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Analytical and Phytochemical Screening of Fresh *Haridra* Rhizome Processed with Different Methods

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ABSTRACT

The demand for Ayurvedic formulations is increasing both in the domestic market as well as internationally. Therefore, it is difficult to procure quality raw drugs for the preparations as there is high potential of receiving adulterated or substituted drugs. *Haridra* (*Curcuma longa*) is one of the important herbal drug used in the treatment of many disorders as a single drug therapy as well as an ingredient in many of the compound formulations. Procuring best quality of *Haridra* which meets the API standards is a challenge to every Ayurveda drug manufacturing industry. Hence an attempt was made to process the freshly procured *Haridra* in 3 different methods: Sample A- Subjecting it for direct drying in drier, Sample B-Steam boiling and drying, Sample C- Water boiling and drying. Later those samples were subjected for analysis. Changes in the Analytical and Phytochemical parameters were noted.The final quantity of yield obtained after subjecting to different methods was 16.5%, 14% & 13.6%, respectively in Sample A, B & C. Physico-chemical parameters complies with that of Pharmacopeial specifications except Volatile oil percentage in Sample A. Curcumin percentage was highest in Sample C.

Key Words *Haridra, Analytical and Phytochemical parameters, Curcumin*

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INTRODUCTION

Since ages man has been using various herbs for the treatment of different disorders. *Haridra* (*Curcuma longa Linn.*) is one of the important plants having potent medicinal values. It is also being used in different ritual practices as well as common spice for culinary purpose. Due to its multi-dimensional pharmacological activities, it is one of the common ingredients in many of the Ayurvedic formulations.

It is botanically identified as Curcuma longa, belonging to Zingiberaceae family.

Distribution[1]: A genus of about 70 species of rhizomatous herbs distributed in India, Thailand,

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Malaysia, Archipelago and N. Australia. About 30 species occur in India of which a few are of economic importance.

The plant is a native of Southern Asia (probably India) and is cultivated extensively throughout the warmer parts of the world. It is grown on a large scale in India, China and East Indies. It is cultivated in almost all the States in India, particularly in Tamil Nadu, Bengal and Maharashtra. The main regions of cultivation in Tamil Nadu are Guntur, Cuddapah, Krishna. In Maharashtra, the chief area of cultivation being Satara district.

Morphology[2]

A tall herb root stock large, ovoid with sessile, cylindric tubers orange coloured inside.

Leaves- Very large, in tufts up to 1.2 meter or more long, including the petiole which is about as long as the blade, oblong lanceolate, tapering to the base.

Inflorescence- Spike, 10-15 cm long, peduncle 15 cm or more, concealed by the sheating petiole. Flowering bracts pale green, bracts of coma tinged with pink.

Flowers- Pale yellow as long as bracts, corolla tube has funnel shaped upper half which is pinkish white.

Rhizome- Rhizome occurs in two forms: long turmeric consisting of lateral branches of the rhizome and round turmeric which is the primary rhizome. The long turmeric is cylindrical in shape, upto 7 cm long and 1.5 cm broad and have a yellowish brown external surface with small round root scars. The round turmeric is ovate, oblong or conical in shape, 4-8 cm in length and 2-3 cm in diameter. Transversely cut portion of both the forms shows a waxy surface of deep orange colour, having a central cylinder twice as broad as the cortex. It has characteristic aromatic odour and bitter taste.

Varities of *Haridra*

Haridra – Curcuma longa Linn.

Daruharidra– Berberis aristata Dc

Amragandhi Haridra– Curcuma amada Roxb

Vana Haridra - Curcuma aromaticum Salisb

Kali Haridra - Curcuma caesia Roxb.

Zedoary - Curcuma zedoaria Rosc.

Harvesting

Harvesting is done after 8 months of planting when lower leaves turn pale and stems are dried[3].The fingers and rhizomes are boiled separately for 30-40 minutes until froth and white fumes appear. They are then drained and dried in the sun for 10-15 days, until they become dry and hard. They are then cleaned and polished mechanically in a drum rotated by hand or by power. Curing of rhizomes is essential for both development of attractive yellow color and characteristic aroma. Unless cured rhizomes lacks both features[4].

Curing of Haridra:Fresh turmeric is cured for obtaining dry turmeric.The fingers are separated from mother rhizomes.Mother rhizomes are usually kept as seed material. Curing involves boiling of fresh rhizomes in water and drying in the sun. In traditional method of curing, the cleaned rhizomes are boiled in water just enough

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to immerse them. Boiling is stopped when froth comes out.

