



Specific Standardization and Determination of Curcuminoid Levels in Turmeric (*Curcuma longa*) Rhizomes from Three Cultivation Centers in Java Using the HPLC Method

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ABSTRACT

Curcuma longa is one of Indonesia's most important medicinal plants, with curcuminoids (curcumin, demethoxycurcumin, and bisdemethoxycurcumin) responsible for its anti-inflammatory, antioxidant, hepatoprotective, and anticancer activities. This study aimed to standardize turmeric rhizomes from Temanggung, Blitar, and Wonogiri and determine curcuminoid levels using a validated HPLC method. Specific standardization parameters were evaluated according to the Indonesian Herbal Pharmacopoeia (FHI) Edition II, while HPLC analysis employed a C18 reversed phase column with UV detection at 425 nm. All samples met FHI physicochemical standards, and the HPLC method showed excellent linearity ($r^2 \geq 0.9996$), precision (%RSD $\leq 0.95\%$), and accuracy (98.7–99.8%). Curcuminoid content differed significantly among cultivation sites ($p < 0.001$), with the highest level in Blitar ($4.48 \pm 0.09\%$), followed by Temanggung ($3.96 \pm 0.07\%$) and Wonogiri ($3.39 \pm 0.06\%$). Curcumin levels ranged from 2.46–3.19% w/w, exceeding the FHI minimum standard ($\geq 1.0\%$). These findings demonstrate that cultivation site significantly influences curcuminoid profiles, emphasizing the importance of region specific quality specifications for herbal raw material procurement.

Keywords: *Curcuma Longa*, Curcuminoids, HPLC, Crude Drug Standardization, Geographical Variation, Java

INTRODUCTION

Curcuma longa L. (Family Zingiberaceae), commonly known as turmeric, is a rhizomatous herbaceous perennial native to South and Southeast Asia and one of the world's most extensively cultivated and traded spice medicinal plants. Indonesia ranks among the top global producers, with cultivation concentrated across Java, Sumatra, and Kalimantan. The rhizome has been used for centuries in Ayurvedic and traditional Indonesian (*jamu*) medicine to treat digestive disorders, inflammatory conditions, hepatic dysfunction, and skin diseases. In recent decades, turmeric has become one of the most studied natural products in global phytomedicine, driven by the broad pharmacological portfolio of its principal bioactive constituents.

The bioactive constituents responsible for the characteristic yellow orange color of turmeric rhizomes are the curcuminoids, a group of polyphenolic pigments. Three curcuminoids are recognized: (1) curcumin (diferuloylmethane), comprising approximately 70–80% of the total fraction; (2) demethoxycurcumin (4 hydroxycinnamoyl feruloylmethane), at 15–25%; and (3) bisdemethoxycurcumin (bis-4-hydroxycinnamoylmethane), the minor component at 1–10%. Structurally, these 1,7-diaryl-1,6-heptadiene-3,5-diones are differentiated by the degree of methoxylation on their phenolic rings. Their shared β -diketone scaffold enables metal chelation, scavenging of reactive oxygen species, and inhibition of multiple inflammatory signaling cascades.

The pharmacological profile of curcuminoids encompasses anti inflammatory activity through inhibition of NF κ B and COX-2 pathways, induction of apoptosis in cancer cell lines, modulation of the Nrf2-antioxidant response element pathway, suppression of amyloid aggregation in Alzheimer's disease models, and broad spectrum antimicrobial activity (Aggarwal et al., 2007; Chattopadhyay et al., 2004). Despite extensive pre clinical documentation, clinical translation has been hampered by poor aqueous solubility, rapid metabolism, and limited systemic bioavailability—driving active research into nanoformulation and novel delivery system development (Anand et al., 2007; Lopresti, 2018).

Quality assurance of turmeric raw material is a prerequisite for the reproducibility of biological effects and the safety of herbal preparations. The curcuminoid content of turmeric rhizomes varies considerably depending on genotype, agroclimatic conditions (altitude, temperature, rainfall, soil composition), agricultural practices, and harvest maturity. In Indonesia, quality standards are defined in the *Farmakope Herbal Indonesia* (FHI) and *Materia Medika Indonesia*, specifying a minimum curcuminoid content of 1.0% (w/w)—a threshold that does not capture the geographic diversity of domestic production.

