Pharmacognostic evaluation of *Curcuma longa* Linn and its standardization by IR, HPLC and HPTLC

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***Abstract*: Medicinal plants are a significant asset for all major systems of medicine or healthcare, nutraceuticals and cosmetics. The therapeutic plant-based medications have the additional benefit of being simple, effective and offering a broad spectrum of activity with an emphasis on the prevention of diseases. The present research work provides significant insight into a quantitative estimation of curcuminoids which is present in turmeric and is a mixture of curcumin, desmethoxycurcumin [4-hydroxycinnamoyl-(4-hydroxy-3-methoxycinnamoyl) methane] and bisdemethoxycurcumin [bis-(4-hydroxy cinnamoyl) methane] in the sample using analytical techniques like TLC, HPLC, HPTLC and IR spectral analysis. The preliminary phytochemical analysis was carried out which confirmed the presence of alkaloids, saponins, flavonoids, glycosides, tannins and terpenoids. Microscopic powder characteristics of *Curcuma longa* Linn were also analyzed.**

***Keywords*: *Curcuma longa*, Curcumin, Desmethoxycurcumin, Bisdemethoxycurcumin**

Introduction

Turmeric has been used throughout human history for various purposes all over the world [1]. It is the most significant spice used as the main ingredient of dishes in South Asian countries and is thus known as the "Golden spice" [2]. Turmeric has been in use as a therapeutic agent for about 4000 years. A significant number of scientific studies have been undertaken due to the expanding interest in Turmeric and its therapeutic properties [3]. The perennial rhizomatous herb contains dried cured rhizomes of *Curcuma longa* Linn, which pertains to the family Zingiberaceae [4]. The medicinal herb attains a height of 60-150m, consists of aromatic tubers which are yellow in color [5]. The harvesting time is 9-10 months and is hand-picked between October-April. *Curcuma* is amply grown across the warmer regions and subtropical regions of the world [6]. Turmeric is grown in almost all states of India, especially West Bengal, Maharashtra, Tamil Nadu and other North-Eastern states [7]. For over 2500 years, Turmeric has been extensively used in India for traditional systems of medicine under the name Indian saffron [8].

Table 1 Taxonomy

Kingdom	Plantae
Division	Magnoliophyta-Flowering plant
Class	Lilliopsida-monocotyledons
Subclass	Zingiberidae
Order	Zingiberales
Family	Zingiberaceae
Genus	Curcuma
Species	Curcuma longa L

Table 2 Vernacular Names

Language	**Name**
English	Indian saffron
Sanskrit	Haldi
Hindi	Haldi
Bengali	Halud
Telugu	Haridra
Tamil	Manjal
Kannada	Arisina
Gujrati	Halada

The phytochemical evaluation of various extracts of turmeric indicated the presence of polyphenols like flavonoids and curcuminoids, whereas curcumin contributes 90% to curcuminoids along with desmethoxycurcumin and bisdemethoxycurcumin [Figure 1,2,3] [9].

