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ANALYTICAL QUANTIFICATION OF CURCUMIN IN CURCUMA CAESIA ROXB. W.S.R TO H.P.T.L.C

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ABSTRACT

The three foundations of Ayurveda, the science of life, are Hetu, Linga, and Aushada. The study of Aushada dravyas in all of its facets is included in Aushada jnana. An extensive macroscopic, microscopic, physicochemical, and phytochemical screening, as well as standardization for herbal drugs used in the formulation, are required to determine the purity of herbal raw materials and to detect adulteration among them. This will help to avoid any ambiguity and will also serve as a reference for future research. In the present study genuine botanically identified Krishna Haridra rhizome was collected in Sharad rutu and its Macroscopic, Microscopic, Pharmacognostic evaluation, Phytochemical evaluation and quantification of Curcumin was done through hyphenated technique HPTLC.

INTRODUCTION

For many years, plants have been one of the world's richest sources of bioactive substances, and they have been providing humans with novel therapeutic options.[1] Pharmaceutical items invariably contain medicinal herbs. They have a variety of bioactive chemicals that have different medicinal effects.[2] India is well-known for its traditional medical practices, which include Unani, Siddha, Yoga, and Ayurveda. Even the ancient Vedas and other writings mention these medical systems. The reason Ayurveda is

referred to be the "science of longevity" is that it offers all the necessary advice to lead a long, healthy life, including concepts such as dinacharya and rutucharya.[3]

Roughly 90% of Ayurvedic remedies are made from plants and have the ability to balance the doshas and reverse pathophysiological processes. The majority of traditional medicines are polyherbal, utilizing anywhere from three to thirty different herbs. These ingredients are precisely mixed together to create a balanced, repeatable composition.[4]

Traditional medical systems have made extensive and frequent use of plants in the zingiberaceae family. Black turmeric, or *Curcuma caesia Roxb*., is a Zingiberaceae family member. The essential oils identified as camphor, ar-turmerone, (Z)-β- ocimene, ar-curcumene, 1,8-cineole, β-elemene, borneol, bornyl acetate, tropolone, ledol, β-elemenone, and α-bulnesene are responsible for the plant's aromatic rhizomes and leaves. This plant's varied chemical makeup supports a range of biological processes that could be advantageous to the food, cosmetic, and health industries.[5] *Curcuma caesia*, often known as Krishna Haridra, is a superb natural antiseptic, antifungal, and analgesic. They have potent antibacterial, anti- inflammatory, and anti-mutagenic qualities as well. The biological action of curcuminoids, which include curcumin and two related compounds, demethoxycurcumin and bisdemethoxycurcumin, has been primarily linked to the pharmacological activity of Krishna Haridra. These compounds may have advantages for the food, cosmetic, and health industries.[6] The most significant part of this plant, the rhizome, is frequently used in traditional medicine to treat a variety of conditions, including rheumatoid arthritis, leukaemia, fever, wounds, allergies, leprosy, bronchitis, haemorrhoids, and fever. The results of the various pharmacological studies showed that the extract from Curcuma caesia had anti-inflammatory, anti-asthmatic, anthelmintic, anti-acne, hepatotoxic, nephrotoxic, neurotoxic, anti-bacterial, anti-fungal, anti-diabetic, antiproliferative, anticancer, antiulcer, and cytotoxic properties.[7] Texts state that Krishnaharidra is Saundarya Prasadak, Swedajanana, and that in the Bengal region, it is used in place of haladi.[8] Research using herbal products cannot be deemed scientifically legitimate unless the product under investigation is authenticated and characterized. Herbal medicine standardization typically relies on one or more recognized active biochemical compounds. For the qualitative and quantitative examination of curcuminoids in Curcuma species, many analytical or hyphenated techniques have been developed recently, such as HPLC, HPTLC, NMR, GC-MS, LC-MS, and UV-Visible Spectrophotometry.

OBJECTIVES

1) Pharmacognostical study of Krishna Haridra Rhizome (*Curcuma caesia Roxb.*)
2) Complete phytochemical analysis of Krishna Haridra Rhizome powder (*Curucma caesia Roxb*.)
3) Quantification of Curcumin in *Curucma caesia Roxb.* rhizome extract with special reference to H.P.T.L.C

REVIEW OF LITERATURE

Very less references of Krishna Haridra has been found in Samhitas.

