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Evaluation of influence of Rutu (Seasons) on quality of Haridra (*Curcuma longa* Linn.) by pharmacopoeial tests and HPTLC fingerprinting

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ABSTRACT

Medicinal herbs are to be harvested during their optimal season to obtain herbal products of the best possible quality. In the criteria for standardization, season of collection of the used part of a plant plays an important role to assure the quality of the drug. Ayurvedic classics have advocated certain season for the collection of used parts of the medicinal plants. *Haridra kanda* (rhizome of *Curcuma longa* Linn.) has wide range of applications as antioxidant, antiinflammatory, anti diabetic, anticarcinogenic, hypocholesterolemic, wound healing, antitumor and hepatoprotective agent. It is one of the important drug in Ayurveda used therapeutically in charmadosa, prameha, raktavikara, sotha, pandu, vrana etc. This study was carried out to evaluate the effect of *sangraha kala* (seasons of collection) of *Haridra* on quality indicating Pharmacopoeial constants. *Haridra* was cultivated and collected at different seasons and the procured rhizomes were powdered and subjected to qualitative and quantitative analysis. Macroscopic, microscopic, physicochemical, solvent extraction and chromatographic studies carried out suggest that Haridra collected during *Sharad rutu* (autumn season) contain more active principles to meet Pharmacopoeial requirements. It may be concluded that *Sharad rutu* is the ideal season for collection of *Haridra* rhizomes, which revalidates the classical reference in ancient Ayurvedic texts.

Keywords: Ayurvedic Pharmacopoeia of India, *Sangraha kala*, Pharmacopoeial constants, Quality control, Standardization.

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INTRODUCTION

Medicinal herbs should be harvested during optimal season to obtain herbal products of the best possible quality. Ayurveda have advocated particular season for the collection of used parts of medicinal plants. It is mentioned in Ayurvedic classics that drugs become capable of producing maximum therapeutic effects when their potency is augmented by *desha sampat* (collecting the plants from appropriate habitat), *kala sampat* (collecting these plants in appropriate season), *guna sampat* (collecting plants when these are enriched with excellent attributes) and *bhajana sampat* (storing these plants in appropriate containers)[1]. Moreover in the criteria for standardization, season of collection of the used part of a plant plays an important role to assure the quality of the drug. *Curcuma longa* linn.(*C.longa*). commonly known as turmeric is a well-known spice and its rhizomes are used in Ayurveda and Unani system of medicine[2][3]. *Haridra kanda* (rhizomes of *C.longa*, family zingiberaceae) contain curcumin as the main constituent[4] and has broad range of activities including anti oxidant, anti inflammatory, anti diabetic, anti carcinogenic, hypocholesterolemic, wound healing, anti spasmodic, anticoagulant, antitumor and hepatoprotective activities[5][6]. Haridra is an important herb in Ayurveda used therapeutically for charmadosa, prameha, raktavikara, sotha, pandu, vrana and varnya[7]. According to *Charaka samhita* rhizomes are to be collected in *sharad rutu* (autumn season)[8]. But difference of opinion is seen in *Susruta Samhita*[9] and lexicon named *Raja Nighantu*[10]. Hence this study was carried out to evaluate effect of *sangraha kala* (season of collection) of *kanda* (rhizomes) of *Haridra* (turmeric/ *C. longa*) on Pharmacopoeial constants.

MATERIALS AND METHODS

Haridra finger rhizomes were collected from cultivator, authentified and planted in raised beds in the herbal garden of Shri Dharmasthala Manjunatheshwara College of Ayurveda and Hospital (SDMCAH), Hassan. In order to keep the age of the plant constant, the planting and harvesting of rhizomes were planned such that the harvesting time exactly comes around the time of collection of *Haridra* for that

