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Original article

HPTLC method development and quantification of curcumin content in different extracts of rhizomes of *Curcuma longa* L.

N.M.A. Rasheed, G. Sowmya Srividya and K. Nagaiah

Organic and Biomolecular Chemistry, CSIR-Indian Institute of Chemical Technology, Tarnaka, Hyderabad-500007, Telangana State, India

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Abstract

Turmeric is very long known for its potent medicinal values in Indian traditional systems of medicine. *Curcuma longa* L. commonly known as turmeric (Haldi) is a well known plant which is used as a drug in traditional medicine such as Ayurvedic and Unani system of medicine. Curcumin is main active component present in the rhizomes of *C. longa*. The contents of curcuminoids in turmeric rhizomes vary often with varieties, locations, sources, and cultivation conditions, while there are significant variations in composition of turmeric rhizomes with varieties and geographical locations. The present study reported a simple, sensitive and fast HPTLC method for quantification of curcumin in solvent extracts of rhizomes of *C. longa* L. The separation was performed on TLC aluminium plates precoated with silica gel G F_{254}. Good separation was achieved in the mobile phase of chloroform: methanol: formic acid (9.6:0.4:0.1, v/v) at Rf= 0.70 ± 0.05 for curcumin. The method was validated successfully having good resolution of peaks along with precision, accuracy and repeatability. Curcumin capsules (>95% curcumin) have not been emerged as a drug so far but had been taken in food or dietary supplement. In traditional medicine, it is well recommended to use 2 to 3 grams turmeric powder in our daily diet. Therefore, the objective of the study to extract turmeric and assess curcumin content and other constituents by HPTLC with a suggestion to use the whole extract derived from the equivalent 2 to 3 grams of the turmeric powder. Present study is to determine high content of curcumin 95-98% and recommended for many diseases. The HPTLC method can be used for quantification of Curcumin in extracts of rhizomes of *C. longa* L.

Key words: *Curcuma longa* L, turmeric, curcumin, solvent extracts, HPTLC, quantification

1. Introduction

Turmeric is commonly known for its wide range of biological and medicinal values in Indian traditional system of medicine. Turmeric comprises about 70 species and highest diversity is concentrated India, *i.e.*, 40 species. India had been the largest producer, consumer and exporter of *C. longa* L. in the world commonly known as Haldi, is a well- known plant used as a drug in Ayurvedic and Unani system of medicine (Chopra *et al.*, 1956; Kapoor, 2001). It is a pan tropical crop cultivated widely in Southeast Asia, used in the food preparation for its flavour and colour. Turmeric contains a yellow coloured mixture of three phenolic compounds, the main active principle or constituent was curcumin, and other constituents including demethoxycurcumin, bisdemethoxycurcumin, essential oil (2-4%); fixed oil (2-3%) likes turmerone, atlantone, and zingiberone. It also contains sugars, proteins and resins (Antony, 2003). Curcumin 1, 7-bis-(4-hydroxy-3-methoxyphenyl)-1, 6- heptadiene-2, 5-dione (Figure 1) is a yellow colored phenolic pigment obtained from powdered rhizome of *C. longa* L. belongs to family Zingiberaceae. Curcumin possess antioxidant and several other biological activities. The agronomy factors and suitability need to be studied. Estimation of curcumin and its oleoresin need to be researched and propagated. And there is a growing tendency to adulterate turmeric in several ways. So, selection of high yielding varieties and detection of adulteration is extensively required research for turmeric. Studies support curcumin as well as the essential oils and turmerones have similar properties while some favour oleoresin extracts which contain both curcumin and essential oils and turmerones. Turmeric tuberous roots are also considered to be good for the medicinal properties and its derived products (Shameli *et al.*, 2013). Selection of better strains of turmeric having high yields of curcumin oleoresin is desirable. For example, Alleppey (Kerala, India) variety of turmeric has high curcumin (approx 6%) while Telangana, India variety has only about 2% of curcumin. Turmerones also independently have activity against cancer. Several references shown anticancer activity of the whole extract. Further, the other constituents in the extract enhanced the activity. Infact, *ar*-turmerone, exhibits medicinal properties equally effective also these upon biological studies reveal good activity on Alzheimer diseases as mentioned earlier.

Curcumin capsules (95% curcumin) have not been emerged as a drug so far but had been taken in food or dietary supplement, supportive nutritional supplement in the form of capsules and others, *etc.* (Prucksunand *et al.*, 2001). Limited Phase II clinical studies on turmeric extracts were done but not with 95% curcumin as a whole drug or along with some other drug in case of particular disease or diseases. Toxicity effect of high dose of 95% of curcumin

Author for correspondence: Dr. K. Nagaiah
Sr. Principal Scientist, Organic and Biomolecular Chemistry, CSIR-Indian Institute of Chemical Technology, Tarnaka, Hyderabad-500007, Telangana State. India
E-mail: nagaiah@iict.res.in
Tel.: +91-9440485366

was not assessed. Earlier studies from our group studied that the curcumin capsules and curry masala powder of turmeric assessed major bioactive curcuminoids in *C. longa* L. rhizomes and herbal products using non-aqueous capillary electrophoresis (Anubala *et al*., 2014).

