linn.)

Sample	<i>Curcuma longa</i> (Linn.)
R _f value	0.16, 0.41, 0.49, 0.58, 0.63, 0.69, 0.74, 0.78, 0.81, 0.87, 0.94



Fig. No. 3. Thin Layer Chromatography of *Curcuma longa* (Linn.)

DISCUSSION:

Curcuma longa (Linn.) is bitter in taste, characteristics odour and yellow in colour. Powder microscopic study of *Curcuma longa* (Linn.) revealed pitted tracheid, cork cell and starch grains after observation under microscope. Loss on drying is a water holding property of test substance. Moisture content and pH value was found to be 10.01% and 6.6. Extractive value is directly relative to strength or potency of drug which estimates in different solvents. Water soluble extractive value and alcoholic extractive value in sample were found 12.19% and 9.03%. Ash value is the indicator of the presence of inorganic and earthy matter in the plant. The higher ash value is suggestive of thermo – non labile / heat stable or inorganic constituents. The total ash value in sample was 7.53%. The acid insoluble content which indicates the presence of siliceous matter and heavy metals in sample found 0.53%. Water soluble ash estimates the inorganic water soluble salt was found 6.74% in sample. Qualitative analysis of inorganic matter showed the presence of carbohydrate, alkaloid, amino acid, protein, saponin, glycosides and tannin in *Curcuma longa* (Linn.). Thin layer chromatography establishes the phytochemical fingerprint profiling in drug for identity.

CONCLUSION:

Curcuma longa (Linn.) is a well-known *Ayurveda* drug. After performing the work, it was found that the phytochemical screening confirmed the presence of various phytochemical constituents such as carbohydrates, amino acids, protein, tannin, saponin, glycoside and alkaloids. Different physicochemical parameters such as loss on drying value, pH value, water and alcohol soluble extractive value, total ash, acid insoluble ash, water soluble ash, and R_f value were observed. These values can be useful to detect purity, safety and efficacy of the drug. All studied standardization parameters like pharmacognostical, phytochemical and physicochemical analysis provide the knowledge in the identification and authentication of *Curcuma longa* (Linn.).

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