High Performance Liquid Chromatography (HPLC) is the gold standard technique for simultaneous quantification of individual curcuminoids, offering superior selectivity and precision compared to spectrophotometric methods (Jayaprakasha et al., 2002). HPLC based methods are adopted in the United States Pharmacopeia (USP), European Pharmacopoeia (Ph. Eur.), and British Pharmacopoeia (BP). Despite this, comprehensive comparative HPLC data integrated with specific standardization parameters across major Javanese cultivation centers have not been systematically reported.



This study was designed to: (1) perform specific standardization of turmeric rhizomes from Temanggung, Blitar, and Wonogiri, encompassing organoleptic, macroscopic, microscopic, and physicochemical parameters; (2) develop and validate an HPLC method for simultaneous determination of all three curcuminoids per ICH Q2(R1); (3) compare curcuminoid profiles across cultivation sites by statistical analysis; and (4) generate a regional dataset to support development of more comprehensive national *Curcuma longa* crude drug monographs.

METHODS

1. Plant Material and Botanical Identification

Fresh turmeric rhizomes (*Curcuma longa* L.) were collected from three geographically distinct cultivation centers: Temanggung Regency (altitude 800–1,000 m asl, volcanic andosol soil, Central Java), Blitar Regency (altitude 150–250 m asl, andosol from Kelud volcanic deposits, East Java), and Wonogiri Regency (altitude 300–500 m asl, clay loam lithosol soil, Central Java). Sampling was conducted in March 2024, at 9–10 months post planting (optimal harvest maturity). Each site was represented by three independent batch replicates from geographically separated plots within the same site. Botanical identification was performed at the Herbarium Bogoriense, National Research and Innovation Agency (BRIN), Bogor, and voucher specimens were deposited under accession numbers HB 2024-0127 (Temanggung), HB-2024-0128 (Blitar), and HB-2024-0129 (Wonogiri).

2. Chemicals and Instruments

Reference standards of curcumin (purity 98.5%), demethoxycurcumin (98.2%), and bisdemethoxycurcumin (97.8%) were obtained from Sigma-Aldrich (St. Louis, MO, USA). HPLC-grade acetonitrile, glacial acetic acid (pro analysis, 100%), and methanol were purchased from Merck KGaA (Darmstadt, Germany). Ultrapure water was prepared in house using a Millipore Milli Q system. Instrumentation included a Shimadzu LC-20A HPLC system (Shimadzu Corporation, Kyoto, Japan) equipped with an LC-20AD pump, SIL-20AC autosampler, and SPD-M20A photodiode array (DAD) detector. Additional equipment included a Mettler Toledo XS205 analytical balance, Branson 5800 ultrasonic bath, Nabertherm L 3/11 muffle furnace, Buchi R-300 rotary evaporator, and Olympus CX43 binocular microscope.

3. Preparation of Crude Drug

Fresh rhizomes were washed, peeled, sliced transversely to approximately 2 mm thickness, and air dried at 27–30°C for 48 hours, followed by oven drying at 50°C to constant weight (moisture ≤10%). Dried rhizomes were pulverized and sieved through a No. 40 mesh (425 µm) to yield fine grade powdered crude drug. Powders were stored in sealed amber glass containers at room temperature, protected from light and moisture.

4. Specific Standardization

Specific standardization followed the general principles of the WHO Guidelines for Herbal Drug Standardization (WHO, 2011) and specific methods described in *Farmakope Herbal Indonesia* (FHI) Edition II (BPOM RI, 2020). Parameters assessed included: (a) organoleptic evaluation (form, color, odor, taste); (b) macroscopic examination (10× hand lens); (c) powder microscopy at 40–400× magnification; (d) moisture content (gravimetric, 105°C, 5 h); (e) total ash (600°C, 3 h); (f) acid-insoluble ash (10% HCl); (g) water-soluble extractable matter (cold maceration, 24 h); and (h) ethanol-soluble extractable matter (96% ethanol, cold maceration, 24 h).

5. Standard and Sample Solution Preparation

Stock standard solutions (1,000 µg/mL) of each curcuminoid were prepared in HPLC-grade methanol. Working standards were prepared by serial dilution to concentration series of 0.5, 1.25, 2.5, 5.0, 10.0, 15.0, 20.0, and 25.0 µg/mL (curcumin) and 0.5–20.0 µg/mL (demethoxycurcumin and bisdemethoxycurcumin) in triplicate.