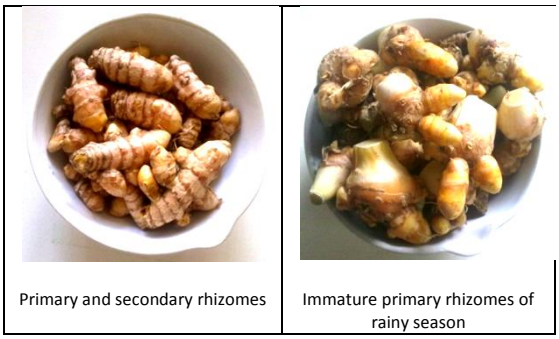
particular season (*rutu*) and each plant harvested had completed six months of growth (Table 1). After harvest and cleaning few entire rhizomes were kept aside for macroscopic and microscopic studies at Department of Dravyaguna, SDMCAH, Hassan. Others were sliced, shade dried and made to coarse powder for analysis of parameters like loss on drying, total ash, acid insoluble ash, water soluble extractive, alcohol soluble extractive TLC and HPTLC studies following standard methodology at S.D.M. Centre for Research in Ayurveda and Allied Sciences, Udupi. The above mentioned parameters were calculated for all the six seasonally collected samples and the procedures recommended as per World Health Organization (WHO) guidelines were followed[11]. For HPTLC, one gram of powdered samples were dissolved in 20 ml ethanol and kept for cold percolation for 24h and filtered. 2,4 and 6µl of the above samples of were applied on a pre-coated silica gel F_{254} on aluminum plates to a band width of 7 mm using Linomat 5 TLC applicator. The plate was developed in Toluene:Ethyl acetate(8.0:2.0). The developed plates were visualized in UV 254, 366 and 540nm and then derivatised with vanillin sulphuric acid reagent and scanned under UV 254 and 366 and 540nm. R_f, colour of the spots and densitometric scan were recorded.

Table 1: Planning and harvesting of Haridra (*Curcuma longa* Linn.)

S.N.	Planting date	Harvesting date	Harvesting *rutu* (season)
1	May 8th 2013	November 8th 2013	*Sharad* (autumn) Mid-September to mid-November
2	July 8th 2013	January 8th 2014	*Hemanta* (early winter) Mid-november to mid-january
3	September 8th 2013	March 8th 2014	*Sisira* (winter) Mid- January to mid- march
4	November 8th 2013	May 8th 2014	*Vasanta* (spring) Mid-march to mid -may
5	January 8th 2014	July 8th 2014	*Grishma* (summer) Mid- may to mid- july
6	March 8th 2014	September 8th 2014	*Varsha* (rainy) Mid-july to mid- september

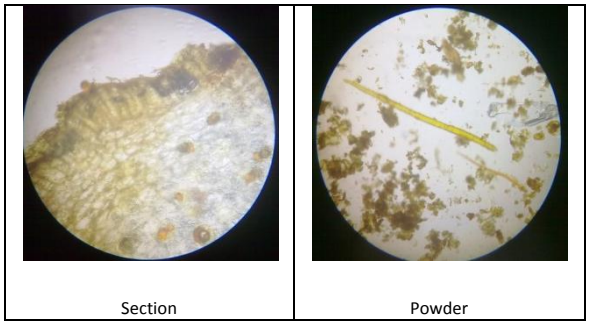
RESULTS AND DISCUSSION

In macroscopy it was noted that all the samples had matured primary rhizomes (Figure 1) giving rise to secondary rhizomes except *varsha ritu* (rainy season) sample which had immature primary rhizomes with complete absence of secondary rhizomes and larger bulbs indicating that the growth of plant take place in this season. Section microscopy and powder microscopy (Figure 2) was almost similar in all the samples and indicated the presence of oleoresin cells and starch cells , fibres respectively.



Primary and secondary rhizomes | Immature primary rhizomes of rainy season

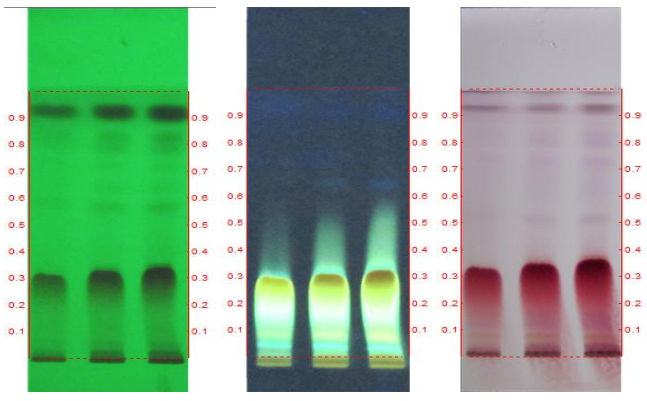
Figure 1: Macroscopy of Haridra *Curcuma longa* Linn.