Therefore, the purpose of the study is to extract high content of curcumin (95-98%) to be available and recommended for many diseases. However, up to phase II clinical studies were conducted only on 98% curcumin. Toxicity studies were not done on such high content of curcumin (98%) which will be equivalent to allopathy drug. Without toxicity studies and multi-centered clinical trials, it is not advisable to use as a drug. On the other hand, it is well recommended in traditional medicine to use 2 to 3 grams turmeric powder in our daily diet. Therefore, the objective of the studies to extract turmeric and assess curcumin content and other constituents by HPTLC with a suggestion to use the whole extract derived from the equivalent 2 to 3 grams of the turmeric powder. The literature surveys reviewed that whole extract of turmeric which contains curcumin exert better activity. It is also reported that other extractives of turmeric (volatiles and turmerones) will enhance the bioactivity of curcumin. Turmerones component of oleoresin of turmeric have been used to enhance the absorption of curcumin. These turmerones have strong anti-inflammatory and anticancer properties. In fact, recent studies shows turmerones are as potent as curcuminoids as mentioned below. Volatile oils and turmerones of turmeric are principle flavouring compounds, α-turmerone, and β-turmerone, *ar*-turmerone are the primary components of oleoresin of turmeric rhizome and give characteristic pungent smell to turmeric. Aromatic (*ar*-) turmerone is a natural antimicrobial and antifungal compound and possess antivenom properties. Alpha and beta turmerones act as antibacterial agents against disease-causing bacterium that cause serious infections and prevent cavities. They also possess anticancer properties and work against liver cancer, leukemia, and breast cancer cells. For *e.g*., *ar*-turmerone help in preserving cognition. They suppress amyloid-beta considered responsible for the plaques and tangles in the brain that stop neural connections which ultimately lead to Alzheimer's disease. *ar*-turmerone also inhibit inflammatory cytokines that begin the spiral of cognitive decline in the brain. Therefore, incorporating whole extract of turmeric which contains volatile oils, curcumins and turmerones into our daily regimen may be one of the best and simplest ways to keep as in good health for years to come. It is better to use curcumin with turmerones and essential oils that is the whole extract of turmeric rather than curcumin alone (Anonymous, 2017a). *ar*-Turmerone impaired the Aβ-induced inflammatory response of microglial cells by inhibiting the NF-κB, JNK, and p38 MAPK signaling pathways. Lastly, *ar*-turmerone protected hippocampal HT-22 cells from indirect neuronal toxicity induced by activated microglial cells. These novel findings provide new insights into the development of *ar*-turmerone as a therapeutic agent for the treatment of neurodegenerative disorders (Park *et al*., 2012) and (Anonymous, 1990).

In market, there are several formulations available in combination of curcuminoids and essential oils such as Finest nutrition-turmeric Curcumin capsules (1000 mg) as healthy nutrition; NutriFlair-Turmeric Curcumin with BioPerine® Black Pepper 1300mg (120 Capsules) as dietary supplement; Terry Naturally-CuraMed with BCM-95 750 mg. (120 Soft gels) as Healthy inflammation response; Nature's Best-Turmeric Tablets 10,000 mg (as 500 mg of extract) standardized at 95% curcumins which act as 20 times more concentrated than culinary turmeric and used as food supplement ; SBR Nutrition-liquid turmeric curcumin, pure turmeric extract-fulvic acid used as a antioxidant support liquid dietary supplement; Nature's answer-Turmeric-3 supplying three active constituents 5,000 mg; Tinctures-concentrated herbal extracts offer 1000 mg dose. Tinctures are not to be confused with liquid extracts. The therapeutic value of a plant lies in its holistic balance of natural ingredient present in it and the quality of the herb (Anonymous, 2017b).

Stronger, more effective curcumin capsules, liquid extracts, used as dietary supplements, healthy nutrition, food supplement provides, healthy inflammation response through multiple pathways in the body, providing remarkable health benefits. Cellular health: studies showed curcumin's ability to support cellular health protect DNA and RNA from oxidative stress and support multiple cellular processes. Mood: curcumin supports healthy levels of serotonin and dopamine in the brain, key neurotransmitters for optimal mental health. Clinical studies show curcumin's effectiveness for enhancing mood and well being. Kidney support: curcumin supports functioning of healthy kidney. Joint health: curcumin maintains flexibility and comfort in joints while supporting cartilage structure. Brain health: curcumin helps maintain focus and concentration while protecting brain cells from oxidative stress. Turmeric: the intelligent choice for cognitive health. Intestinal health: curcumin supports healthy bile secretion, bowel motility, and fermentation processes while supporting the gut mucosal lining. Liver detoxification: curcumin promotes healthy levels of the body's own detoxifiers, such as glutathione and superoxide dismutase. Cardiovascular health: Studies indicated that curcumin supports vascular integrity and healthy cholesterol balance and supports heart function. Antioxidant: curcumin powerfully protects the body from damaging oxidative stress (Anonymous, 2017c) skin diseases, skin care agent: Raw turmeric is applied when there is a cut or wounds also, recommended internally and externally to cure or prevent the spread of skin diseases like eczema, ringworm, antibacterial activity. Many skin care lotions and ointments. Turmeric is a common ingredient due to antibiotic effect of turmeric principles are responsible.

