IAMJ

Research Article *International Ayurvedic Medical Journal* *ISSN:2320 5091*

ANALYTICAL EVALUATION OF AQUEOUS EXTRACT OF *HARIDRA*

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ABSTRACT

Curcuma longa, Linn.-Haridra (Turmeric), an *Ayurvedic* herb is useful for treatment of Diabetes, Skin diseases, Jaundice, Menstrual disorders, Hematuria, Hemorrhage, and Colic pain. *Curcuma longa* used in wide Varity of *Ayurvedic* formulations, a well known and main ingredient of *Haridra khanda*, an established anti-ictiric, anti-allergic and anti inflammatory formulation indicated in *Sheetapitta* (Urticaria). Pharmaceutical industry, there exists a potential and growing market export for the herbal extract. Here an attempt has been made to standardize the aqueous extract of *Curcuma longa* in particular to *Curcumin, Germplasm* of Duggirala, Guntur district of Coastal Andhra Pradesh, a traditional turmeric belt, subjected for Standardization parameters viz. Physico–Chemical, Organoleptic and Chromatographic Analytical techniques. Analysis of the extract shows values of particle size through 40 mesh 100%, Loss on Drying 6.08%, pH 6.35, Water Soluble Extractive 87.04%, Alcoholic soluble Extractive 35.68%, Total ash 31.45%, Acid insoluble ash 3.08%, Bulk Density (gm/ml) 0.69 and Trapped density (gm/ml) 0.90. Heavy metal and Microbial values are also within the prescribed limits of Ayurveda Pharmacopeia of India. HPTLC graph shows peak value of the total height 381.5 and total area 4991.8. *Rf (Retention fraction)* value of *Curcumin* was 0.32. Estimation of Value of Marker Compound shows *Curcumoids* by Spectrometric Method and Gallic acid By HPLC method were 0.30% on d/b and 9.19% respectively. The HPTLC method was a simple, precise, specific, sensitive and accurate, used for routine quality control of mono herbal extract as well as a formulation.

Keywords: *Haridra, Curcuma longa Linn,* HPTLC

INTRODUCTION

Plant and herbal materials are using widely in the developing and developed countries as a mainstream of medicine or as an alternative medicine. In recent times, plant research has been increased all over the world and larger evidence has been accumulated to highlight the immense potential of the medicinal plants used in various traditional systems of medicine. [1, 2] Because of a great diversity and variability of crude drugs, regional identification, Species selection is a critical point for the efficacy. Analytical techniques like High Performance Thin Layer Chromatography (HPTLC) finger printing, Physical analysis, pH, Total Ash, Acid insoluble ash, Bulk density, Trapped density, Heavy metals,

Assay of marker compound by HPLC (High Performance Liquid Chromatography) Method has a pivotal role in quality control and standardization. [3, 4] Standardization and quality control of herbal as well as the Ayurvedic products are essential for the acceptance on the modern parameters. [5] The World Health Assembly - in resolutions WHA31.33 (1978), WHA40.33 (1987), WHA42.43 (1989) and WHA56.31 (56th session –May 2003) has emphasized the need to ensure the quality of medicinal plant products by using modern control techniques and applying suitable standards and urges Member States, where appropriate, to ensure safety, efficacy and quality of herbal medicines by determining national standards for, or issuing monographs on herbal raw materials and traditional medicine formulae. [6, 7] Naturally derived herbal and botanical extracts will experience some of the fastest growth among the major nutraceutical ingredient groups, according to "World Nutraceutical Ingredients to 2015". Turmeric renowned for its anti-inflammatory properties is a perfect example. Between 2010 and 2011, India exported nearly 70 million kilograms of turmeric, according to the Market News Service (MNS) report.[8,9]

Standardized extracts believe that they represent a trend towards higher technological refinement, will provide a more consistent, stronger and more effective product backed by chemical analysis to confirm the presence and ratio quantity of one or a number of characteristic plant constituents.