Following Acharayas have mentioned Krishna Haridra under the heading of Haridra as follows.

Under Haritakyadi Varga, Bhavaprakash have mentioned different types of Haridra. Amongst them while mentioning Vanaharidra (Wild Turmeric); Bhavaprakash has mentioned Krishna Haridra (Kalihaladi; English- Black turmeric; Latin- Curcuma caesia Roxb.) The morphology of rhizome is described here as greyish black in colour with circular margins. The inner aspect of the rhizome is bluish in colour. The taste and smell of the Krishna Haridra rhizome is similar to that of camphor. The properties have been described as similar to that of Kachoor. It is used for cosmetic purpose hence described as Saundarya Prasadak. The Udvaratana (paste) is used to promote sweating. The fresh rhizomes are used as substitute of Haladi (Curcuma longa) in some parts of Bengal.[8]

Under Ardrakaadi Varga, Adarsha Nighantu have mentioned Curcuma caesia and mentioned its various vernacular names as Black Zeodary in English, Narakachoor in Hindi and Bengal and Kala Haladi in Marathi. Further he continues to explain various other types of Haridra.[9]

COLLECTION OF THE SAMPLE

The Rhizome sample of Curcuma caesia was collected from herbal garden of Shri Shivayogeeshwar Rural Ayurvedic Medical College & Hospital, Inchal.

PLACE OF WORK

Study was carried out at the KLE's Shri B. M. Kankanawadi Ayurveda Mahavidyalaya, Department of Central Research Facility, Deemed-to-be- University, Belagavi, Karnataka,

India and further H.P.T.L.C and quantification of Curcumin was carried out at KLE's Basic Science Research Center, Belagavi, Karnataka, India.

STUDY WAS DESIGNED UNDER FOLLOWING HEADINGS

1. Pharmacognostical study of Krishna Haridra Rhizome (*Curcuma caesia Roxb*.)
2. Physicochemical analysis of Krishna Haridra Rhizome (*Curcuma caesia Roxb*.)
3. Preliminary phytochemical analysis of Krishna Haridra Rhizome powder (*Curcuma caesia Roxb*.)
4. Different Solvent Extraction for H.P.T.L.C - Methanolic Extract
5. Quantitative Analysis by Instrumental method - H.P.T.L.C 6.

RESULTS

MACROSCOPIC FEATURES IN TABULAR PRESENTATION

Tests	Results
Form	Powder
Colour	Light brown
Taste	Bitter
Odour	Pleasant

MICROSCOPIC STUDIES OF CURCUMA CAESIA

The transverse sections of Curcuma casiea shows: **(a)** Rhizome hairs **(b)** Cork cell **(c)** Stele region, Xylem, Phloem, pericycle & Endodermis **(d)** Starch grain, Xylem, Phloem, pericycle& Endodermis which were observed and different histological parameters determined under microscope.

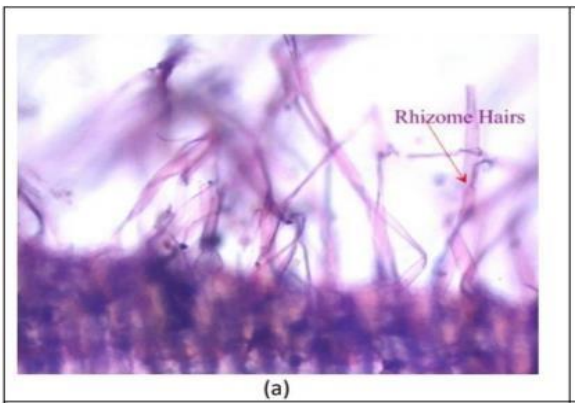


Fig No.1: Rhizome Hairs.