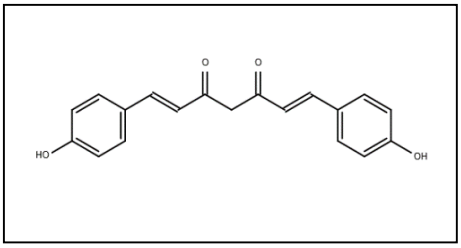

Figure 1 Chemical structure of Curcumin

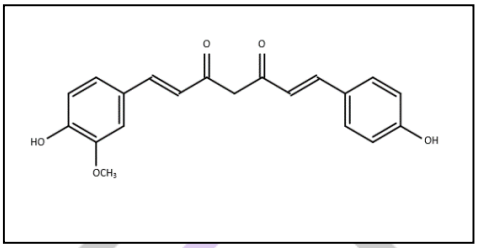

Figure 2 Chemical structure of desmethoxycurcumin

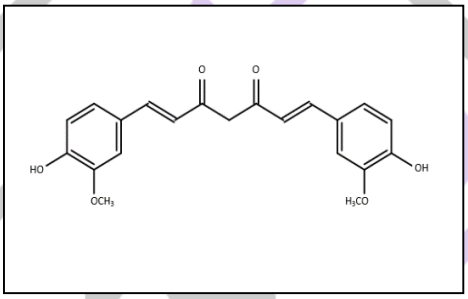

Figure 3 Chemical structure of bis-demethoxycurcumin

Curcumin was first isolated in 1815 and its chemical structure was determined by Roughley and Whiting in 1973. [10,11]. It has a melting point at 176–177°C; forms a reddish-brown salt with alkali and is soluble in ethanol, alkali, ketone, acetic acid and chloroform. The curcuminoids impart yellow colour to Turmeric [12]. Curcumin is used in Ayurvedic and Chinese medicine as an anti-inflammatory [13], antioxidant, antibacterial, antifungal [14,15]. It was used as a digestive aid, and to treat wounds, infection and jaundice. In Unani, it is used as a natural antiseptic [16]. Pre-formulation studies such as organoleptic, macroscopic and microscopic evaluation were performed to identify the drug and its purity [17]. In the present study, a complete and systematic study of drug is done, which comprise of origin, common names, scientific nomenclature, family, pre-formulation studies such as macroscopical, microscopical and organoleptic characters. Phytochemical analysis, spectral analysis, qualitative estimations are carried to identify the drug and its purity. Quantification of curcumin using chromatographic techniques like HPTLC, HPLC in Curcuma longa extract.

2. Material and Methods

Procurement & Authentication of Plant material

2.1 Procurement of the plant material

Curcuma longa (Rhizomes) was purchased from Khari Baoli, Chandni chowk, Delhi-110006, Delhi, India.

Authentication of the plant material

The collected plant material was authenticated by AIMIL Pharmaceuticals (I) limited, New Delhi-110028, India against COA number: A/RM/2021/0033250

2.2 Equipment: CAMAG Wincat V Switzerland [HPTLC Applicator], CAMAG TLC Scanner III, Switzerland [HPTLC Scanner], Perkin Elmer, Germany [IR Spectrophotometer], HPLC System Shimadzu, (Japan), HPLC Column Li Chrospher 100 RP-18e Column, AND Moisture Analyzer, Heating plate, Autoclave and common glassware.

2.3 Chemicals and Reagents: The reagents such as Mayer's reagent, Dragendroff's reagent, Wagner's reagent, Hager's reagent and chemicals such as methanol, ethanol, sodium hydroxide, glycerine, phloroglucinol and acetone

2.4 Morphological studies: Morphological details of the drug are given by observing it with the naked eye or with the aid of a magnifying lens. In this description general condition of the drug, size, shape, outer surface, the inner surface along with sensory characters like color, odor and taste are given

2.5 Microscopy: Transverse section microscopy: For anatomical examination of *Curcuma longa* rhizome transverse sections were cut after the rhizomes were softened, the rhizomes were dipped in glycerol solution overnight. The softened rhizomes were

cut into thin, small sections using a razor. The best selected section was stained using phloroglucinol and conc. Hydrochloric acid and examined under the microscope. [18]

Powder microscopy: The powdered drug was passed through sieve number (80) and then soaked in distilled water for 24 hours at room temperature. For the examination of the microscopic characters, a small amount of powder was placed on a slide and 1-2 drops of phloroglucinol was added along with 1 drop of concentrated hydrochloric acid. Safranine was used for staining and then the powder sample was covered with a coverslip and excess fluid was drawn off from one side of the slide using filter paper and observed under microscope at 10x. [19]

2.6 Physicochemical studies: As per the reference, quantitative standard procedures were followed for studying characteristics like total ash, acid-insoluble ash, water-soluble ash, alcohol-soluble extractive value, water-soluble extractive value, moisture content. Alcoholic extract of *Curcuma longa* was subjected to qualitative chemical analysis for detecting the secondary metabolites present in it. [20]

Identification of Curcuminoids in the sample

2.7 Phytochemical Analysis: Preliminary phytochemical examination to confirm the presence of alkaloids, saponins, flavonoids, glycosides, tannins and terpenoids was performed using standard procedure. [21]

2.8 Qualitative Analysis:

2.8.1 Thin layer chromatography: TLC analysis of the sample with respect to standard curcumin was performed on pre-coated plates with Silica gel 60 F254 of 0.2mm thickness as stationary phase and using Toluene: Ethyl Acetate: Formic Acid (7:2.5:0.5) as mobile phase. Detection was done under UV 366 nm. The Rf value of the sample was calculated and compared with the Rf value of the standard. [22]