Standardization of extract will assess the qualitative and quantitative of marker compound. [10]

MATERIAL AND METHODS

Collection of Plant Material

Curcuma longa, Linn. Rhizomes purchased from the local market and identified by the Taxonomist of the R & D Division of the Laila Impex, Vijayawada. *Curcuma longa, Linn*. Belongs to the family *Zingeberaceae* is commonly known as the turmeric, known for its edible rhizome (*Haldi).*

Sample Location*: Samples purchased from Duggirala* Turmeric Market yard, Guntur District, a known Traditional Turmeric belt of Coastal Andhra Pradesh, India.

Collection Month: November

Issues related to trade: *Curcuma longa, Duggirala* trade variety selected for the study.

All the chemicals used in the present study are of analytical reagent quality.

Description: Botanical Features of *Curcuma longa, Linn* [11, 12, 13, 14]

- Trunk: grows to just over 1 meter.
- Leaves: lengthy and rectangular in shape;
- Flowers: lengthy white spike flowers.
- Root System: rhizomes (2.5 - 7.5 cm in length by 1 cm in diameter).

Sibling Species of the Curcuma Genus: there are about 80-130 species of Curcuma longa, a perennial herb, is a member of the Zingiberaceae (ginger) family. The plant grows to a height of three to five feet, and is cultivated extensively in Asia, India, China, and other countries with a tropical climate. It has oblong, pointed leaves and bears funnel-shaped yellow flowers.

a) Macroscopic [11, 12, 13, 14]

Rhizomes ovate, oblong or pyriform (round turmeric) or cylindrical, often short branched (long turmeric), former about half as broad as long, latter 2-5 cm long and

about 1-1.8 cm thick, externally yellowish to yellowish-brown with root scars and annulations of leaf bases, fracture horny, fractured surface orange to reddish brown, central cylinder twice as broad as cortex: odour and taste characteristic.

b) Microscopic [11, 12, 13, 14]

Transverse section of rhizome shows epidermis with thick-walled, cubical cells of various dimensions, cortex characterized by the presence of mostly thin-walled rounded parenchyma cells scattered collateral vascular bundles, a few layers of cork developed under epidermis and scattered oleo-resin cells with brownish contents; cork generally composed of 4-6 layers of thin-walled, brick-shaped parenchyma, cells of ground tissue contain starch grains of 4-15 µ in diameter, oil cell with sub raised walls containing either orange-yellow globules of volatile oil or amorphous resinous matter, vessels mainly spirally thickened, a few reticulate and annular.

Part Used: Rhizome [11, 12, 13, 14]

Authentication: A voucher specimen of the sample (No.21) Raw drug (Serial No: 3322) has been identified. Authenticity matched with the raw material specimen R.D. No. 211 in house museum and deposited in the institute. The water extract of *Curcuma longa* kept in an airtight double foiled package in a cool temperature for further studies. (Batch Number: L 10060519).

METHODOLOGY

The Physico-chemical, organoleptic and Spectrographic studies conducted at Laila impex, R&D division Vijayawada. Physico-chemical parameter of the *Curcuma longa*, *Linn*.was determined as per Guidelines of Indian system of Medicines and Homeopathty and Ayurveda Pharmacopeia. Total Ash values, Loss on drying, Water soluble ash, Acid insoluble ash, Heavy metals, Alcohol soluble extractive and Water soluble extract values were determined. [4, 5, 6 14, 15, 16]

Microbial screening: Microbial screening carried out as per Guidelines of Indian system of Medicines and Homeopathty and Ayurveda Pharmacopeia at Laila impex, R &D division Vijayawada for the safe use of the individual plant extract and checked whether total Aerobic count, total yeast and Mould count exceeds the limits.[4,5,6 14, 15, 16]

Preparation of Extracts: The Fresh Rhizome sample of *Curcuma longa* was air dried and powered. The dried powder of the rhizomes was treated for extraction by hot water for 6 hours. The process was repeated twice. The pooled extracted was concentrated and dried under vacuum, until it forms to Dry flakes. Dry flakes pulverized by Multimill/ Micropulviniser and sieved on shifter and packed.