1.1 Turmeric remedies

Curcumin or turmeric extract or turmeric powder is taken as: one teaspoon of turmeric powder in 1 cup of warm milk. Drink three cups a day to reduce arthritis pain and other ailments. (OR) add one teaspoon of turmeric in your food or curry daily (OR) 500 mg of turmeric extract as capsules or tablets two or three times a day (OR) curcumin 400 mg in the form of capsules of tablets two or three times a day (Mukhodapadhyay *et al*., 1982; Chandra and Gupta, 1972; Mowreyl, 1990).

Tea: Pour two cups boiling water over one teaspoon turmeric and steep for 10 min. Strain. Add honey and /or lemon if desired. Capsules: two to three grams of turmeric per day provides 60 to 100 mg curcumin, the daily amount typically consumed in the diet in India. Standardized extract: To replicate the levels of curcumin used in the clinical trials on turmeric, purchase an extract that guarantees a specific level of curcumin (sometimes written as curcuminoid on the label). Most studies used turmeric extracts providing 1 to 2 g per day of curcumin, taken in 2 or 3 divided dosed (Chevallier, 2016; Johnson *et al*., 2017).

Curcumin is an antioxidant, and it appears to work as an anti-inflammatory in the body. Thus, it may benefit inflammatory conditions such as osteoarthritis, rheumatoid arthritis and Alzheimer's disease. It shows promise as a compound to prevent and treat cancer, and ongoing clinical trials continue to investigate curcumin is one of several curcuminoids, which are polyphenolic compounds, present in turmeric. In addition to curcumin, turmeric also contains smaller amounts of the curcuminoids, demethoxy curcumin and bisdemethoxycurcumin. Researchers believe curcumin to be the most biologically active curcuminoids in turmeric. Analogs of curcumin (Cur), such as demethoxycurcumin (DMC), bisdemethoxycurcumin (BDMC), tetrahydrocurcumin (THC) and turmerones, modulate inflammatory signaling and cell proliferation signaling. To some extent, bisdemethoxycurcumin is used as a pigment and nutraceutical with antimutagenic properties. Curcumin molecule has a broad range of activities includes anti-inflammatory, antioxidant, hypocholesterolemia, anticarcinogenic, wound healing, anticoagulant, antispasmodic, antitumor, hepatoprotective activities (Maiti *et al.*, 2007; Anand *et al.*, 2008; Goel *et al.*, 2008; Kunnumakkara *et al.*, 2007) and antidiabetes (Merrell *et al.*, 2009; Reddy *et al.*, 2009; Sandur *et al.*, 2007). The important antioxidant property of curcumin can prevent rancidity of foods and provide foodstuffs containing less oxidized fat or free radicals and plays an important role in keeping curry for a long time without it turning rancid.

The contents of curcuminoids in turmeric rhizomes vary often with varieties, locations, sources, and cultivation conditions, while there are significant variations in composition of essential oils of turmeric rhizomes with varieties and geographical locations. Hence, it is imperative to know the content of main active constituent curcumin in turmeric (Cooper *et al.*, 1994). Authentication of turmeric products can be achieved by chromatographic and other analytical techniques (Li *et al.*, 2011). The rhizomes undergo processing before using, such as boiling separately for about 40-60 min under slightly alkaline condition in earthen vessels which changes the main active content in the sample. Few reports are available in respect of curcumin content in the primary rhizome which increased from September to October in Japan (Kobayashi *et al.*, 2010). However, HPTLC method has been previously reported for quantification of curcumin but no reports have been found to determine curcumin content in different solvent extracts of rhizome of *C. longa* L and this information is critically important for the extraction of active component curcumin and other constituents for manufacturing of herbal products. Hence, it would be very significant to develop an efficient analytical method that can analyze curcumin quantitatively having good efficiency. The present HPTLC method was suitable for rapid screening of plant materials can be performed without any special sample pretreatment.

Name	Formula	Molecular Weight	Nature of compounds present
α-Turmerone	$C_{15}H_{22}O$	218.3346	Turmerones
β-Turmerone	$C_{15}H_{22}O$	218.3346	
ar-Turmerone	$C_{15}H_{20}O$	216.324	
Zingiberene	$C_{15}H_{24}$	204.357	Monocyclic sesquiterpene
Curcumin	$C_{21}H_{20}O_6$	368.39	Curcuminoids (phenolic compounds)
Demethoxycurcumin	$C_{20}H_{18}O_5$	338.359	
Bisdemethoxycurcumin	$C_{19}H_{16}O_4$	308.33	