For sample preparation, 100.0 mg of powdered crude drug was extracted with 20 mL methanol by ultrasonic bath (30 min, 25°C), centrifuged (3,000 rpm, 10 min), and the residue re-extracted twice with 2.5 mL methanol. Combined supernatants were made up to 25.0 mL with methanol and filtered through 0.45 µm nylon membrane prior to injection. Each sample batch was analyzed in triplicate ($n = 3$ independent extractions per batch), and each extract was injected twice, with the mean value used for quantification.

6. HPLC Method Validation

The HPLC method was validated per ICH Q2(R1) (ICH, 2005) for: (a) Specificity DAD spectral overlay at 350–550 nm; (b) Linearity eight-point calibration curves, assessed by linear regression (r^2); (c) LOD and LOQ calculated as $3.3\sigma/S$ and $10\sigma/S$, respectively; (d) Precision six replicate injections of a mid range standard (%RSD); (e) Accuracy standard addition at 80%, 100%, 120% of nominal sample concentration ($n = 3$ per level), expressed as % recovery; and (f) Robustness—assessed by small deliberate variations in mobile phase composition ($\pm 2\%$ acetonitrile), flow rate (± 0.1 mL/min), and column temperature ($\pm 2^\circ\text{C}$).

7. Statistical Analysis

Inter site differences in curcuminoid content were evaluated by one way ANOVA ($\alpha = 0.05$). Significant ANOVA results were followed by Tukey's HSD post hoc test. The primary statistical analysis was performed on batch means ($n = 3$ per site) to avoid pseudoreplication. All analyses were performed with IBM SPSS Statistics version 26.0. Data are expressed as mean $\pm$ SD.



RESULTS

1. Specific Standardization

Organoleptic examination revealed characteristic but regionally differentiated profiles. Blitar samples exhibited the most intensely orange yellow coloration, consistent with their higher curcuminoid content. Temanggung samples showed deep yellow-orange hues, while Wonogiri samples appeared paler. All samples displayed the characteristic pungent-aromatic odor and slightly bitter, spicy taste typical of turmeric, confirming species-level identity.

Powder microscopy confirmed species identity across all samples. Diagnostic features observed included: oval starch grains with a central hilum (10–35 μm diameter), parenchyma cells containing yellow-brown oleoresin droplets (the curcuminoid-bearing cells), spiral and reticulate vascular bundles, thin-walled parenchymatous cells, and prismatic calcium oxalate crystals. No adulteration or foreign organic matter was detected by microscopy.

Physicochemical results are presented in Table 5. Moisture content (7.85–9.04%) satisfied the FHI requirement of $\leq 10\%$. Total ash (5.97–6.72%) was within the FHI limit of $\leq 8\%$, indicating acceptable mineral content without excessive soil contamination. Acid-insoluble ash (0.74–0.91%) was well below the FHI ceiling of $\leq 1\%$, confirming adequate field cleaning. Ethanol-soluble extractable matter (16.34–19.26%) followed the same rank order as total curcuminoid content (Blitar > Temanggung > Wonogiri), serving as a useful surrogate predictor for curcuminoid level in routine quality control. All standardization parameters for all three sites satisfied FHI requirements.

Table 1. Physicochemical Standardization Parameters of Turmeric Crude Drug from Three Cultivation Centers in Java

Parameter	Temanggung	Blitar	Wonogiri	FHI Limit
Moisture content (%)	8.21 ± 0.14	7.85 ± 0.22	9.04 ± 0.18	$\leq 10\%$
Total ash (%)	6.34 ± 0.11	5.97 ± 0.15	6.72 ± 0.13	$\leq 8\%$
Acid-insoluble ash (%)	0.82 ± 0.04	0.74 ± 0.03	0.91 ± 0.05	$\leq 1\%$
Water-soluble extractable (%)	11.53 ± 0.28	12.14 ± 0.31	10.87 ± 0.24	$\geq 10\%$
Ethanol-soluble extractable (%)	17.82 ± 0.35	19.26 ± 0.42	16.34 ± 0.38	$\geq 15\%$
Total curcuminoids (%)	3.96 ± 0.07	4.48 ± 0.09	3.39 ± 0.06	$\geq 1.0\%$

Note: Data expressed as mean $\pm$ SD ($n = 3$ batches). FHI = Farmakope Herbal Indonesia. Values expressed as % w/w on dry-weight basis.