Spectroscopic screening: [15, 16]

Thin layer Chromatography/High performance Thin Layer Chromatography method for identification *Curcuma longa*, *Linn* extract dry powder (DP B.NO: C 10060519) had been performed at Research and Development wing of Laila Impex, Vijayawada.

HPTLC aluminum plates pre-coated with silica gel 60 F_{254} (10x 10) with 200 µm thickness (E. Merck, Germany) were used as the stationary phase. The plates were washed with methanol and activated at 110^0 C for 10 minutes prior to chromatography.

a) Composition of the Mobile phase was a mixture of *Tolune* with *Ethylacetate Formic Acid* (50: 50: 5).

b) Preparation of test solution: Weighed 1 gram of extract powder in to a 50 ml round

flask. Refluxed and Make up with the volume with methanol.

c) Procedure: applied separately to chromatoplate 5 µl of test solution and develop the chromatoplate in above mobile phase about 8 cm from point of application. After development of spots the plate removed and dried with hot air blower. The separated bands on the HPTLC plates were scanned over the wavelenghth of 500 nm by using LINOMAR IV (CAMA G, Sonnemattstise, 17, Switzerland).

RESULTS

Six Samples in 3 batches *of Curcuma longa, Linn* water extract were studied for its Physico - chemical, Organoleptic, Microbial screening and Heavy metal limits.

Organoleptic Characters of Curcuma Extract:

Colour: Brown
Texture: Dry Powder
Odour: Aromatic
Taste: Bitter

Physico-chemical parameters of the aqueous extract of the *Curcuma longa, Linn*.was assessed viz. total ash, water soluble ash, acid insoluble ash, water soluble extractive, ethanol soluble extractive and moisture content and shown in [Table 1].

Table1: Physico Chemical Standards of Curcuma Aqueous Extract

S.No.	Physico chemical parameter	Values of Aqueous extract *Curcuma longa, Linn*.
1.	Particle size through 40 mesh	100%
2.	Loss on Drying	6.08%
3.	Water soluble Extractive	87.04%
4.	Alcoholic soluble Extractive	35.68%
5.	*pH*	6.35
7.	Total ash	31.45%
8.	Acid insoluble ash	3.08%
9.	Bulk Density(gm/ml)	0.69
10.	Tapped density (gm/ml)	0.90

Arsenic, Lead and cadmium heavy metals Limits in *Curcuma longa* water extract assessed and shown in [Table 2].

Table 2: Limits of Heavy Metals of Curcuma Aqueous Extract

S.No.	Heavy metal	Values in Aqueous extract *Curcuma longa*
1	Arsenic	< 2ppm
2.	Lead	< 5ppm
3	Cadmium	< 1ppm

Analysis reveals a minor presence of some of Heavy metals but the sample does not exceed the limits given according to Ayurveda pharmacopeia.

Microbial Screening assessed for *Yeast, Moulds, Escherichia coli, Salmonellae, Pseudomonas aeruginosa and Staphylococcus aureus* [Table 3].

Microbial count is within the Ayurveda pharmacopeia and safe for the formulation.

Table 3: Microbial Screening of Curcuma Aqueous Extract

S.No.	Microbial type	Values in Aqueous extract of *Curcuma longa, Linn.*
1	Total plate count	<1000 CFu/gm
2.	Yeast Moulds	< 10 CFu/gm
3.	*Escherichia coli*	Absent
4.	*Salmonellae*	Absent
5.	*Staphylococcus aureus*	Absent
6.	*Pseudomonas aeruginosa*	Absent

Assay by HPLC and spectrometric Method:

Value of Marker Compound shows *Curcumoids* by Spectrometric Method and Gallic acid By HPLC method were 0.30% on d/b and 9.19% respectively.