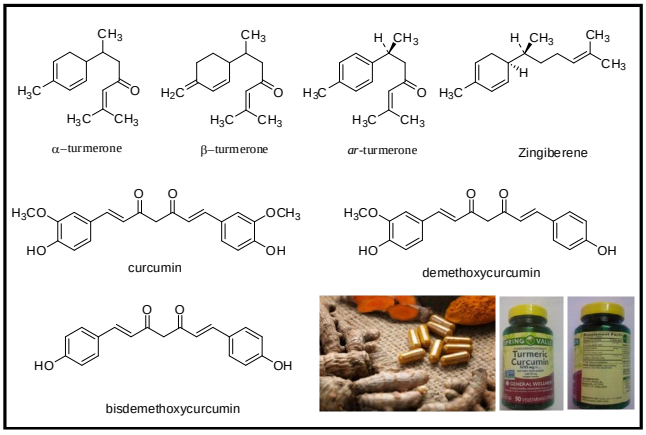

Figure 1: Structures of turmeric, chemical constituents, turmeric capsule and rhizome

2. Materials and Methods

2.1 Reagents

All chemicals and reagents used were of HPLC grade (Fischer scientific). Curcumin was isolated from the rhizome in pure form and was characterized by spectroscopic methods.

2.2 Raw materials and samples

Rhizomes of *C. longa* L. were procured from the local market and were authenticated by Botanist, Central Research Institute of Unani Medicine, Hyderabad. Three *C. longa* L. samples were procured, *viz.*, CS, CLA and CLA.

Marketed spring valley Standardized extract, Turmeric curcumin capsule of 500mg containing (turmeric powder-400 mg, Turmeric extract Standardized to 95% curcuminoids -50 mg, *Zingiber officinale* root powder-50 mg) used as a dietary supplement for general wellness.

2.3 Preparation of standard stock solution

Isolated pure curcumin from turmeric as standard was accurately weighed and suitable dilution was made to give a solution containing 1mg/1ml and 50 µg/ml (50 ng/µl). These solutions of curcumin were further used for analysis.

2.4 Extraction of plant material for HPLTC analysis

Turmeric branded marketed powder (TP), Turmeric secondary rhizome powder (TK), Turmeric big tuber (TBT) was extracted with hexane first followed by methanol; Secondly TP, TK and TBT extracted with methanol and ethanol; later TBT ethanol was washed with Xylene (Tbt etxy); and turmeric methanolic extracts of 4 different samples were taken. Labeled as 1-16 (1: Turmeric marketed powder (TPh)- hexane ext; 2: Turmeric secondary rhizome (Tkh)-hexane ext; 3: Turmeric big tuber (Tbth) - hexane ext; 4: (TPhm)Turmeric marketed powder- methanol ext after hexane ext; 5: (Tkhm)Turmeric secondary rhizome - methanol ext after hexane ext; 6: (Tbthm)Turmeric tuber - methanol ext after hexane ext; 7: (TPm)Turmeric marketed powder- methanol ext; 8: (Tkm)Turmeric secondary rhizome - methanol ext; 9: (Tbtm)Turmeric tuber - methanol ext; 10: (Cur) Pure curcumin std; 11: (CS)Turmeric methanolic extract sample 1; 12: (Tcap)-Turmeric capsule extract sample 2; 13: (TbtEt) Turmeric tuber - ethanol ext; 14:

(TbtEtXy)Turmeric tuber-ethanol ext followed by xylene wash; 15: (CLA)-Turmeric methanolic extract sample 3; 16: (CLE)-Turmeric methanolic extract sample 4)

One hundred grams or amount taken as given in Table 1 was finely powdered separately, and extracted with boiling methanol for six hours using soxhlet extraction apparatus. Later, the extract was evaporated to dryness. The amount of residue obtained is calculated and percentage of extract found is represented in the Table 1. From this, each extract 10 mg was taken in a 10 ml volumetric flask and dissolved with the help of methanol under sonicator and then the solution is made up to 10 ml and, thus obtained solution was used for HPTLC analysis. Turmeric curcumin capsule was taken 500 mg and extracted with 500 ml methanol (ratio 5 g : 5 ml) filtered, dried to constant and finally dissolve with methanol to make up to 5 ml. Similar manner each sample extract was prepared and stock solutions of 1 mg/ml was made and used for further analysis.

2.5 TLC instrumentation and conditions

The samples are spotted in the form of bands of width 5 mm with an automatic TLC applicator of Desaga Sarstedt Gruppe (Germany) having 10 µl syringe on precoated silica gel aluminium plate 60 F_{254} (20 cm × 10 cm with 0.2 mm thickness; E. Merck, Dermstad, Germany). A constant application rate of 5 sec/µl was employed and distance between two bands was 10 mm. The slit dimension was kept at height 0.2 mm and width 4.0 mm, resolution at 0.1mm measurement, 16 No. of measurements per position, smoothing value at 15. These instrumental parameters were kept constant throughout the samples analyses. The mobile phase consists of chloroform, methanol and formic acid (9.6:0.4:0.1, v/v) for the development of TLC plate. The plates were developed in ascending order in a twin through TLC chamber which was pre-saturated with the mobile phase for 30 min; the length of each run was 80 mm. The TLC runs were performed under the laboratory conditions (temperature: 25 ± 2^0C and %RH: 60 ± 5). The plates were dried in air before detection. Densitometric analysis was performed at wavelength 366 nm with a Desaga Sarstedt Gruppe by ProQuant 1.6 version software. The source of radiation utilized was deuterium and tungsten lamp. The mobile phase composition for TLC was optimized using different solvents of varying polarity and good resolution was achieved by above stated solvent system. The Rf value for curcumin was found to be 0.70 ± 0.05