The boiling should last for 40-60 minutes when rhizome turn soft. The stage at which boiling is stopped largely influences the colour and aroma of the final product. Over cooking spoils the colour of the final product while under-cooking renders the dried product brittle[5].

Polishing:Dried turmeric has a poor appearance and a rough dull outer surface with scalesand root bits. The appearance is improved by smoothening and polishing the outer surface by manual or mechanical rubbing.

Manual polishing consists of rubbing the dried turmeric fingers on a hard surface. The improved method is by using a hand operated barrel or drum mounted on a central axis, the sides of which are made of expanded metal mesh. When the drum filled with turmeric is rotated, polishing is effected by abrasion of the surface against the mesh as well as by mutual rubbing against each other as they roll inside the drums. Turmeric is also polished in power operated drums. The yield of polished turmeric from the raw material varies from 15-25%[6].

Procuring the best quality of *Haridra* rhizomes which meets the Pharmacopeial standards in Ayurveda pharmaceutical industries is really a challenging situation. Theoretically, when a drug is boiled in water all the water soluble active principles gets extracted from the drug which may hamper the quality of drug. Hence the attempt was done to check the analytical parameters of freshly procured *Haridra* rhizomes after processing it with different methods.

MATERIALS & METHODS

Freshly procured *Haridra* from the localities of Sullia was subjected to 3 different methods of processing viz Direct drying in dryer, Steam boiling and drying, Water boiling and drying.

1st method of processing Haridra (Sample A)

Freshly procured *Haridra* rhizomes after washing thoroughly were sliced into small pieces (As shown in Fig. 1& 2) and subjected for drying in Tray drier at 60°C. After it gets completely dried (As shown in Fig. 3) change in weight was noted and analytical tests were carried out.

2nd method of processing Haridra (Sample B)

Freshly procured *Haridra* rhizomes were subjected to washing with tap water for removal of physical impurities. It was weighed and cut into smaller pieces. Those rhizomes were then taken in idli cooker and boiled under steam for 30 minutes (As shown in Fig. 4 & 5). The properly cooked rhizomes were spread over the tray (As shown in Fig. 6) and placed in Tray drier at 60°C until it gets completely dried (As shown in Fig. 7). Change in weight were noted. Later analytical tests were carried out.

3rd method of processing Haridra (Sample C)

Freshly procured *Haridra* rhizomes were washed with tap water for removal of physical impurities. It was weighed and then taken in an open vessel and boiled with sufficient quantity of water till the rhizomes gets cooked completely (As shown

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in Fig. 8). These rhizomes were then cut into small pieces (As shown in Fig. 9) and dried in Tray drier at 60^{o}C till it got completely dried (As shown in Fig. 10). Change in weight was noted and later analytical tests were carried out.

Fig. 1: Fresh Haridra Rhizomes

Fig. 2:Fresh Haridra Rhizomes sliced for drying in Drier (Sample A)

Fig. 3:Dried Haridra Rhizomes (Sample A)

Fig. 4: Fresh Haridra Rhizomes taken for Steam boiling (Sample B)

Fig. 5: Steam boiling of Fresh Haridra Rhizomes (Sample B)

Fig. 6: Fresh Haridra Rhizomes after subjecting to steam (Sample B)

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Fig. 7:Dried Haridra Rhizomes (Sample B)

Fig. 8: Boiling of Fresh Haridra Rhizomes in water (Sample C)

Fig. 9:Fresh Haridra Rhizomes after boiling in water (Sample C)

Fig. 10:Dried Haridra Rhizomes (Sample C)

Analytical study

All the 3 samples of *Haridra* processed by different methods were subjected to evaluate their Organoleptic properties, physico-chemical analysis, Qualitative estimation of Phytoconstituents, TLC, Quantitative estimation of Curcumin percentage and detected phytoconstituents qualitatively with UV- VIS Spectroscopy by following standard protocol. These studies were carried out at In house Quality Control Laboratory of GMP Certified KVG Ayurveda Pharma & Research Centre, Sullia. The results obtained are depicted in Tables below.

