

A HPTLC method for chemotaxonomic evaluation of some *Curcuma* Species and their commercial samples

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A simple, rapid, and selective HPTLC method has been established for separation and determination of curcumin, extracted from rhizomes of different *Curcuma* species, which are sold in herbal drug markets of India by the name of *Amraharidra*. Chromatography was performed on silica gel 60F₂₅₄ plates with chloroform: ethanol: acetic acid, 95: 4: 5 (v/v), as mobile phase. Detection and quantification were performed densitometrically at $\lambda_{\max} = 260$ nm with curcumin as external standard. This method was also found suitable for rapid screening of plant materials for chemotaxonomic.

Keywords: Curcumin, *Curcuma*, Densitometry, HPTLC

Introduction

Curcuma species (*Zingiberaceae*) are widely used as a traditional medicine for treatment of a variety of ailments – as a stomachic, calmate, bitter tonic for indigestion and liver disorder¹. Acetone extract of plant contains curcumin, an important diaryl heptanoids with multiple biological activities^{2,3}. High-performance thin-layer chromatography (HPTLC) is commonly used for identification, assay and purity testing, and for determination of stability, dissolution, and content uniformity of raw materials (herbal and animal extracts, fermentation mixtures, drugs and excipients) and formulated products (pharmaceuticals, cosmaceuticals, nutraceuticals). In HPTLC, densitometry has been used to test purity^{4,5}, for assay of pharmaceutical dosage forms⁶, and to obtain a chromatogram for herbal fingerprinting^{7,8}.

This study presents a simple, rapid and selective HPTLC method for separation and determination of *Curcuma* species, sold in Indian markets as *Amraharidra*.

Experimental

Plant Material, Reagents and Standard Solutions

Rhizomes, collected from Palghat of Kerala, India, were authenticated by NBRI, Lucknow (LWG 221248,

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CA; LWG 221249, CAr; LWG 221250, 1999 CZ), India and nine commercial samples procured from Bangalore, Mumbai, Delhi, Hyderabad, Lucknow, Nagpur, Poojapura, Pune, and Varanasi markets of India were also used for detailed evaluation. Crude rhizome samples were submitted to drug depository of NBRI, Lucknow. All reagents used were of analytical grade. Reagents used were from Merck (Germany) and standard curcumin (Fig. 1A) procured from Sigma-Aldrich (Steinheim, Germany).

Accurately weighed curcumin standard (1 mg) was dissolved in acetone (1 ml) to prepare a standard solution (1 mg/ml).

Sample Preparation

Powdered (100 mesh) dried rhizomes (5 g) were soaked in acetone (4 × 20 ml, each for 1 h). Extracts were combined, filtered, and evaporated to dryness by rotary evaporation. Accurately weighed acetone extract (10 mg) was dissolved in acetone (10 ml) to prepare a solution (1 mg/ml).

Chromatographic Conditions

Chromatography was performed on glass-backed HPTLC plates (20 cm × 10 cm) coated with 0.25 mm layers of silica gel Si 60 F₂₅₄ (E. Merck, Germany). Standard solution (2, 4, 6, 8 and 10 μ l) and sample extract solution (5 μ l) were applied to plates as bands