High performance Thin Layer Chromatography

High performance Thin Layer Chromatography (HPTLC) of *Curcuma longa*, *Linn* (DP B.NO: C 10060519) under Spectrum M: TEF3_3 produced 2 peaks with peak values of Total height is 381.5 and Total area is 4991.8 [Figure 1]

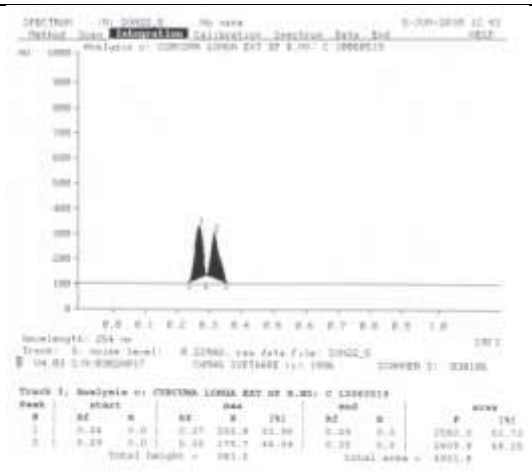

Figure 1: HPTLC of Aqueous Extract of *Curcuma longa*

Peak values of HPTLC of *Curcuma longa Rf* value assessed shown in Table 4.

Table 4: High performance Thin Layer Chromatography of Curcuma Aqueous Extract

Peak	Start		Max			End		Area	
#	*Rf*	H	*Rf*	H	%	*Rf*	H	F	%
1	0.24	0.0	0.27	205.8	53.96	0.29	0.0	2582.0	51.72
2	0.29	0.0	0.32	175.7	46.04	0.35	0.0	2409.9	48.28

The above results show the *Curcuma longa Linn* aqueous extract had the prescribed limits in the Pharmacopoeia and was of standard quality and the microbial limits and the heavy metals were in safe limits.

DISCUSSION

The main aim of the paper is to standardize the aqueous extract rather than the Hydro alcoholic of methanol extracted. *Curcuma longa* purchased from the *Duggirala*, Guntur district of Andhra Pradesh, a traditional turmeric belt of Coastal Andhra had under gone standardization methods and evaluated the percentage of yield of Curcumin from this regions. Those who like to use the aqueous extract of curcuma as an ingredient in formulation this study will guide to standardize the formulation. The aqueous extract of *Curcuma longa Linn.- Haridra* was studied for Organoleptic characters, Physicochemical analysis and HPTLC method for quality control and utility of

means of comparison with crude drug in particular to efficacy and bio availability. Simple aqueous extract had taken and Curcuma was not supposed to percolate with highly toxic and strong solvents like hexane, benzene, and methyl chloride acetate. Analysis reports will reveal a minute quantity of toxic solvents if percolated and extracted with other solvents rather than water. Efficacy, Qualitative, quantification and percentage of active compound in aqueous extract of Curcuma may study for the future clinical studies.

The Physico-chemical Analysis, Microbial screening values of *Curcuma longa* water extract were within the prescribed limits of Ayurveda Pharmacopoeia and of standard quality.[14] Even though a number of species of *Curcuma* are available in the market genuine of *Curcuma longa* can be standardized by using the marker compound identification. Adulteration of *curcuma longa*, with organic and in organic compounds and *Curcuma zedoaria* (White Turmeric), a toxic substance, wild variety of curcuma genus can be identified by HPTLC finger printing. The HPTLC finger print of the drug is also useful to verify the quality and determine the same drug in compound formulations.[15,17] Value of Marker Compound shows *Curcuminoids* by Spectrometric Method and Gallic acid By HPLC method were - 0.30% on d/b and 9.19% respectively. Microbial and Heavy metals values were within the limits of Ayurveda Pharmacopeia.[4,5,6 14,15,16]

Further studies will conduct to standardize a method to yield a better percentage of Curcumin from the aqueous extract of *Curcuma longa*.