2.6 Calibration

From the stock solution of curcumin (50 ng/µl) in methanol, an aliquots of 2 µl, 4 µl, 6 µl, 8 µl and 10 µl was applied four times (n = 4) over the silica gel TLC plate separately to obtain final concentration range of 100-500 ng/spot for construction of calibration plots. TLC plate was developed and analyzed as described earlier. Further dilutions with the lowest concentration in the calibration curves were carried out to provide a series of standard solutions for evaluating the limits of detection (LOD) and the limits of quantification (LOQ) of the component.

3. Method validation

The developed method is validated as per the ICH guidelines. Method validation is carried out to confirm that the analytical method employed for this specific analysis is suitable for its intended use. Results from method validation can be used to check its quality, reliability, and consistency. The method was validated by determining linearity, precision, accuracy, limits of detection (LOD), limits of quantification (LOQ) and repeatability.

3.1 Calibration curve

Calibration graph was found to be linear over the concentration range of 100-500 ng/spots. Linearity was evaluated by determining five standard working solutions for four times (n = 4). The peak area and concentration was subjected to linearity analysis to calculate the calibration equation.

3.2 Precision and accuracy

The intra-day precision and accuracy of the assays were evaluated by performing replicate analyses (n = 4) of samples (5, 100 and 500 ng/spot). The inter-day precision and accuracy of the assay was determined by repeating the intra-day assay on three consecutive days. Precision was expressed as the percentage relative standard deviation (%RSD) of measured concentrations where as accuracy was expressed as percent recovery.

3.3 Limit of detection (LOD) and limit of quantification (LOD)

In order to estimate LOD and LOQ for curcumin, we spotted blank methanol (n=4) following the same method as explained under the chromatographic conditions and the standard deviation (σ) of the magnitude of analytical response was determined. The LOD was expressed as (LOD=3.3 σ/S), whereas LOQ was expressed as (LOQ=10 σ/S) where S= Slope of curcumin calibration curve.

3.4 Specificity

The specificity of the method was ascertained by analyzing standard drug and sample extracts. The spot for curcumin in sample was confirmed by comparing Rf value and UV spectrum of spot with that of standard.

3.5 Quantification of curcumin in sample extracts

Quantification of curcumin content in rhizomes of *C. longa* L. and its extracts were carried by application of 2 µl each of the sample extract from the stock solutions on the same TLC plate using an automatic TLC applicator of Desaga Sarstedt Gruppe with 10 µl syringe. The plates were developed and scanned as mentioned earlier. The peak areas were recorded and amount of curcumin was calculated corresponding to the peak area of curcumin standard (Rasheed *et al*., 2010; 2011a; 2012a; 2012b and Naikodi *et al.*, 2011b).

4. Results

4.1 Selection and optimization of mobile phase conditions

Initially toluene: ethyl acetate: methanol, toluene: ethyl acetate: formic acid, chloroform: methanol in varying ratios was investigated. These solvent systems did not provide the satisfactory separation and resulting streaking and typical peak shape was obtained with lesser Rf value close to the loading spot. Finally, chloroform: methanol: formic acid (9.6:0.4:0.1, v/v/v) gave a sharp and well-defined peak at Rf value of 0.70 (Figure 2). Well defined spots were obtained when the chamber was saturated with the mobile phase for 30 min at room temperature before the development of plate.