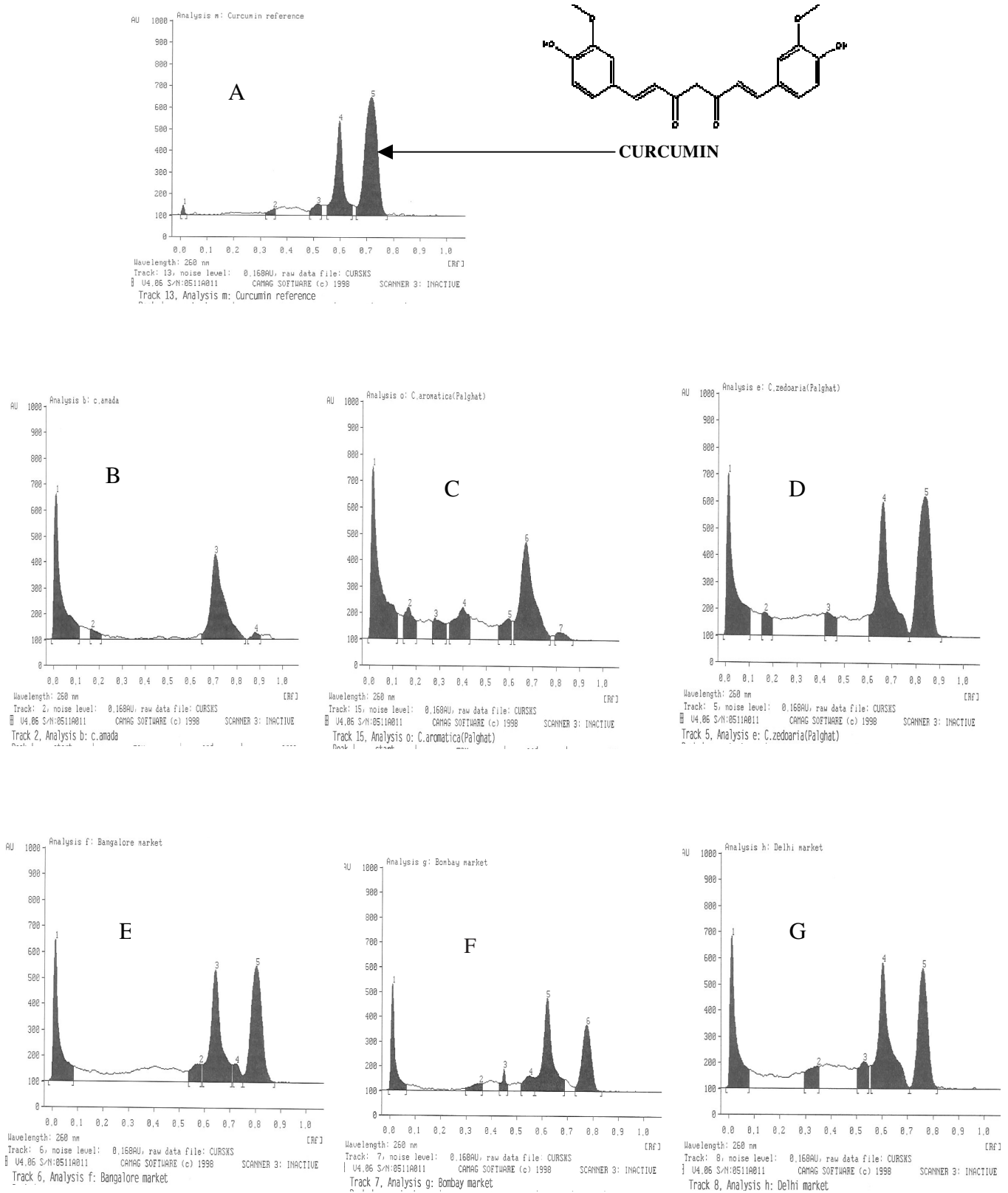


Fig. 1— Chromatograms of: A, Curcumin; B, *Curcuma amada*; C, *C. aromatic*; D, *C. zedoaria*; E, Bangalore sample; F, Bombay sample; G

