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VALIDATED METHOD FOR ESTIMATION OF CURCUMIN FROM DIFFERENT VARIETIES OF CURCUMA LONGA

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ABSTRACT

Curcumin, the active molecule present in *Curcuma longa* is known for its antitumour, antioxidant, antiarthritic, anti-amyloid, anti-ischemic, anti-inflammatory activities. In addition, it may also be effective in treating malaria, prevention of cervical cancer, etc. High-performance thin-layer chromatography (HPTLC) method has been employed for determination of curcumin in four varieties of turmeric and compared with standard curcumin. The HPTLC separation was performed on precoated aluminium backed HPTLC plates of 0.2 mm layer thickness with silica gel 60 F254 chloroform: methanol (9.5:0.5) employed as mobile phase for the identification and quantification of Curcuminoids in the turmeric varieties. The plate was developed up to 80 mm at temperature of 20 ± 4^{o}C for 10 min. of chamber saturation. The proposed method may be useful as an accurate, simple, cost effective and sensitive for quantitative estimation of curcumin in *Curcuma longa* extracts.

KEYWORDS : High-performance thin-layer chromatography, Curcumin, spices, Curcuma longa, finger,

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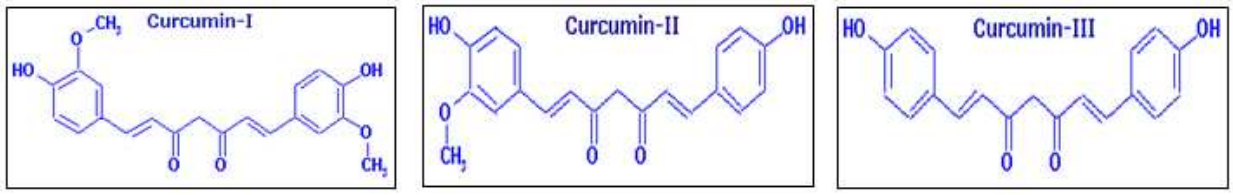
INTRODUCTION

The turmeric (Curcuma longa Linn) plant, a perennial herb belonging to the family of *Zingiberaceae*, is cultivated extensively in south and southeast tropical Asia. The rhizome of this plant is also referred to as the "root" and is the most useful part of the plant for culinary and medicinal purposes. Curcumin is the most important fraction of turmeric which is responsible for its biological activity. Literature reveals its anti-inflammatory, cholagogue, hepatoprotective, blood-purifier, antioxidant, detoxifier and regenerator of liver tissue, antiasthmatic, anti-tumour, antiprotozoal, stomachic, carminative properties. It reduces high level of cholesterol in plasma. Its antiplatelet activity offers protection to heart and vessels. It also prevents DNA damage in lymphocytes[1]. It is also a potent free radical scavenger, having superoxide anions, singlet oxygen, hydroxyl radicals scavenging and lipid peroxidation inhibitory activities[2]. Several constituents present in this plant include curcumin is shown in Fig.1. demethoxycurcumin, bisdemethoxycurcumin, volatile oils like turmerone, atlantone, zingiberene, sesquiphellandrene, terpinolene, phellandrene, p-cymene, cineol, caryophyllene, nerolidol, curlone, dehydrozingerone, zerumbone, germacrene, (and)sesquiterpenes [3-10]. Curcumin has a molecular formula $C_{21}H_{20}O_6$, molecular weight is 368.91. HPTLC shows advantages of low operating cost, high sample throughput and need for minimum sample clean-up. Another major advantage is simultaneous application of several samples using small quantity of mobile phase. HPTLC makes scanning in situ and repeated detection of the chromatogram with the same or different parameters possible[11]. HPTLC analysis of many plants used in Indian Systems of Medicine has been performed [12-14]. The aim of the present work was to attempt to the method for determination of curcumin in different extracts.

Figure1
The active constituents of curcuma long.



Curcumin I: Curcumin - 1, 7-bis (4-hydroxy-3-methoxyphenyl) hepta-1, 6-diene- 3, 5-dione
Curcumin II: de-methoxy curcumin - 1-(4-Hydroxyphenyl)-7-(4-hydroxy-3-methoxyphenyl) hepta-1, 6-diene-3, 5-Dione
Curcumin III: bis-de-methoxy Curcumin - 1, 7-bis (4-hydroxyphenyl) hepta-1, 6-diene-3, 5-Dione

MATERIALS AND METHODS

Chemicals, Reagents and Standards

All solvents & chemicals employed for extraction and separation of Curcumin from Turmeric varieties were of AR grade (Merck) and standard Curcumin (Sigma, USA) was used in HPTLC for analysis.