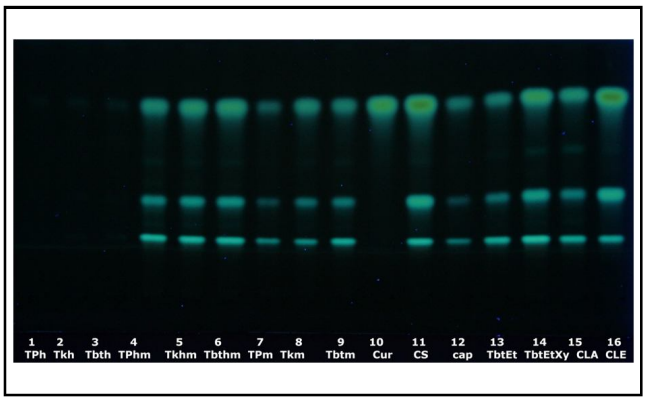

Figure 2: Thin layer chromatography plate of standard and sample. (1: Turmeric branded marketed powder (TPh)- hexane ext; 2: Turmeric secondary rhizome (Tkh)- hexane ext; 3: Turmeric big tuber (Tbth) - hexane ext; 4: (TPhm)Turmeric marketed powder- methanol ext after hexane ext; 5: (Tkhm)Turmeric secondary rhizome - methanol ext after hexane ext; 6: (Tbthm)Turmeric tuber - methanol ext after hexane ext; 7: (TPm)Turmeric marketed powder- methanol ext; 8: (Tkm)Turmeric secondary rhizome - methanol ext; 9: (Tbtm)Turmeric tuber - methanol ext; 10: (Cur) Pure curcumin std; 11: (CS)Turmeric methanolic extract sample 1; 12: (Tcap)-Turmeric capsule extract sample 2; 13: (TbtEt) Turmeric tuber - ethanol ext; 14: (TbtEtXy)Turmeric tuber - ethanol ext followed by xylene wash; 15: (CLA)-Turmeric methanolic extract sample 3; 16: (CLE)-Turmeric methanolic extract sample 4).

4.2 Validation of method

4.2.1 LOD and LOQ

LOD and LOQ were calculated by the method as described in validation section. Detection limit of curcumin was determined by plotting a series of concentrations on the plate and scanning at 366 nm. The lowest amount of curcumin, which could be detected (LOD) was 0.69 ng/spot. The lowest amount of curcumin which could be quantified was found to be 2.10 ng/spot which indicates the ample sensitivity of the method (Table 2).

Table 2: Linear regression data for the calibration plot (n=4)

Parameters	Observation for curcumin
Retention factor (Rf)	0.70
Linearity range (ng/spot)	100-500
Linearity (Correlation coefficient)	R^2 = 0.997
Regression equation	y = 427.7x - 111.6
SE of intercept	40.2468
SD of intercept	89.99184
LOD (ng/spot)	0.69
LOQ (ng/spot)	2.10
Precision (%RSD, n=4)	1.55
Repeatability (%RSD) n=4	1.38

4.2.2 Calibration curve

The calibration curve was plotted as amount of analyte versus average response (peak area) and was linear over the concentration range of 100-500 ng/spot, as shown in Figure 3. The regression equation was obtained y= 427.7x - 111.6 with respect to the peak area with a regression coefficient of R^2=0.997 with high reproducibility and accuracy (Table 2). The set of calibration parameters are summarized in Table 2.

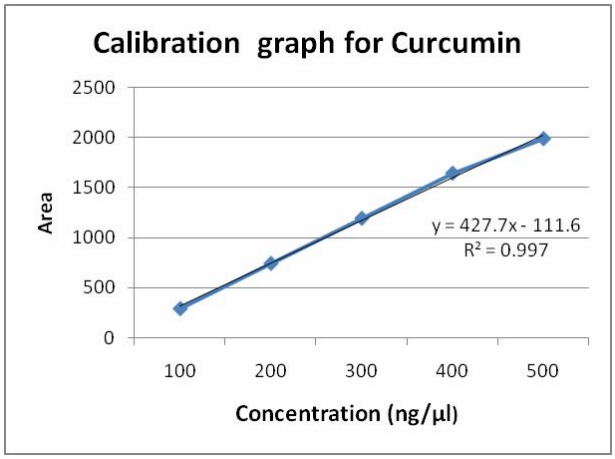

Figure 3: Calibration graph of curcumin

4.2.3 Precision and accuracy

The intra and inter day precision, as percentage relative standard deviation (% RSD) and accuracy of the assay were chosen to determine the precision of the developed assay at curcumin concentration of 5 ng, 100 ng and 500 ng/spot. Intra-day precision was validated with three concentrations: low, medium and high of standard solutions under the optimized conditions for four times within 1 day. Inter-day precision was validated with the standard solutions, used above once a day for 3 consecutive days. Inter- and Intra-day precisions for investigated component was expressed as percentage relative standard deviation (% RSD) and found less than 1.81% for curcumin as summarized in Table 3.

Table 3: Intra-day and inter-day precision

Concentration	Intra day		Inter day	
	Average (n = 4)	%RSD	Average (n = 4)	%RSD
5	0.45	1.94	0.45	1.81
100	294.60	1.19	294.85	1.02
500	1954.16	1.31	1966.16	0.80

4.2.4 Repeatability

The repeatability of the method was studied by assaying six samples of curcumin at same concentration under the optimized conditions. The values were within the acceptable range and so we concluded that the method was accurate, reliable and reproducible.

4.2.5 Specificity

The specificity of the proposed method was determined by comparing the sample and standard peak for its Rf and UV spectrum. A densitogram of *C. longa* L. extracts and sample extracts and UV spectrum of curcumin as shown in Figure 4. A good resolved curcumin spot was observed with Rf value 0.70 ± 0.05 in the chromatogram of the samples extracts as shown in Figure 5.