2. HPLC Method Development and Validation

Mobile phase optimization evaluated several solvent systems; the isocratic system of acetonitrile:1% acetic acid (50:50, v/v) was selected for superior peak symmetry, optimal resolution ($R > 4.97$ for all peak pairs), and stable baseline over 30 minutes. Acetic acid at 1% suppressed ionization of the phenolic hydroxyl groups of curcuminoids, improving retention and reducing peak tailing. Detection at 425 nm corresponds to the UV absorption maximum of the conjugated diketone chromophore common to all three curcuminoids, providing maximal sensitivity.

The optimized HPLC operational conditions are presented in Table 2. The elution order (bisdemethoxycurcumin → demethoxycurcumin → curcumin; Table 3) is consistent with increasing lipophilicity driven by methoxy substitution: bisdemethoxycurcumin (no methoxy groups) is most polar with lowest C18 affinity; curcumin (two methoxy groups) is most lipophilic. Capacity factors (k' = 1.82–4.71) and resolution values (R = 4.97–5.28) confirmed adequate chromatographic separation. Tailing factors (1.04–1.07) indicated excellent peak symmetry

Table 2. Optimized HPLC Operational Conditions for Simultaneous Curcuminoid Determination

HPLC Parameter	Operational Condition
Column	C18 reversed-phase (250 mm × 4.6 mm, 5 μ m)
Mobile Phase	Acetonitrile : 1% Acetic acid (50:50, v/v), isocratic
Flow Rate	1.0 mL/min
Detection Wavelength	425 nm (UV-Vis DAD)
Injection Volume	20 μ L
Column Temperature	30°C
Run Time	30 minutes
Quantification Method	External standard calibration

Table 3. Chromatographic Parameters for Curcuminoids Under Optimized HPLC Conditions

Compound	tR (min)	Capacity Factor (k')	Resolution (R)	Tailing Factor
Bisdemethoxycurcumin	9.41 ± 0.05	1.82	–	1.04
Demethoxycurcumin	14.63 ± 0.06	3.22	5.28	1.07
Curcumin	19.87 ± 0.08	4.71	4.97	1.06

Validation results are summarized in Table 2. Coefficients of determination (r^2 : 0.9996–0.9998) exceeded the ICH threshold of ≥ 0.999 for all analytes. LOD values (0.08–0.13 μ g/mL) and LOQ values (0.25–0.42 μ g/mL) confirmed adequate sensitivity for low-content samples. Intra-day precision (%RSD: 0.72–0.95%) was well within the ICH acceptance criterion of $\leq 2\%$. Accuracy (recovery: 98.7–99.8%) was within the accepted range of 98–102% at all spiking levels. These validation data confirm the method is fit for purpose for routine quantification of curcuminoids in turmeric crude drug.

Table 5. Summary of HPLC Method Validation Parameters for Simultaneous Curcuminoid Determination

Sample	Origin	Curcumin (%)	Demethoxycurcumin (%)	Bisdemethoxycurcumin (%)	Total Curcuminoids (%)
T-1	Temanggung	2.83 ± 0.04	0.71 ± 0.03	0.42 ± 0.02	3.96 ± 0.05



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T-2	Temanggung	2.79 ± 0.03	0.69 ± 0.02	0.39 ± 0.01	3.87 ± 0.04
T-3	Temanggung	2.85 ± 0.05	0.73 ± 0.04	0.44 ± 0.03	4.02 ± 0.07
Mean ± SD	Temanggung	2.82 ± 0.03	0.71 ± 0.02	0.42 ± 0.03	3.95 ± 0.08
B-1	Blitar	3.14 ± 0.06	0.88 ± 0.03	0.51 ± 0.02	4.53 ± 0.07
B-2	Blitar	3.19 ± 0.04	0.91 ± 0.02	0.53 ± 0.03	4.63 ± 0.06
B-3	Blitar	3.11 ± 0.05	0.86 ± 0.04	0.49 ± 0.02	4.46 ± 0.07
Mean ± SD	Blitar	3.15 ± 0.04	0.88 ± 0.03	0.51 ± 0.02	4.54 ± 0.09
W-1	Wonogiri	2.46 ± 0.03	0.58 ± 0.02	0.33 ± 0.01	3.37 ± 0.04
W-2	Wonogiri	2.51 ± 0.04	0.61 ± 0.03	0.35 ± 0.02	3.47 ± 0.06
W-3	Wonogiri	2.48 ± 0.03	0.59 ± 0.02	0.34 ± 0.01	3.41 ± 0.04
Mean ± SD	Wonogiri	2.48 ± 0.03	0.59 ± 0.02	0.34 ± 0.01	3.42 ± 0.05
Overall Mean ± SD	All Sites	2.82 ± 0.29	0.73 ± 0.13	0.42 ± 0.08	3.97 ± 0.47

Note: LOD = Limit of Detection; LOQ = Limit of Quantification; %RSD = Relative Standard Deviation. Acceptance criteria per ICH Q2(R1).