Preparation of Standard Stock Solution

A standard stock solution containing 1mg/10ml of curcumin was freshly prepared and appropriate dilutions of this standard stock solution were made for making the calibration curve by applying different volume of standard to get different amount of standard per spot.

Preparation of Extracts

Four turmeric varieties were used in this study and it was subjected to extraction with n-Hexane, Chloroform, Ethyl acetate, Acetone and Methanol by Soxhlet apparatus. The extract was concentrated under reduced pressure; air

dried and stored in the desiccator's was used for subsequent experiments.

Separation of Curcumin by HPTLC

For HPTLC studies of curcumin, precoated Silca gel 60F254 aluminium plates (10 X 20cm) were used[15]. The extract of turmeric varieties and standard Curcumin was separated by run the spots in chloroform: methanol (9.5:0.5) employed as mobile phase for the identification. The colour and RF values were recorded using spraying the plates with 1 % alcoholic KOH solution.

Chromatographic Conditions

The spots were applied as bands with a band length of 5 mm and the distance between the tracks as 10 mm with the help of Camag HPTLC applicator Linomat IV. Stationary phase was used precoated silica gel 60F254 plates (10cm X 20cm) from E-Merck. The plate was developed in a Camag twin trough chamber after a chamber saturation time for mobile phase. The densitometric analysis was performed on a Camag TLC scanner in the absorbance mode at 421 nm. Chromatogram of standard curcumin (Fig. 2) and the sample has been presented (Figs. 4 -8).

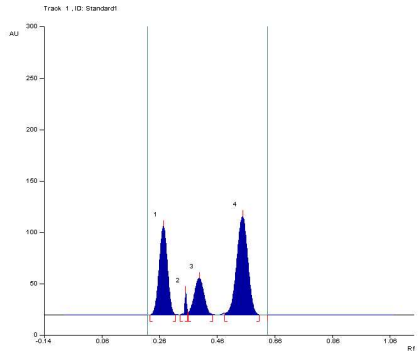
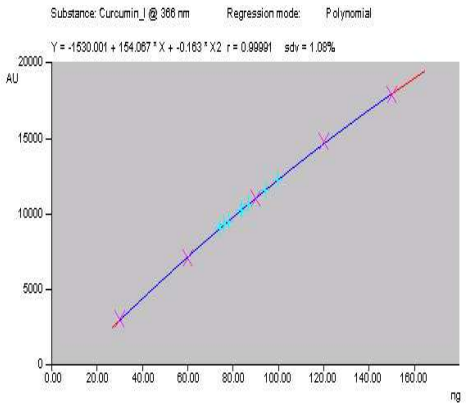


Figure.2
Chromatogram of standard curcumin

Validation Procedures

Calibration of Instrument

Calibration curves for the standard curcumin were prepared with respect to height and area vs. amount per spot by applying a series of standard with different volume. (Graph 1)



Graph 1
Calibration Graphs

Linearity Range

The linearity of the standard graph was obtained with a five-point calibration range between 30–150 ng/spot and the three curcuminoids separated, were quantified in the samples.The presence of the investigated compounds was determined according to their Rf (R_F) values.

Retention Factor (R_F)

$$R_F = \frac{\text{Distance moved by the analyte from origin}}{\text{Distance moved by the solvent front from origin}}$$

Limit of Detection and Limit of Quantization

Limit of detection (LOD) and quantization (LOQ) were determined based on the standard deviation of the response and the slope as per ICH guideline[16]. They were calculated based on the following:

$$\text{Detection Limit} = \frac{3.3\,\sigma}{S}$$

$$\text{Quantification Limit} = \frac{10\,\sigma}{S}$$

Where σ = standard deviation of the response
S = slope of the calibration curve
σ was determined from the responses of a number of blank samples.
The limits of detection (LOD) and limit of quantification (LOQ) were found to be 8 -25 ng per spot, respectively.