Table 1 Results of Processed *Haridra* Samples

Sl. No.	Observations	Sample A	Sample B	Sample C
1.	Initial weight of Fresh Rhizome	2 kg	2 Kg	2 Kg
2.	Weight obtained after its processing	-	1.836 Kg	1.538 Kg
3.	Weight obtained after drying	330 gm	280 gm	272 gm
4.	Percentage of yield	16.5%	14%	13.6%

Table 2 Rresults of Organoleptic Characters of processed *Haridra*

Sl. No.	Organoleptic Characters	Sample A	Sample B	Sample C
1.	Colour	Orange Yellow	Orange Yellow	Orange Yellow

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2.	Taste	Bitter	Bitter	Bitter
3.	Odour	Characteristic	Characteristic	Characteristic
4.	Appearance	Dried rhizomes	Dried rhizomes	Dried rhizomes

Table3 Results of Physico-chemical analysis of processed *Haridra*

Test samples	PHYSICO-CHEMICAL PARAMETERS					
	Loss On Drying	**Total Ash**	**Acid Insoluble Ash**	**Water Soluble Extractive**	**Alcohol Soluble Extractive**	**Volatile Oil**
Sample -A	9.180%	8.871%	0.202%	12.32%	17.04%	3.65%
Sample -B	6.910%	8.200%	0.268%	17.04%	17.84%	4.07%
Sample -C	6.456%	8.900%	0.157%	14.48%	13.6%	5.18%
Pharmacopeial specifications	*NMT 80%*	*NMT 9%*	*NMT 1%*	*NLT 12%*	*NLT 8%*	*NLT 4%*

RESULTS & DISCUSSION

The results of processing of fresh Haridra rhizomes are shown in Table No.1 & Table No.2. Among 3 different processing methods adopted, the final quantity of yield obtained was 16.5%, 14% & 13.6% respectively in Sample A, B & C. The second method of processing was adopted with a view to analyze the changes in analytical parameters that may obtain when compared to third method of processing which is widely practiced commercially.

The results of Physico-chemical Analysis of processed fresh Haridra rhizomes is shown in the Table No.3

Loss on drying- Loss on drying of a drug indicates the presence of moisture content which affects the quality of a drug. In this study the test values of all the three processed samples was very minimal.

Total Ash- Total ash value is important in identification and standardization of the drug or the prepared product. A high ash value is indicative of the presence of inorganic matter which may indicate the contamination, substitution, adulteration of the drug or the prepared product. In this study the total ash value of all the three processed samples was found to be within standard limits.

Acid insoluble Ash-The presence of acid insoluble ash indicates mainly the presence of silica. The acid insoluble ash of all the three processed samples was found to be within standard limits.

Water & Alcohol Soluble Extractive-Extractive values determine the amount of active constituents in a given amount of medicinal plant material when extracted with a solvent thereby indicating the quality as well as purity of the drug. These values provide an indication of the extent of polar, medium polar and non-polar components present in the plant material. Less extractive value indicates addition of exhausted material, adulteration or incorrect processing during drying or storage or formulating. Higher the extractive value, indicates the presence of more of water or alcohol soluble contents in the plants.

In the present study, extractive values were found to be higher in all the 3 samples which complies the API standards.

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Volatile Oil-Volatile oils are known for their antioxidant activity due to their free radical scavenging ability. In this study, Sample A had low volatile content when compared to other two samples and also it fails to comply the pharmacopeial standard. Volatile oil content value in Sample C was found to be more than Sample B, however both these samples comply the standard specifications. Low volatile oil content in Sample A might be due to the processing method adopted for it as exposing to high temperature can lead to decrease in volatile content of a drug.

Phytochemical Analysis

Table 4 Results of Qualitative Phyto-chemical analysis of processed *Haridra*

Sl. No.	Phyto-Chemical Constituents	TEST SAMPLES		
		SAMPLE –A	SAMPLE –B	SAMPLE –C
1.	Carbohydrates	+	+	+
2.	Lipids	-	-	-
3.	Starch	-	-	-
4.	Glycosides	+	+	+
5.	Alkaloids	+	+	+
6.	Tannins	+	+	+
7.	Saponins	-	-	-
8.	Flavonoids	+	+	+
9.	Phenols	+	+	+
10.	Proteins	+	+	+

Table 5 Results of Quantitative estimationby UV Spectroscopy of detected phytoconstituents of processed *Haridra*

SL. NO.	Phyto-Chemical Constituents	Concentration (µg/ml)					
		SAMPLE – A		SAMPLE – B		SAMPLE –C	
		In Ethanolic extract	In Aqueous extract	In Ethanolic extract	In Aqueous extract	In Ethanolic extract	In Aqueous extract
1.	Carbohydrates	573.548	151.405	616.881	308.786	584.976	122.119
2.	Glycosides	4.939	1.159	8.289	1.309	7.199	0.620
3.	Alkaloids	87.414	8.517	96.379	10.862	48.793	5.690
4.	Tannins	137.300	10.800	219.300	15.675	145.550	23.800
5.	Flavonoids	259.439	39.283	259.439	62.475	186.281	56.982
6.	Phenols	656.300	238.300	521.300	321.300	607.300	271.300
7.	Proteins	2.389	0.201	2.526	0.793	1.325	0.839