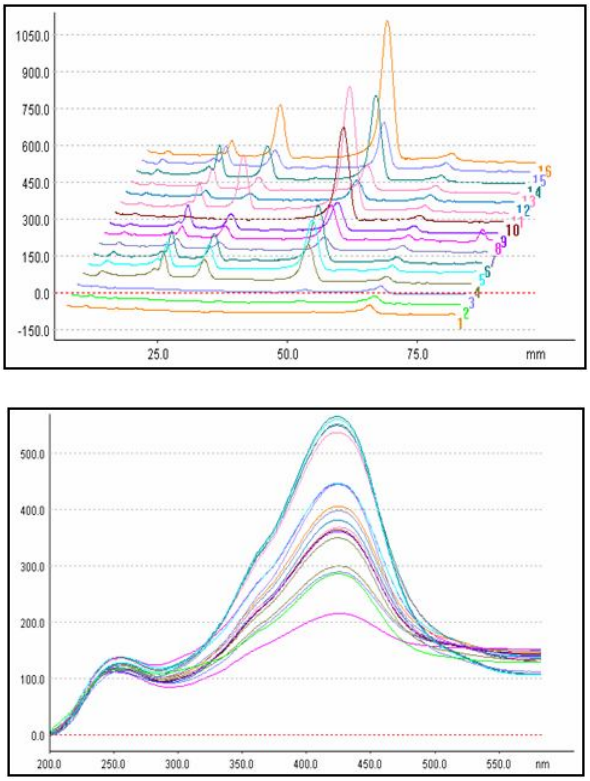

Figure 4: (A) Densitogram of *Curcuma* samples extracts 1-16. (B) Overlapping UV spectrum of curcumin standard and *Curcuma* samples and different solvent extracts.

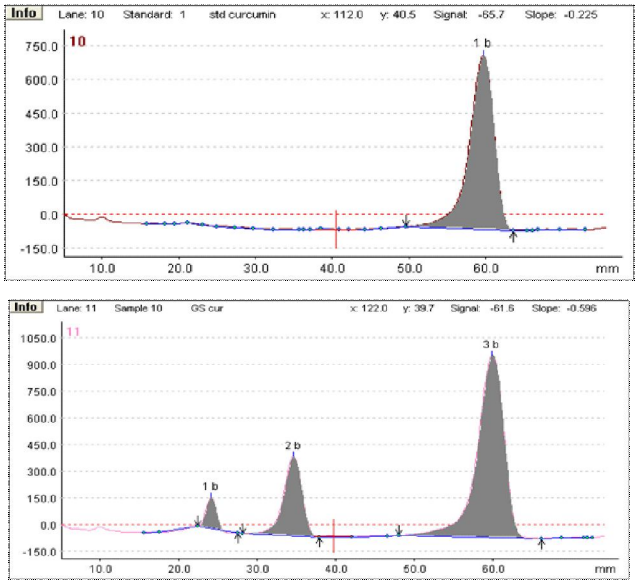

Figure 5: Densitogram of (A) *Curcumin standard*, (B) sample *C. longa* L. methanol extract.

4.2.6 Sample analysis

The established method was subsequently applied for determination of curcumin in *C. longa* L. different solvent extracts and samples methanolic extracts. All samples were analyzed under the optimized TLC conditions. Each sample was analyzed for amount of curcumin found in the samples and extracts as shown in Table 1 and Figure 6.

Table 1: Determination of curcumin in *C. longa* L. samples extracts

Determination of curcumin in *Curcuma longa* L. sample and extracts						
Sample name	**Amount of Powder sample taken (g)**	**Extract obtained (g)**	**Extract yield from powder (%w/w)**	**Area**	**Curcumin content in amount of extract applied (in µg)**	**Curcumin content in 100% extract (g%)**
Pure curcumin std	-	-	-	2847.10	2.0	-
Tph	100	4.00	4.00	0	0	0
Tkh	100	4.00	4.00	0	0	0
Tbth	100	2.00	2.00	0	0	0
Tphm	100	11.00	11.00	1028.96	0.723	3.976
Tkhm	100	8.00	8.00	1520.04	1.068	4.271
Tbthm	100	12.00	12.00	699.12	0.491	2.947
Tp meth	100	10.00	10.00	371.62	0.261	1.305
Tk meth	100	13.00	13.00	1033.15	0.726	4.718
Tbt meth	100	11.00	11.00	797.64	0.560	3.082
Tbtet	100	4.00	4.00	654.26	0.460	0.919
Tbtetxy	100	4.00	4.00	2284.39	1.605	3.210
CS meth sample 1	100	10.00	10.00	1507.7	1.059	5.296
TCap meth sample 2	5.0	4.97	99.4	296.08	0.208	0.052
CLA meth sample 3	100	8.00	8.00	1133.09	0.796	3.184
CLE meth sample 4	100	9.90	9.90	1712.22	1.203	5.954