2.8.2 High pressure thin layer liquid chromatography: Qualitative estimation of curcumin in sample and standard was done by HPTLC. The mobile phase selected for best separation was Toluene: Ethyl Acetate: Formic Acid: Ethanol (6:4:0.3:0.3 v/v) and examined under 254nm and 366nm

Preparation of standard: 10 mg of standard curcumin (Natural Remedies Pvt. Ltd., India) in 10 ml of methanol and homogenize by sonication for 5 minutes

Preparation of sample: 1g of the powdered drug was extracted with 100ml of ethanol consecutively 3 times in a Soxhlet apparatus. Filter and concentrate the extract and make up the volume to 10ml with ethanol in a volumetric flask. [23]

2.9 Spectral Analysis: The sample was subjected to IR spectral analysis (Perkin Elmer, Germany) using KBr pellet technique. IR Spectra of the sample was compared with that of IR Spectra of standard curcumin. The IR Spectra were compared based on different peaks obtained at different Wavenumber [cm^{-1}] representing different functional groups. The sample was analyzed by IR Spectral analysis to confirm the presence of the curcumin.

2.10 Quantitative Analysis of Curcuminoids by High pressure liquid chromatography:

Analytical chromatography was performed on Agilent HPLC system (Infinity 1260). Chromatographic separation of curcumin was achieved on Eclipse XDB-C18 (4.6 x 150 mm, 5 µ particle size) column at a temperature of 70 °C±0.02 °C. The mobile phase is comprised of acetonitrile: water: methanol (50:10:40 v/v/v). The flow rate was at 1.2 ml/min., drug peaks were detected at 430 nm with an injection volume of 20 µl.

Preparation of standard: 1g of coarsely powdered RS with 5ml methanol for 10 minutes, cool and filter.

Preparation of sample: 1g of coarsely powdered *Curcuma longa* rhizome mixed with 5ml of methanol for 10 minutes and slightly heated. Filter and use the filtrate. [24]

3. Results and Discussion:

3.1 Description

3.1.1 Macroscopic: Drug is mainly composed of primary and secondary rhizomes. Primary rhizomes are short pieces, pear-shaped or ovate, known as 'bulb' or round turmeric, vertically growing condensed and swollen, 3 to 7 cm in length, 2 to 3 cm in width. Longitudinally wrinkled and also marked with rows of circular. Secondary rhizomes are also known as long turmeric are, 0.5-1.5 cm in thickness, and their shape is elongated which may be branched. Rhizomes are hard, heavy with short fractures. Yellowish to orange in color, internally dull yellowish [Figure 4]. Aromatic in odor, bitter in taste.

Figure 4 (A) Habit of plant (B) Fresh rhizome (C) Dried cured bulb

3.1.2 Microscopic:

TS: Outer region of the matured rhizome shows 3-5 rows of cork, subsequently 15-20 rows of parenchymatous cortical cells confined with vascular bundles, oleoresin cells and simple starch grains. The ground tissue of the stele is parenchymatous and it is identical to the cortical region embedded with vascular bundles, oleoresin cells and starch grain [Figure 5].