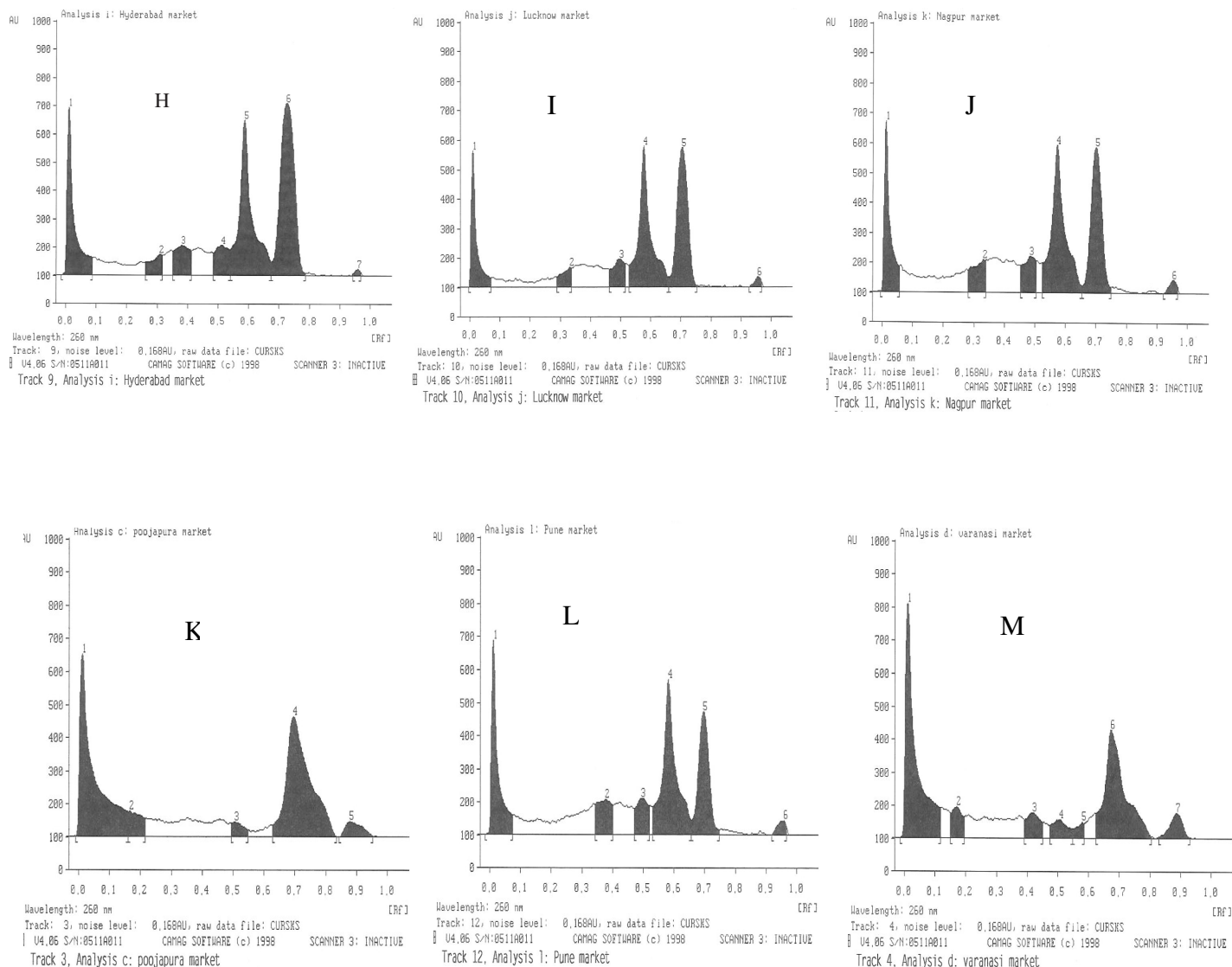


Fig. 1— Chromatograms of: A, Curcumin; B, *Curcuma amada*; C, *C. aromatica*; D, *C. zedoaria*; E, Bangalore sample; F, Bombay sample; G, Delhi sample; H, Hyderabad sample; I, Lucknow sample; J, Nagpur sample; K, Poojapura sample; L, Pune sample; M, Varanasi sample

(6 mm long and 8 mm apart) by using Camag Linomat IV (Switzerland) sample applicator equipped with a 100 μ l micro syringe.