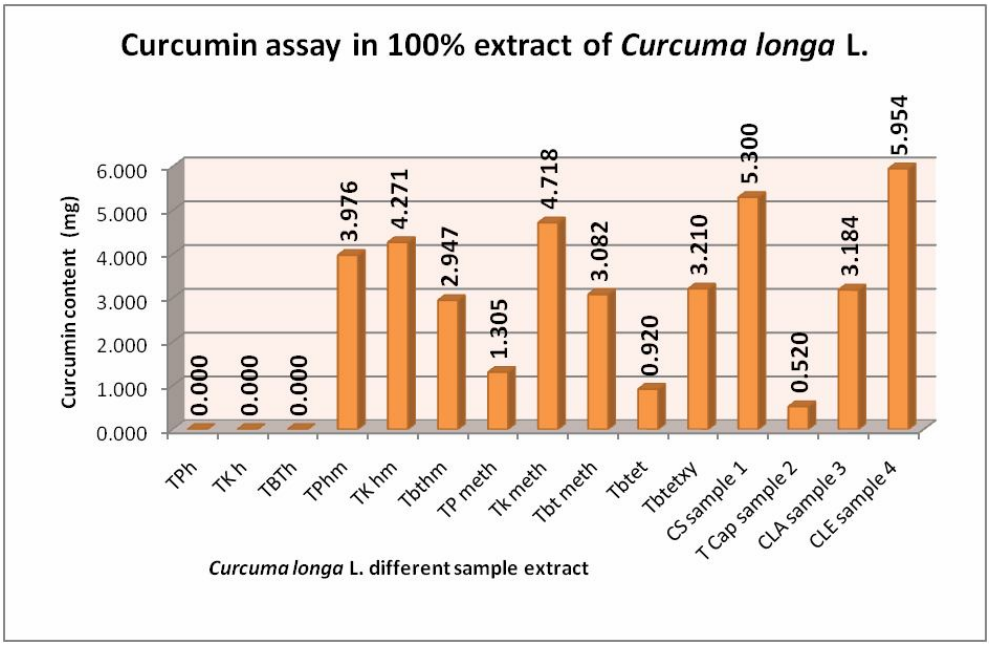

Figure 6: Curcumin assay in *C. longa* L. different solvent extracts.

5. Discussion and Conclusion

A validated HPTLC method has been developed for the determination of curcumin in *C. longa* L. different solvent extracts and samples extracts. The proposed method is simple, precise, specific, less time consuming and cost effective. The analysis proved that the method is evitable for the analysis of curcumin. The method was found suitable for rapid screening of plant materials, can be performed without any special sample pretreatment. HPTLC method will help the manufacturers in determining the quality and standardization of herbal formulations. Such method or fingerprinting is useful in extraction of curcumin from the turmeric and act as a biochemical marker for this medicinally important plant in the pharmaceutical industry. Thus, there is a lot of variation in the content of curcumin when it gets processed or extracted in different solvents, so a vital care is needed for any drug in the manufacturing process if curcumin content is required. Thus, successfully optimized method developed by HPTLC for identification and quantification of curcumin in *C. longa* different solvent extracts.

The present study revealed from turmeric different solvent extracts from *C. longa* L. showed high curcumin content by HPTLC assay in Turmeric secondary rhizome (Tkm) extracted with methanol (100 g of tkm powder yielded about 13 grams extract containing volatile essential oils, turmerones and curcuminoids). The percentage of curcumin in this extract was found to be 4.718 g% and the rest contains essential oil and turmerones. This indicates that the high amount of curcumin can be obtained through methanolic extract of tuber. Turmeric secondary rhizome (Tkhm) methanolic extract extracted after hexane extract (100 g powder yielded about 8 grams extract containing volatile essential oils, turmerones and curcuminoids) The percentage of curcumin in this extract was found to be 4.271 g% and the rest contains essential oil and turmerones. This indicates that the second high amount of curcumin can be obtained by turmeric methanol extract which was carried out after hexane extract. Similarly, turmeric tuber - ethanol ext followed by xylene wash (100 g Tkm powder yielded about 4 grams extract). The percentage of curcumin in this extract was found to be 3.210 g% followed by turmeric tuber - methanol extract (Tbt meth) with 3.082 g%. Similarly in other solvent extracts as shown in Table 1.

Three *C. longa* L. samples 1, 3 and 4 were extracted with methanol and found the curcumin content as 5.296 g%, 3.184 g%, 5.5954 g%, respectively with highest amount in sample 4. *C. longa* L. sample-2 is (Turmeric capsule of 500 mg containing (turmeric powder-400 mg, turmeric extract standardized to 95% curcuminiods - 50 mg, *Zingiber officinale* root powder-50 mg) found the curcumin content as 0.052 g% in 500 mg capsule, confirms the standardized extract containing with 95% Curcuminoids extract. Turmeric powder contains 5.5954 g% the best powder sample. It may be used for the usually recommended powder dose of 2 g per day.

Therefore, the present study helps to obtain the high curcumin content with HPTLC assay from turmeric secondary rhizome and turmeric tubers with methanolic/ethanolic extract. This whole extract contains curcumins, turmerones (essential oil from turmeric) will be more potent activity. Incorporating whole extract of turmeric which contains volatile oils, curcumins and turmerones into our daily regimen may be one of the best and simplest ways to keep as in good health. Our future formulations would be turmeric extract from tuber with daily dose of formulation (capsules or tablets) with equivalent amount of extract obtained from 2 to 3 gm turmeric powdered per day as a health promoting agent and efficacious with respect to the topical or oral formulations to provide the good efficacy in the desired formulation.

Conflict of interest

We declare that we have no conflict of interest.

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