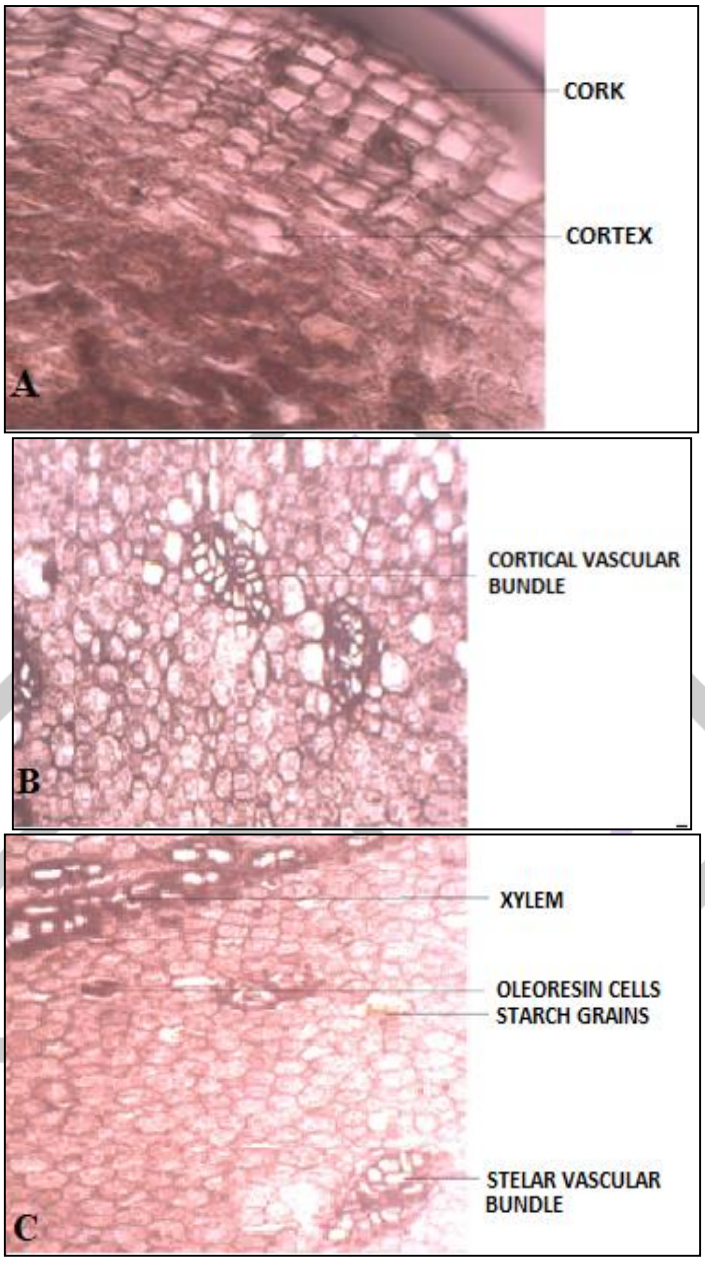

Figure 5 (A&B) TS of outer region of Curcuma longa (C) TS of inner region of Curcuma longa

Powder: The surface view shows a fragment of cork, fragments of cortical and stelar parenchymatous cells embedded with oleoresin cells and simple starch grain. The longitudinal view shows spiral vessels and starch grains dissipated throughout [Figure 6].