Section | Powder

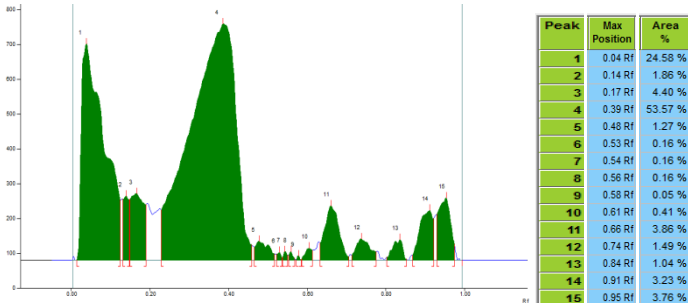
Figure 2: Microscopy of *Curcuma longa* Linn.

Loss on drying was noted maximum in *vasanta ritu* and minimum in *sisira rutu*. *Haridra* contain volatile principles hence it may indicate the moisture content as well as the volatile principles of the drug. Total ash indicates the presence of inorganic mineral content or soil in the drug and was found to be maximum in *vasanta rutu* and minimum in *varsha rutu*. Acid insoluble ash values were maximum in *grishma rutu* and minimum in *sisira rutu*. This may be due to the use of uncured/ unpeeled rhizomes for the study[12]. Alcohol soluble extractive value of Sharad ritu sample shows the highest value (8.76%) compared to all other *rutu* which indicates that the alcohol soluble active constituents are higher in *sharad rutu* compared to other *rutu.* Water soluble extractive value of *sharad rutu* sample shows the highest value (20.14%) compared to all other *rutu* which indicates that the water soluble active constituents are higher in *sharad rutu* compared to others. Among both the extractives obtained, the water soluble extractives were higher which indicates that the drug can be used in classical Ayurvedic preparations like *kashaya, hima, phanta.* Physico chemical constants along with the standards as per Ayurvedic Pharmacopoea of India (API)[13] have been enlisted in Table 2. In TLC (Figure 3) each R_f value indicate a particular chemical component present in a drug. So the obtained results (Table 3) indicate that *sharad rutu* sample have more number of chemical constituents compared to others. In HPTLC each chemical constituent is represented by a peak with its area. HPTLC (at 254 nm) of the samples indicates that *sharad riutu* samples (Table 4) have more number of chemical constituent (Figure 4) compared to other *rutu* samples (Table 5).



At 254 nm | **At 366 nm** | **Post derivatisation**

Figure 3: Photo documentation of sharad ritu samples of *Curcuma longa* at 254nm



Peak	Max Position	Area %
1	0.04 Rf	24.58 %
2	0.14 Rf	1.86 %
3	0.17 Rf	4.40 %
4	0.39 Rf	53.57 %
5	0.48 Rf	1.27 %
6	0.53 Rf	0.16 %
7	0.54 Rf	0.16 %
8	0.56 Rf	0.16 %
9	0.58 Rf	0.05 %
10	0.61 Rf	0.41 %
11	0.66 Rf	3.86 %
12	0.74 Rf	1.49 %
13	0.84 Rf	1.04 %
14	0.91 Rf	3.23 %
15	0.95 Rf	3.76 %

Figure 4: Densitometric scan of sharad ritu samples of *Curcuma longa* at 254nm

Hence the values of *Sharad rutu* sample stand high compared to all other samples in relation to water soluble extractive value, alcohol soluble extractive value, TLC and HPTLC. Similarly a study by Pamplona *et al*. on seasonal variation and analgesic properties of *Curcuma zedoaria* Roscoe (Zingiberaceae) grown in Brazil reported that the rhizomes exhibited about three times more terpenoids in autumn season (sharad rutu) than in other seasons studied[14].

This study indicates that *Haridra* samples collected at *Sharad rutu* (mid-September to mid-November/ Autumn season) have higher quantity of active chemical constituents assuring the therapeutic efficacy of the drug. Many studies were carried out on the seasonal variation in active constituents[15-19] of herbal drugs but this is the first study to evaluate Ayurvedic reference for collection season of *Haridra*.

Table 2: Physico chemical constants of Haridra (*Curcuma longa Linn*.)