DISCUSSIONS

Keeping in view of the ethno-pharmacological importance *C. longa*, the quantitative analysis Curcuminoids in varieties of samples were performed using the reference Curcumin standard. The studies were undertaken for different active constituents such as Curcumin I, Curcumin II and Curcumin III were successfully extracted using Hexane, Chloroform, Ethyl acetate, Acetone and Methanol solvent and separated with HPTLC method. On the basis of these profiles, the percentages of curcumin present in the different brands of turmeric powder have been presented in Table 1. The curcumin (Curcuminoids) content in Chloroform, Ethyl acetate, Acetone and Methanol extract of four varieties of turmeric was found respectively in the range of 1.93- 2.34 %, 2.58 – 3.14 %, 2.24 – 2.72 % and 2.36 – 2.87 %. In this study, comparison of Curcumin content in turmeric varieties which are extracted using different solvent was revealed that the Curcumnoids content were slightly change or very much closer in all extracts. We observed that, Ethyl acetate extract of the all turmeric varieties had higher extractability of Curcuminoid than Methanol, Acetone and Chloroform extracts. The n-Hexane extract does not show any bands corresponding to Curcuminoids in all varieties; it may be the percentage of curcumin content is less than the LOD of value but all other extract show notable content in all varieties of turmeric.The quantity of Curcumin I, Curcumin II and Curcumin III in Chloroform extract, Ethyl acetate extract,

Acetone extract and Methanol extract of Brahumpur finger, Erode finger, Erode hybrid and Salem finger turmeric varieties are shown in Table-1. It was evident that the total Curcuminoid present in the extracts of four turmeric varieties shows the higher extractability in Ethyl acetate. The quantity of Curcumin I, Curcumin II, Curcumin III and total Curcuminoid present in all the extracts except from n-Hexane solvent. Moreover, among the four turmeric varieties, the Erode hybrid finger offer high curcuminoids content next to Salem, Erode, and Brahumpur finger varieties.

Table 1

The percentages of type of curcumin present in the different varities of turmeric powder.

	Curcumin type & content (in %)*															
	Brahumpur finger				Erode finger				Erode hybrid finger				Salem finger			
Name of the Extracts	I	II	III	Total	I	II	III	Total	I	II	III	Total	I	II	III	Total
HE	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
CE	0.87	0.39	0.67	1.93	0.98	0.44	0.76	2.18	1.05	0.47	0.82	2.34	1.00	0.45	0.78	2.23
EE	0.78	0.39	1.42	2.58	0.88	0.44	1.61	2.92	0.94	0.47	1.73	3.14	0.90	0.45	1.65	2.99
AE	0.74	0.38	1.12	2.24	0.83	0.43	1.26	2.53	0.90	0.46	1.36	2.72	0.86	0.44	1.30	2.59
ME	0.78	0.40	1.18	2.36	0.88	0.45	1.33	2.67	0.95	0.49	1.44	2.87	0.90	0.46	1.37	2.74

HE – Hexane Extract: CE – Chloroform Extract: EE – Ethyl acetate Extract: AE – Acetone Extract: ME – Methanol Extract