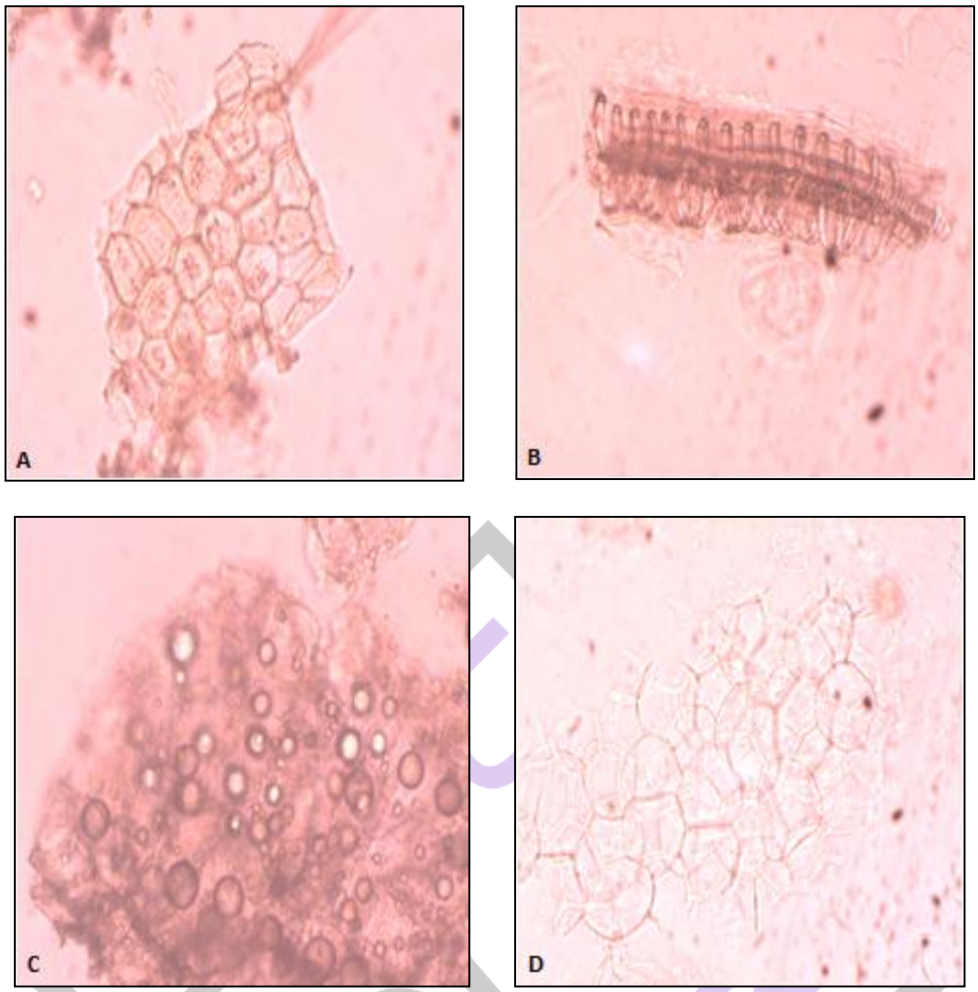

Figure 6 Powder Microscopy: (A) Cork, (B) Vessels, (C) Starch granules (D) Parenchyma cells

3.2 Physicochemical studies: ***Foreign matter:*** NIL ***Total ash:*** 0.23% w/w ***Acid-insoluble ash:*** 0.12% w/w ***Ethanol-soluble extractive:*** 35% w/w ***Water-soluble extractive:*** 40%w/w, ***Moisture content:*** 1.86% w/w.

3.3 Phytochemical screening: The experimental data of preliminary phytochemical evaluation of sample are summarized in Table 3. The data indicated the presence of carbohydrates, proteins and amino acids, alkaloids, terpenoids and flavonoids, tannins and saponins in the alcoholic extract of *Curcuma longa*.

Table 3 Phytochemical Screening

Test	**Observation**
Carbohydrates	+
Proteins and Amino acids	+
Alkaloids	+
Glycosides	+
Terpenoids and Steroids	+
Flavonoids	+
Tannins	+
Saponins	+

3.4 Qualitative Identification:

3.4.1 Thin layer chromatography: When the plate was visualized under UV light 366nm, the Rf value of the sample (0.56) and standard curcumin (0.56) matched completely, which indicated the presence of curcuminoids in the sample [Figure 7].

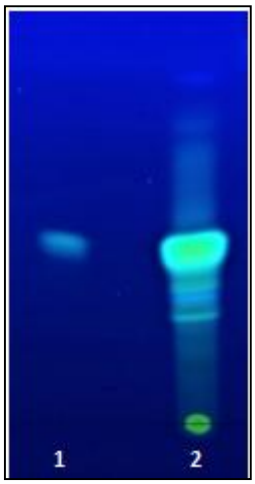

Figure 7: TLC profile of test solution of Curcuma longa rhizome. (1) Curcumin standard and (2) Test solution at 366 nm

3.4.2 High pressure thin layer liquid chromatography: When the plates were visualized under UV light 254nm and 366nm, standard curcuminoids showed the presence of three components i.e curcumin, desmethoxycurcumin and bis-demethoxycurcumin. The Rf values was found to be curcumin (0.44), desmethoxycurcumin (0.471) and bisdemethoxycurcumin (0.314). In the sample, a band corresponding to Rf (0.46), Rf (0.472), Rf (0.315) suggests the presence of curcumin, desmethoxycurcumin and bis-demethoxycurcumin in the sample [Figure 8 & 9].

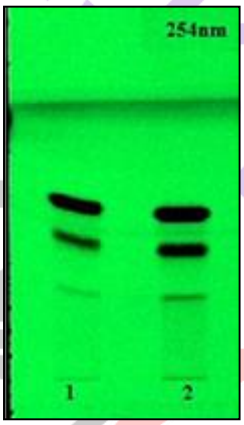

Figure 8 HPTLC chromatogram of (1) Curcumin standard and (2) Test solution at 254 nm

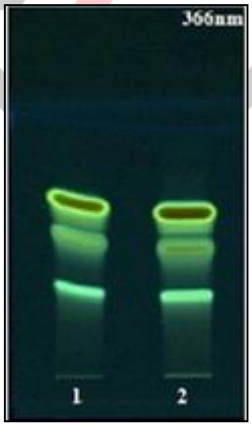

Figure 9 HPTLC chromatogram of (1) Curcumin standard and (2) Test solution at 366

3.4 Spectral Analysis: The IR spectra of the sample confirmed the presence of curcumin in the *Curcuma longa* rhizome. The IR spectrum gave absorption at 3419.17cm-1 indicating the presence of a phenolic group. A small peak is observed at 2862 which shows C-H asymmetric stretching. A peak at 1595 showed significant C=C Stretching and C=O stretching. A peak was seen at 1023 which indicates C-O stretching. A major peak at 611 indicates C-CH out of the plane bending. A standard curcumin sample was also analyzed and it showed a similar spectrum indicating the presence of curcumin in the sample [Figure 10].