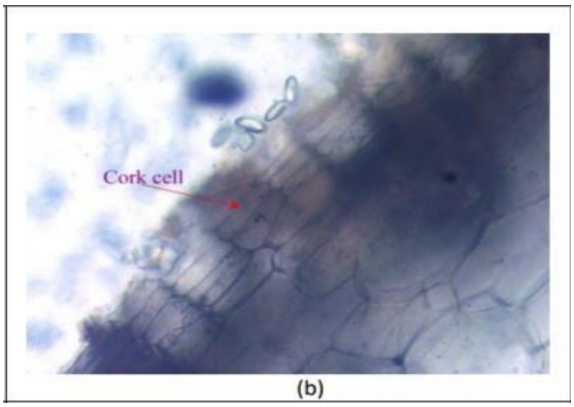


Fig. No. 2: Cork Cell.

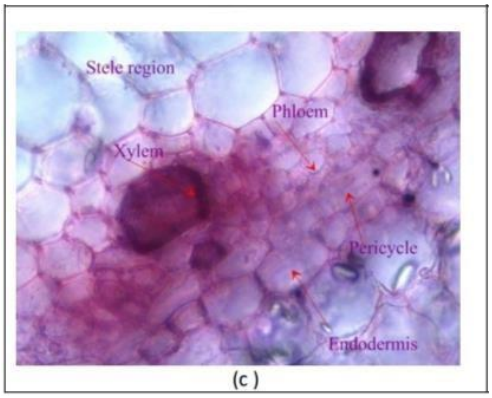


Fig. No. 3: Stele Region, Xylem, Phloem, Pericycle& Endodermis.

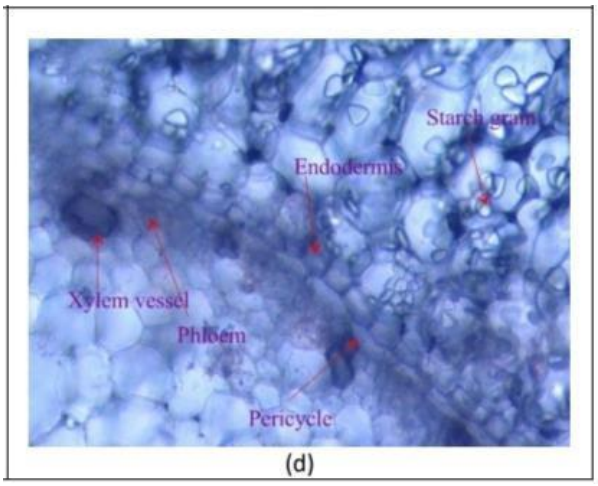


Fig. No.4: Starch Grain, Xylem, Phloem, Pericyclic & Endodermis.

PHYSICOCHEMICAL STANDARDS IN TABULAR PRESENTATION

Parameters	**% (w/w)**
Ash Value	
Total ash value	5.534 %
Acid insoluble ash value	2.069 %
Water soluble ash value	1.286 %
Extractive Value	
Loss on Drying	2.851 %

PRELIMINARY PHYTOCHEMICAL ANALYSIS

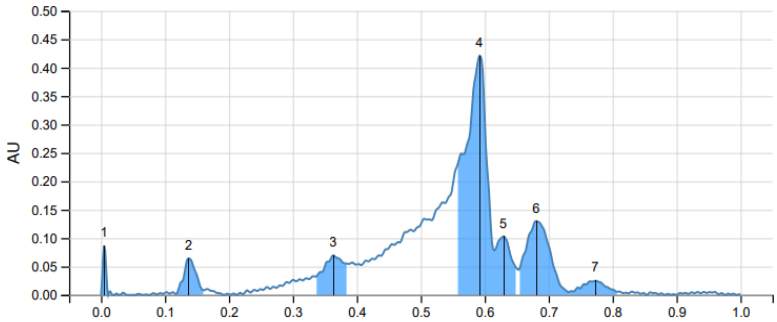
Preliminary phytochemical screening to detect the different chemical principles present viz., carbohydrates, proteins, amino acids, steroids, flavonoids, alkaloids, tannins, and glycosides were analyzed. In the methanolic extract Carbohhydrates, flavonoids and Cardiac Glycosides were found. Whereas in the aqueous extract Carbohydrates, reducing sugars, steroids, flavonoids, alkaloids and saponin glycosides were found. Results in detail are summarized in table below.