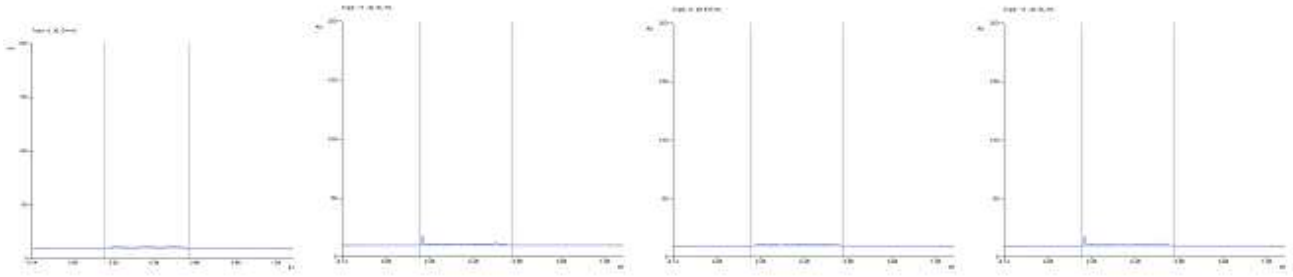


Figure 4

HPTLC chromatogram of Hexane extract of four verities of turmeric powder

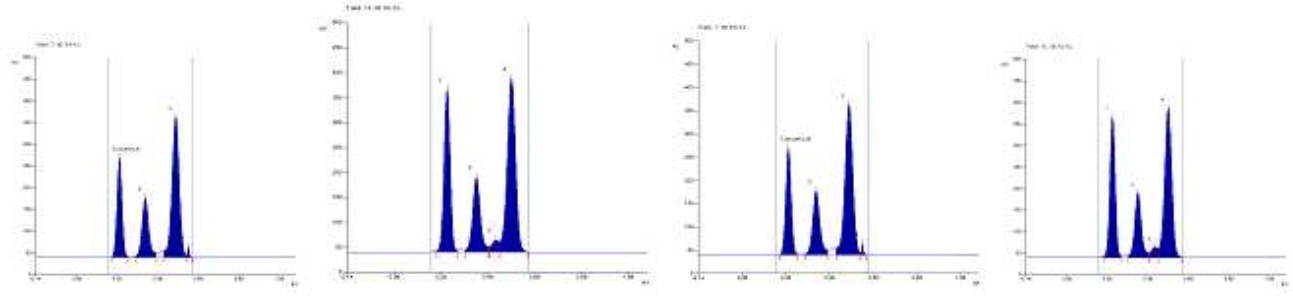


Figure 5

HPTLC chromatogram of Chloroform extract of four verities of turmeric powder

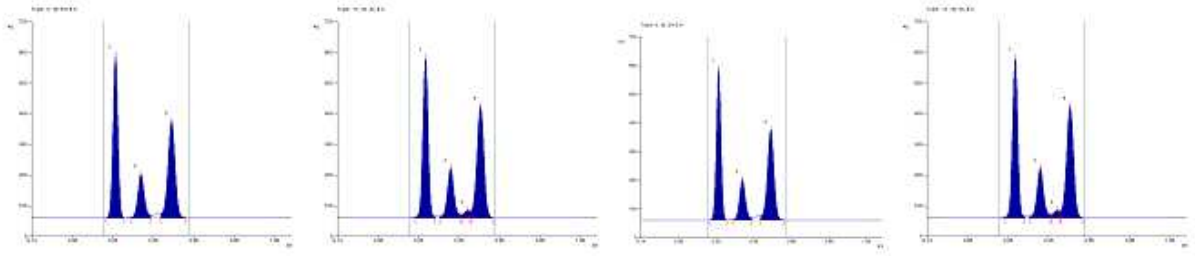


Figure 6
HPTLC chromatogram of Ethyl acetate Extract of four verities of turmeric powder

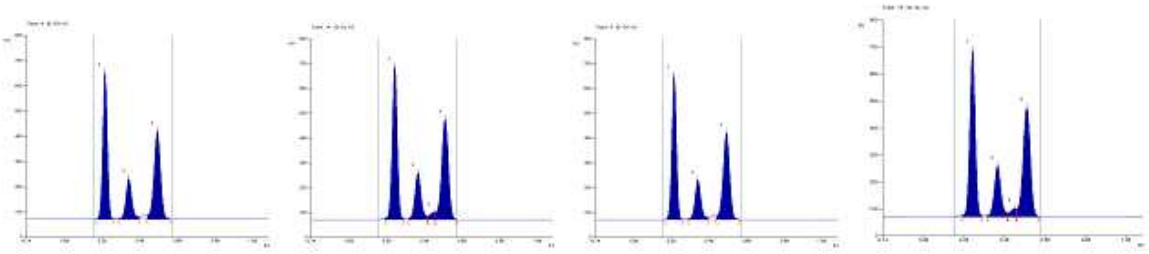


Figure 7
HPTLC chromatogram of Acetone Extract of four verities of turmeric powder

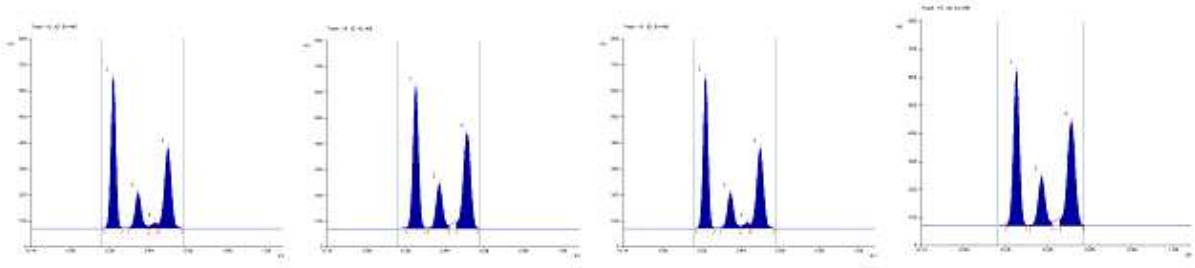


Figure 8
HPTLC chromatogram of Methanol Extract of four verities of turmeric powder

CONCLUSION

HPTLC method was represented as an excellent technique for simultaneous determination of curcuminI, curcumin II and curcumin III in the varieties of *C.longa* extract with good sensitivity and precision. Running time and cost per analysis are relatively low in comparison with other methods. Furthermore, the method can be used as quality control for curcumin in *C, Longa* and will play a reference role on the determination of active constituent in other medicinal plants or pharmaceutical herbal preparations.

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