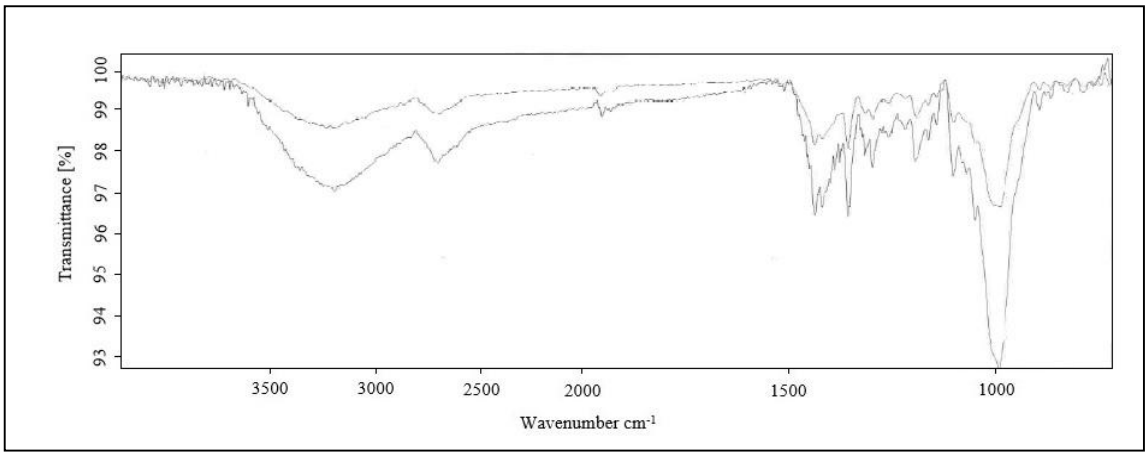

Figure 10 IR spectral comparison of Curcumin standard and *Curcuma longa* test solution

3.5 Quantitative Analysis of Curcuminoids:

Quantification of Curcuminoids in sample and standard curcumin was carried out through the HPLC method. HPLC studies were performed on the sample and standard curcumin to establish the presence of curcuminoids in the sample [Figure 11 & 12].

Table 4 HPLC of standard curcuminoids

NAME	RT	AREA (µV. sec)	% AREA	RESOLUTION	ASSYMETRY	PLATES	HEIGHT
Curcumin	14.325	4539291.000	78.660	0.000	1.69	12287.81	202289
Desmethoxycurcumin	16.575	1072148.750	18.579	4.110	1.63	13055.28	42387
Bisdemethoxycurcumin	19.108	159302.500	2.761	4.136	0.91	13937.76	7113