HPTLC RESULTS

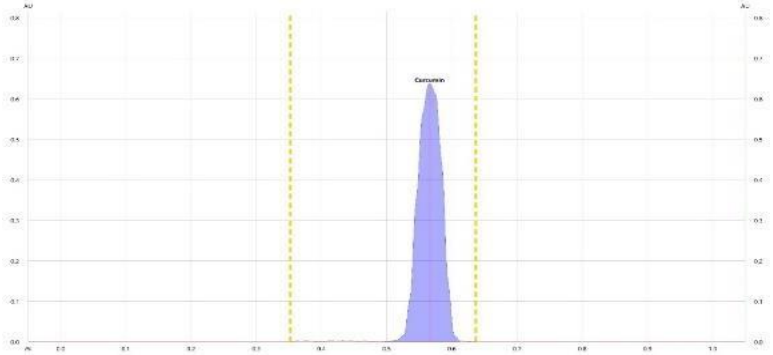
The results of HPTLC finger print for methanolic extract of Black turmeric are given in below table. The Rf value range between 0.1 to 0.811 confirmed the presence of different phytoconstituents in the extract.

Peak #	Start		Max			End		Area	
	R_F	H	R_F	H	%	R_F	H	A	%
1	0.000	0.0000	0.005	0.0864	9.61	0.011	0.0000	0.00052	1.69
2	0.118	0.0015	0.137	0.0643	7.15	0.161	0.0083	0.00146	4.78
3	0.337	0.0327	0.363	0.0697	7.75	0.387	0.0544	0.00278	9.08
4	0.558	0.2311	0.592	0.4210	46.82	0.615	0.0786	0.01594	52.11
5	0.615	0.0786	0.629	0.1030	11.46	0.652	0.0448	0.00299	9.78
6	0.653	0.0443	0.681	0.1297	14.43	0.731	0.0051	0.00574	18.77
7	0.731	0.0051	0.773	0.0250	2.78	0.806	0.0056	0.00116	3.79

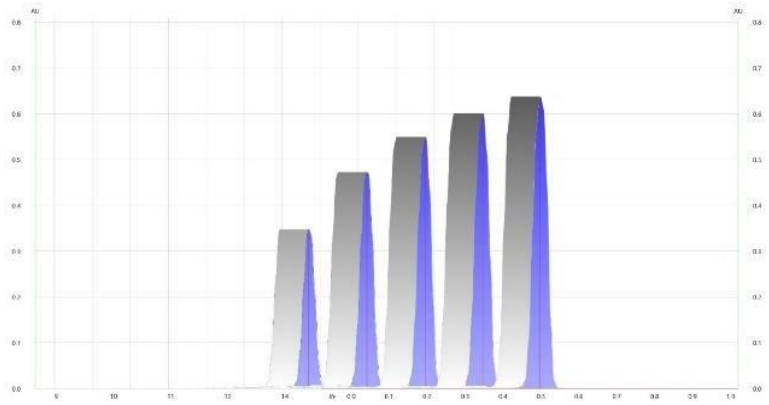
The H.P.T.L.C results of extract for sample size 2 µl, under 254nm revealed the presence 7 spots with Rf max values. Among all 7 detected peaks, 1 spot (spot no. 4) the corresponding Rf max 0.558 contain comparatively very high percentage area of 52.11 (may be the marker compounds or active principle). The Rf value range between 0.1 to 0.811 confirmed the presence of different phytoconstituents in the extract. It was compared with the standard curcumin. To determine the concentration of the curcumin standard present in the extract, triplicate measurements were performed. The concentration of the curcumin was determined to be 0.652 mg/ml.



FINGERPRINTING OF PLANT EXTRACT



CHROMATOGRAM PEAK OF STD CURCUMIN



LINEARITY PEAK OF STD CURCUMIN

SUMMARY

HPTLC, or high-performance thin layer chromatography, is an advanced instrumental method. It can be a highly effective analytical tool for chromatographic information of complex mixtures of inorganic, organic, and biomolecules due to its benefits in automation, scanning, full optimization, selective detection principle, minimum sample preparation, hyphenation, processing, etc. The goal of analytical chemistry is to increase the accuracy of current methods in order to satisfy the ever-increasing demands on chemical measurements in contemporary society. With this background the study was undertaken to quantify Curcumin in Curcuma caesia.

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