S.N.	*Rutu* (season)	Loss on drying (%)	Total ash (%)	Acid insoluble ash (%)	Alcohol soluble extractive value (%)	Water soluble extractive value (%)
1	*Sharad*	19.36	11.07	1.047	8.76	20.14
2	*Hemanta*	17.67	10.67	1.20	3.56	14.47
3	*Sisira*	17.10	9.06	1.00	3.99	18.10
4	*Vasanta*	22.79	12.35	1.49	6.09	18.74
5	*Greeshma*	22.42	11.02	1.90	6.62	11.92
6	*Varsha*	19.19	8.86	1.09	7.73	18.88
API Standard[14]		NMT 12	NMT 9	NMT 1	NLT 8	NLT 12

API - Ayurvedic Pharmacopoeia of India; NMT - Not more than; NLT - Not less than

Table 3: R_f values of extracts by photodocumentation of six samples of *Curcuma longa* Linn. at 254 nm

S.N.	R_f value	*Sharad*	*Hemanta*	*Sisira*	*Vasanta*	*Grishma*	*Varsha*
1	0.07	+LG	+DG	_	_	_	_
2	0.27	_	_	_	_	+DG	+DG
3	0.30	_	+DG	_	_	_	_
4	0.31	_	_	+DG	+DG	_	_
5	0.32	+DG	_	_	_	_	_
6	0.50	_	_	_	_	+LG	_
7	0.52	_	_	_	_	_	+LG
8	0.56	+DG	_	_	_	_	_
9	0.63	+LG	_	_	_	_	_
10	0.64	_	_	_	+LG	_	_
11	0.67	_	_	_	_	+LG	+LG
12	0.71	+LG	+LG	+LG	+LG	_	_
13	0.78	LG	_	_	_	_	LG
14	0.82	+LG	_	_	_	_	_
15	0.90	_	_	_	_	_	+DG
16	0.92	+ DG	_	_	_	_	_
Total	**16**	**8**	**3**	**2**	**3**	**3**	**5**

D=dark, L=light, G=green

Table 4: Rf values of extracts by denaitometric scan of six samples of *Curcuma longa* Linn. at 254 nm

S.N.	R_f value	*Sharad*	*Hemanta*	*Sisira*	*Vasanta*	*Grishma*	*Varsha*
1	0.01						+
2	0.02		+	+	+		
3	0.03					+	
4	0.04	+					
5	0.05						+
6	0.06			+			
7	0.08		+	+	+		
8	0.09						+
9	0.10		+	+	+		
10	0.14	+					
11	0.17	+					
12	0.31					+	
13	0.33						+
14	0.35		+	+			
15	0.36				+		
16	0.39	+					
17	0.48	+					
18	0.53	+					
19	0.54	+					
20	0.56	+				+	
21	0.58	+					
22	0.59						+
23	0.61	+					
24	0.64					+	
25	.65			+			
26	0.66	+			+		
27	0.71		+	+			
28	0.72				+		
29	0.74	+					
30	0.75					+	+
31	0.80		+	+		+	
32	0.81				+		
33	0.84	+					
34	0.87				+		+
35	0.91	+					
36	0.95	+					
37	0.97		+	+			
Total	**37**	**15**	**7**	**9**	**8**	**6**	**7**

Table 5: Total number of spots/ peaks of all samples of *Curcuma longa* Linn. at different wavelengths

Season		Sarad	Hemanta	Sisira	Vasanta	Grishma	Varsha
TLC/photo documentation	254nm	8	3	2	3	3	5
	366nm	6	5	5	5	3	3
	Derivatised	10	4	4	4	5	8
	Total	24	12	11	12	11	16
HPTLC/ Densiometry	254nm	15	7	9	8	6	7
	366nm	5	3	3	3	3	4
	Total	**20**	**10**	**12**	**11**	**9**	**11**

CONCLUSION

Haridra is a very common, abundantly available and highly used drug medicinally, traditionally and commercially. The best time for harvest (quality peak season) should be determined according to the quality and quantity of biologically active constituents rather than the total vegetative yield of the targeted medicinal plant parts. The present study discloses the fact that quantity of phytoconstituents, thickness of rhizomes, extractive values etc. varies in different *rutus*. The study shows that both the aqueous and alcoholic extractive values of all the six *rutus* are different and was noted highest in *sharad rutu* sample. Macroscopic, water soluble extractive value, alcohol soluble extractive value TLC and HPTLC studies shows that *Sharat ritu* sample contain comparatively higher active principles than any other *ritu*. Hence the present study revalidates the classical reference in Ayurveda, which states *Sharad rutu* as the ideal season for collection of *kanda* (rhizomes). Further studies may be carried out similarly with the quantitative estimation of curcumin in different season for its better commercial supply.

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CONFLICTS OF INTEREST

No conflicts of interest.

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Nil.

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