The results of Phytochemical Analysis of processed fresh *Haridra* rhizomes is shown in the Table No.4 & Table No.5. The medicinal value of the plant lies in the phytochemical constituents of the plant which shows various physiological effects on human body. Plants contain lots of free radical scavenging molecules some of which include alkaloids, amines, terpenoids, phenolic acids, tannins and other secondary metabolites with high level of anti-oxidant activity. Most of the phytochemicals are anti-oxidant agents which essentially reduce the damages caused in tissue during physiological process[7].

In the present study, Qualitative phytochemical analysis showed the presence of Carbohydrates, Glycosides, Alkaloids, Tannins, Flavonoids, Phenols & Proteins in all the three samples (Shown in the Table No.4). These detected phytoconstituents when subjected to quantitative estimation by UV vis Spectroscopy showed

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varied concentrations in ethanolic and aqueous extracts of all the 3 samples (Shown in the Table No.5 and Fig. 11,12,13,14,15 &16).

There was increased concentration of all the compounds in alcoholic extract of Sample B except Phenols which was more in Sample A. The compounds when evaluated with water extract of all samples also showed increased concentration in Sample B except Tannins & Proteins which was more in Sample C.

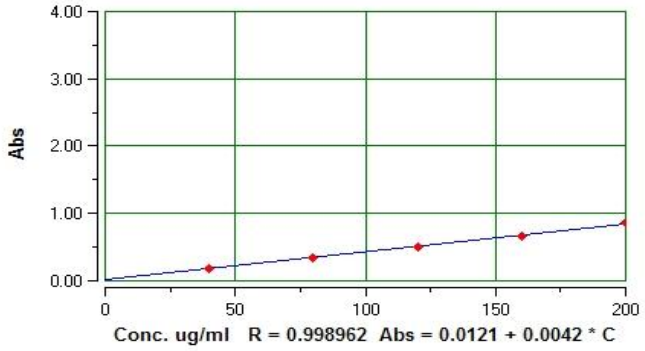

Figure 11 Graph of Carbohydrate Concentration at 620nm Absorbance

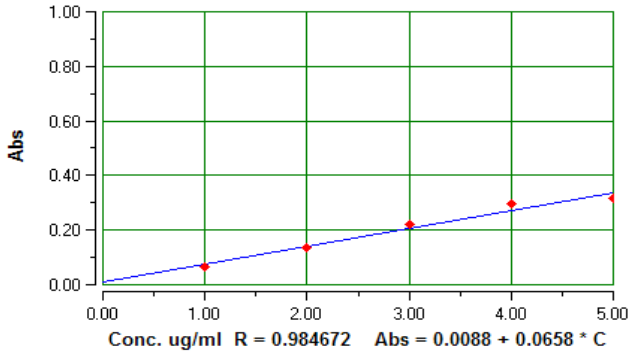

Figure 12 Graph of Protein Concentration at 540nm Absorbance

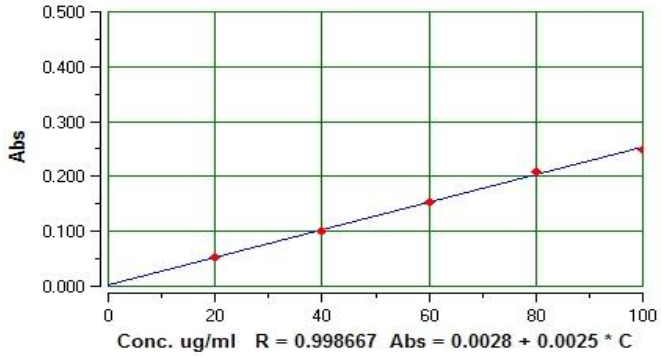

Figure 13 Graph of Alkaloid Concentration at 470nm Absorbance

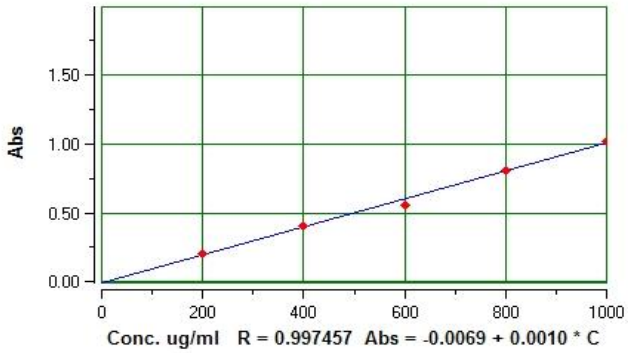

Figure 14 Graph of Phenol Concentration at 760nm Absorbance

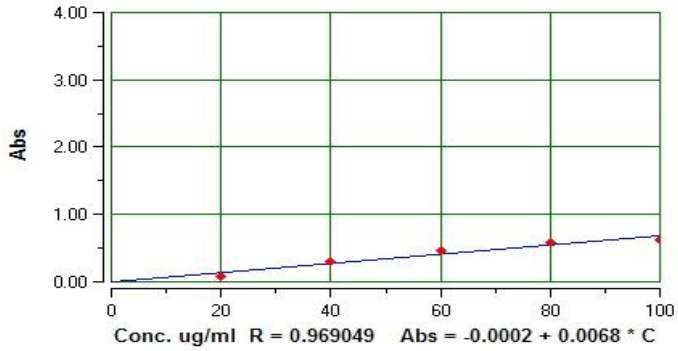

Figure 15 Graph of Flavonoids Concentration at 328 Absorbance

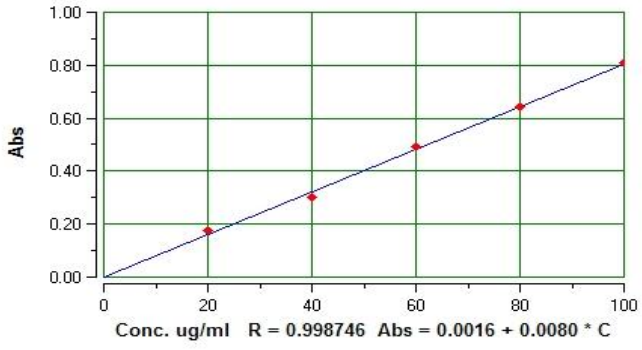

Figure 16 Graph of Tannins Concentration at 760nm Absorbance

The less values in Sample C might be due to the compounds been degraded or extracted into the water & the least values in Sample A is may be due to the compounds being thermolabile. However, there was no complete degradation of compounds noted in any of the samples.

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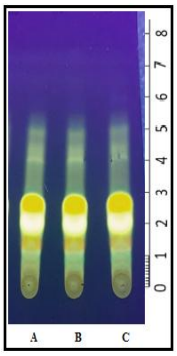

Figure 7 TLC Photodocumentation of *Haridra* Samples

TLC