CONCLUSION

Physicochemical analysis, organoleptic parameters, heavy metal analysis, Microbial overloads analysis carried as for the guidelines of Ayurveda pharmacopeia. Qualitative and quantitative marker compound was assessed by HPTLC and HPLC .The study is useful for standardization of the water extract of *Curcuma longa Linn* - *Haridra* rhizome especially for those using Curcuminoids as a bio-active compound extracted especially devoid of toxic solvents.

ACKNOWLEDGEMENT

The authors are thankful to the Director General- CCRAS, New Delhi for encouragement and support and also to Shri G. Ganga Raju – Chairman & Shri G. Rama Raju -Managing Director, Laila Impex Vijayawada for valuable guidance and help for conducting this study.

REFERENCES

1. Sagar Bhanu P.S., Zafar R., Panwar R. "Herbal drug standardization". *The Indian Pharmacist* 2005; 4(35): 19-22.

2. Patel P.M., Patel N.M., Goyal R.K. "Quality control of herbal products", *The Indian Pharmacist* 2006; 5(45): 26-30.

3. Shrikumar S., Maheshwari U., Sughanti A., Ravi T.K., "WHO guidelines for herbal drug standardization".2006.

4. Anonymous. W.H.O. Geneva Quality Control Methods for Medicinal plants materials. 1st ed, New Delhi: A.I.T.B.S. publishers& Distributors; 2002:11, 18, 61-3.

5. Anonymous. The international pharmacopoeia, General methods of analysis Vol. 1, 3rd ed. Geneva, World Health Organization.1979.

6. Anonymous. WHO guidelines for assessing quality of herbal medicines with

reference to contaminants and residues. WHO Library Cataloguing-in-Publication Data. WHO Press, Geneva, World Health Organization 2007.

7. Lazarowych N.J., Pekos P. "Use of fingerprinting and marker compounds for identification and standardization of botanical drugs: Strategies for applying pharmaceutical HPLC analysis to herbal products". *Drug Information Journal* 1998; 32:497-512.

8. Leung A. *Encyclopedia of Common Natural Ingredients Used in Food, Drugs, and Cosmetics*. New York, NY: John Wiley; 1980:313-314.

9. Julie Dennis, Contributing Writer. 2012-international-herb-botanical-trends. www.nutraceuticalsworld.com/issues/2012-07/view_features/2012-international-herb-botanical-trends/.) (Study published by the Fredonia Group, Cleveland, OH.) (Accessed on 23-4-13).

10. Michael Tierra. Why Standardized Herb al Extracts?*www.planetherbs.com/ phytotherapy/why-standardized-herbal-extracts.html* (Accessed on 23-4-13).

11. Sharma PC, Yelne MB, Dennis TJ. Database on Medicinal plants used in Ayurveda. Vol.1. New Delhi: CCRAS; 2000: 152-55.

12. Vaidya Ratnam Varier PS. Indian Medicinal Plants. Vol.1.1st ed. Madras: Orient Longman; 1996:320-322.

13. Bhavamisra, Bhavaprakasha Vol 1. Srikantha Murthy KR editor. 2nd Ed. Varanasi: Chowkambha Krishnadas Academy; 2004:246.

14. Anonymous. The Ayurveda Pharmacopeia of India part1, Vol. 1.1st Ed. New Delhi: Department of ISM&H, M/O Health welfare: Ayurveda pharmacopeia committee. New Delhi IIHM, CCRAS 2001:45.

15. Current GLP & Guidelines for ISM & Homeopathic Drug Testing Laboratories (Department of ISM&H, M/O Health welfare, New Delhi) 2001.

16. Anonymous. Thin layer chromatographic Atlas of Ayurvedic Pharmacopoeia. Part I, vol. I, 1st Ed. New Delhi: Govt. of India. Health and Family Welfare; 2009:59.

17. Paramasivam, M.; Poi, R.; Banarjee, H.; Bandopadhyaya, A. High-Performance thin layer chromatographic method for quantitative determination of curcuminoids in *Curcuna longa* germplasm. *Food Chem*., 2009, 113,640-644.

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Source of support: Nil
Conflict of interest: None Declared