Detection, Quantification and Calibration of Curcumin

Plates were developed to a distance of 80 mm, with chloroform: ethanol: acetic acid, (95: 4: 5, v/v), as mobile phase, in a Camag glass twin-trough chamber (20 cm \times 10 cm) previously saturated with mobile phase vapor; temperature was 27°C and relative humidity 37%. After removal of plates from chamber, completely dried in air at room temperature (27°C) and peak areas for samples and standard were recorded by densitometry in

absorbance/reflectance mode at $\lambda_{\text{max}} = 260$ nm, by means of a Camag TLC Scanner 3 with WINCATS version 4.06 software.

Validation Parameters

Scanning same spots five times checked precision of scanner and coefficient of variance was calculated. Repeatability of method was also established by applying 5 μ g per spot of each standard solution five times and coefficient of variance was calculated. Limit of detection (LOD) and limit of quantification (LOQ) were also determined.

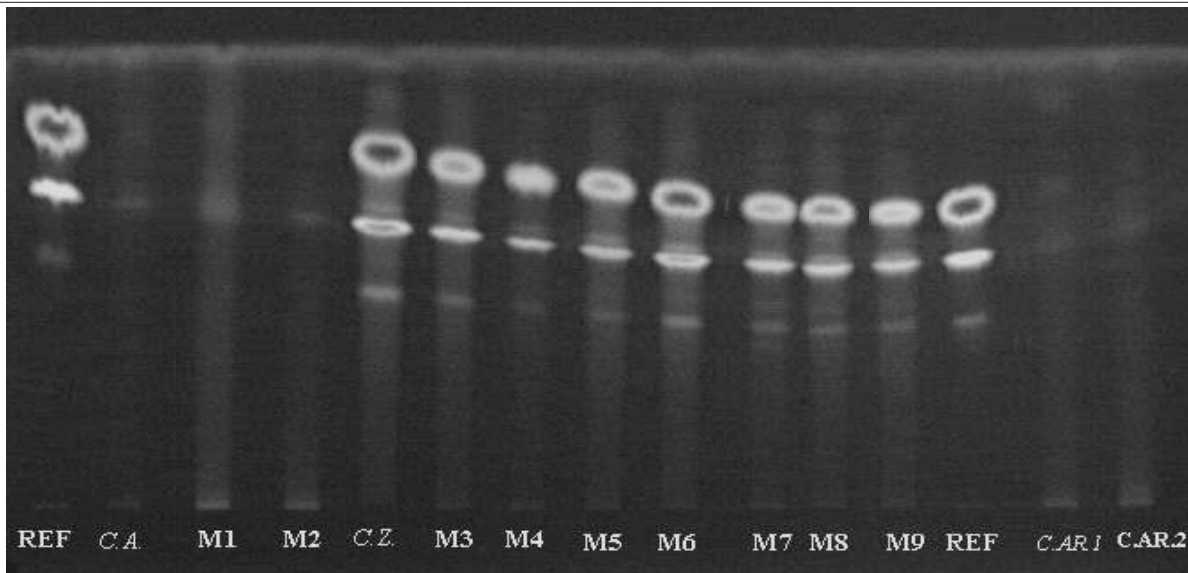


Fig. 2— HPTLC profile of different *Curcuma* rhizome samples (UV 366nm) [REF, Curcumin; CA, *Curcuma amada*; M1, Poojapura; M2, Varanasi; CZ, *C. Zedoaria*; M3, Bangalore; M4, Bombay; M5, Delhi; M6, Hyderabad; M7, Lucknow; M8, Nagpur; M9, Pune; CAR1, *C. aromatica*-Lucknow (UP); CAR2, *C. aromatica*-Palghat (Kerala)]