TLC provides a chromatographic drug finger print. It is therefore, suitable for monitoring the identity and purity of drugs and for detecting adulteration and substitution. It is a powerful and versatile chromatographic technique that has significant importance in various fields due to its ability to separate, identify and quantify compounds in a rapid, cost- effective and sensitive manner. In the present study, as shown in Fig.17, all the three samples of Haridra processed by different means showed 3 different major spots corresponding to each other which suggests there is no complete degradation of compounds in all the samples.

Estimation of Curcumin content by UV-VIS Spectroscopy

Estimation of Curcumin was done with methanolic extracts of the processed Haridra samples. In the present study (As shown in Table No.6, Fig. 18 & Fig. 19), Sample C showed highest amount of Curcumin percentage followed by Sample B and Sample A. Less percentage of Curcumin in Sample B and Sample A is may be due to the thermal decomposition of the constituent when subjected to heat. Curcumin is sensitive to heat & can degrade when exposed to high temperatures during processing & drying. Therefore, low temperature drying methods like Sun drying, Shade drying & Steam drying are preferred to preserve Curcumin content.

CONCLUSION

For the quality assurance of any of the pharmaceutical product it is important to procure best quality of raw drug which compiles to the pharmacopeial standards. Due to wide range of therapeutic properties of *Haridra* there is an increasing demand for its cultivation and supply. Quality of the drug can be maintained and assured if proper measures are adopted during Cultivation, Harvesting and Post harvesting methods.As our *Acharyas* mentioned about the qualities that an ideal drug should possess, it can be only understood by revalidating through sophisticated techniques in the present era. In the present study it can be inferred that if the raw drug has to be used as a whole then method of Steam boiling is suitable for processing the fresh *Haridra* rhizomes. Similarly, when the drug is to be used in its isolated extract form (Curcumin Extract), then Water boiled method of processing the fresh *Haridra* rhizome is ideal. However further sophisticated techniques can be adopted to get more conclusive data. The properties of the drug changes when it is subjected to certain *Samskaras* as claimed by our *Acharyas* and this is evident through the analytical study results obtained in the present study.

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