***Total Area of Peak** = 5770742.250 (µV. sec)*

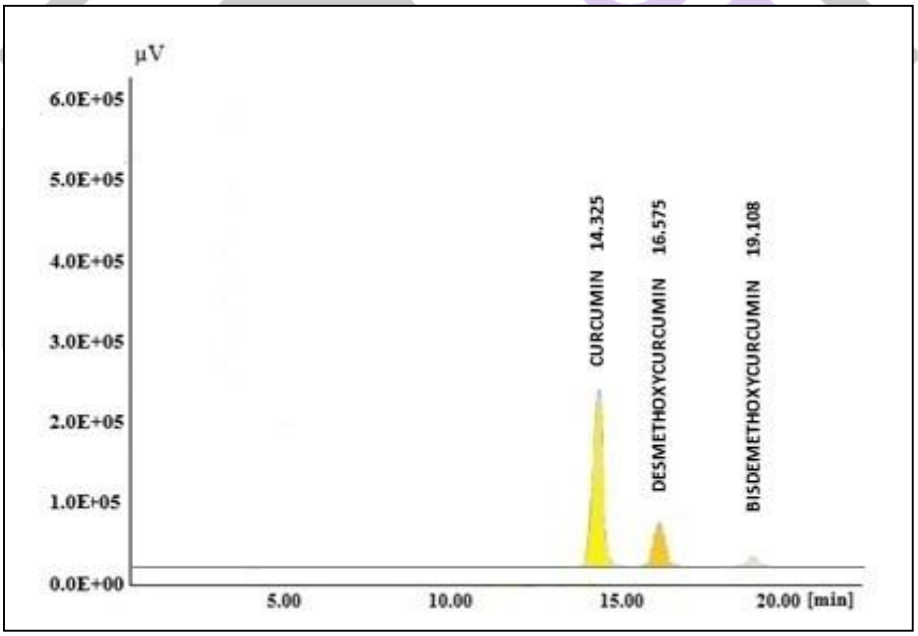

Figure 11 HPLC profile of Standard Curcuminoids

Table 5 HPLC of *Curcuma longa* Powder

NAME	RT	AREA (µV. sec)	% AREA	RESOLUTION	ASSYMETRY	PLATES	HEIGHT
Curcumin	14.025	438068.250	56.264	0.000	1.23	13190.19	22592
Desmethoxycurcumin	16.208	1872097.000	23.388	4.409	1.24	16539.77	8093
Bisdemethoxycurcumin	18.642	158431.250	20.348	4.094	1.55	11770.53	5848

***Total Area of Peak** = 778596.500 (µV. sec)*

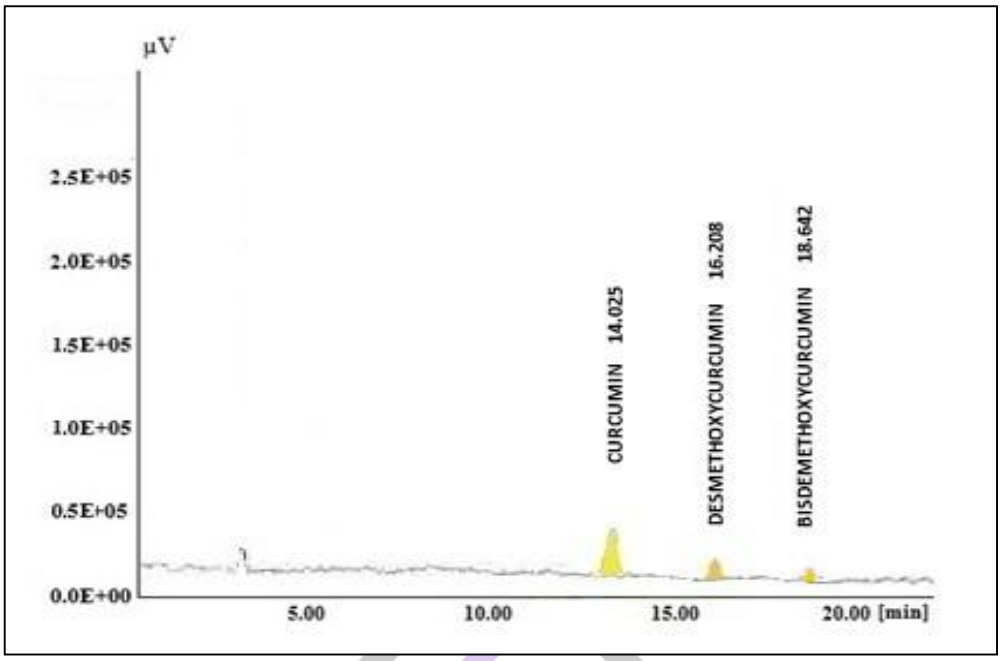

Figure 12 HPLC profile of *Curcuma longa* Powder

4.Conclusion: The present study was pursued to standardize *Curcuma longa* Linn using physicochemical analysis, phytochemical analysis, and analytical techniques like TLC, HPLC, HPTLC and IR spectral analysis confirmed the quantitative presence of curcuminoids which is a mixture of curcumin, desmethoxycurcumin [4-hydroxycinnamoyl-(4-hydroxy-3-methoxycinnamoyl) methane] and bis-demethoxycurcumin [bis-(4-hydroxy cinnamoyl) methane]. Percentage of curcuminoids content in the sample was found to be 3.27% w/w.

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Disclosures of conflict of interest: The authors declare no conflicts of interest.

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