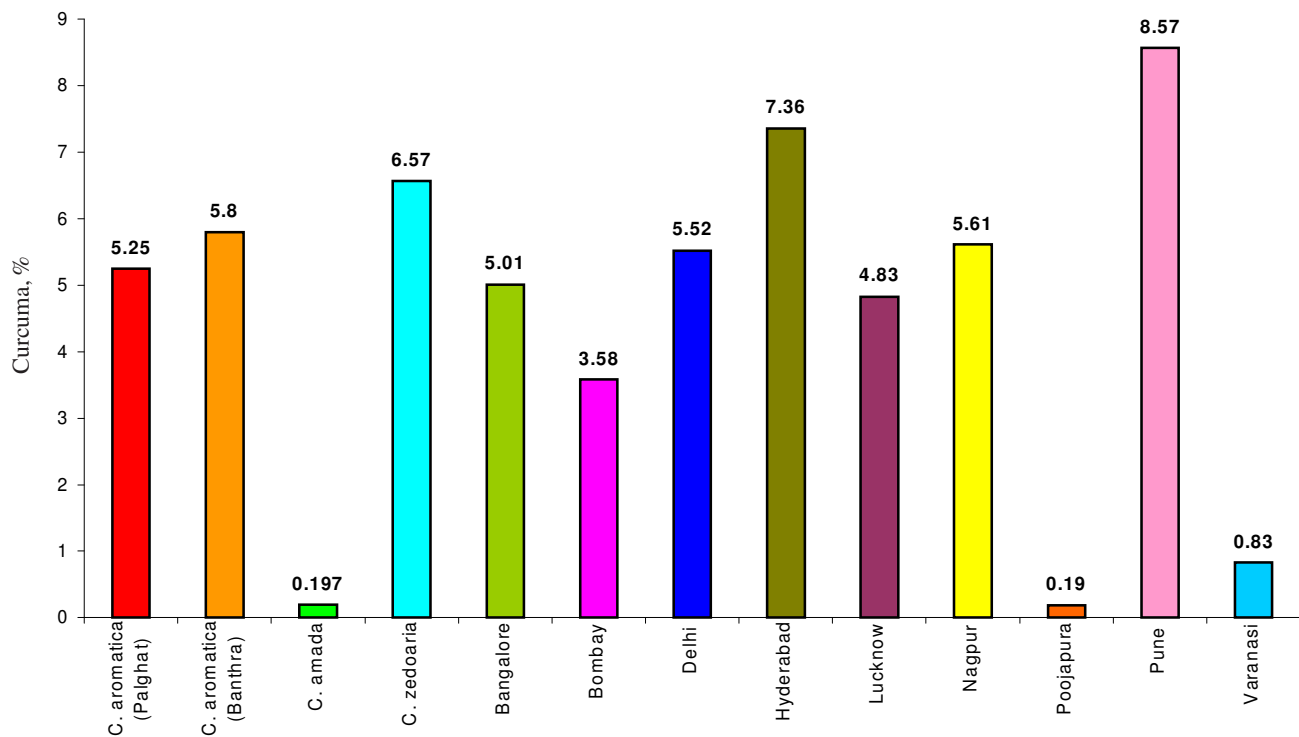


Fig. 3— Quantitative estimation of curcumin in different *Curcuma* species and market samples

Results and Discussion

Different compositions of mobile phase were checked and desired resolution of curcumin with reproducibility of peaks was achieved by using chloroform: ethanol: acetic acid, (95: 4: 5, v/v), as mobile phase. Under these conditions, R_f of curcumin was 0.75 and compound was well resolved from other components

of extract (Figs 1 & 2). Calibration curve (range, 1-20 μg) of curcumin was linear. Different values for curcumin determination were: R_f 0.75; regression equation, $y = 1185.456 + 3602.145x$; and r^2 , 0.943. Curcumin in rhizomes of *Curcuma* species and its market samples is also presented (Fig. 3).

Conclusions

Developed HPTLC method can be used for identification and quantitative determination of curcumin in *Curcuma* species. New method characterized by high sensitivity and linearity over a wide range of concentrations. This method is also suitable for rapid screening of plant materials for chemotypic assessment as well as quality control and batch to batch consistency in raw materials and can be performed without any special sample pretreatment.

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