3. Curcuminoid Content Across Three Cultivation Centers

Individual and mean curcuminoid contents are presented in Table 1. Curcumin was consistently the most abundant curcuminoid (71–72% of total), followed by demethoxycurcumin (18–20%) and bisdemethoxycurcumin (10–11%), consistent with previously reported ratios for turmeric grown in Southeast Asia.

Table 6. Curcuminoid Content (% w/w, dry weight basis) in Powdered Turmeric Crude Drug from Three Cultivation Centers in Java

Sample	Origin	Curcumin (%)	Demethoxycurcumin (%)	Bisdemethoxycurcumin (%)	Total Curcuminoids (%)
T-1	Temang gung	2.83 ± 0.04	0.71 ± 0.03	0.42 ± 0.02	3.96 ± 0.05
T-2	Temang gung	2.79 ± 0.03	0.69 ± 0.02	0.39 ± 0.01	3.87 ± 0.04
T-3	Temang gung	2.85 ± 0.05	0.73 ± 0.04	0.44 ± 0.03	4.02 ± 0.07
B-1	Blitar	3.14 ± 0.06	0.88 ± 0.03	0.51 ± 0.02	4.53 ± 0.07
B-2	Blitar	3.19 ± 0.04	0.91 ± 0.02	0.53 ± 0.03	4.63 ± 0.06

B-3	Blitar	3.11 ± 0.05	0.86 ± 0.04	0.49 ± 0.02	4.46 ± 0.07
W-1	Wonogiri	2.46 ± 0.03	0.58 ± 0.02	0.33 ± 0.01	3.37 ± 0.04
W-2	Wonogiri	2.51 ± 0.04	0.61 ± 0.03	0.35 ± 0.02	3.47 ± 0.06
W-3	Wonogiri	2.48 ± 0.03	0.59 ± 0.02	0.34 ± 0.01	3.41 ± 0.04
Mean	All sites	2.82 ± 0.29	0.73 ± 0.13	0.42 ± 0.08	3.97 ± 0.47

Note: T = Temanggung; B = Blitar; W = Wonogiri; 1–3 denote independent batch replicates. Data expressed as mean ± SD ($n = 3$ analytical replicates per batch, % w/w dry basis). Total curcuminoids = curcumin + demethoxycurcumin + bisdemethoxycurcumin.

Curcumin content was highest in Blitar (range 3.11–3.19%, mean $3.15 \pm 0.04\%$), intermediate in Temanggung (2.79–2.85%, mean $2.82 \pm 0.03\%$), and lowest in Wonogiri (2.46–2.51%, mean $2.48 \pm 0.03\%$). An identical rank order was observed for demethoxycurcumin and bisdemethoxycurcumin. Total curcuminoid content followed the same pattern: Blitar ($4.48 \pm 0.09\%$) > Temanggung ($3.96 \pm 0.07\%$) > Wonogiri ($3.39 \pm 0.06\%$).

One-way ANOVA revealed highly significant inter-site differences for all four response variables (all $p < 0.001$; Table 6). Tukey's HSD post hoc test confirmed that all pairwise site comparisons were statistically significant ($p < 0.05$). The magnitude of variation in total curcuminoids across sites reached approximately 32% (relative difference between Blitar and Wonogiri), underscoring the agronomic significance of cultivation site selection for raw material quality.

Table 7. One-Way ANOVA and Tukey HSD Post Hoc Test Results for Curcuminoid Content Across Three Cultivation Sites

Analyte	F-value	p-value	df	Tukey's HSD Grouping
Curcumin	187.43	< 0.001	2, 24	B > T > W*
Demethoxycurcumin	142.18	< 0.001	2, 24	B > T > W*
Bisdemethoxycurcumin	98.56	< 0.001	2, 24	B > T > W*
Total curcuminoids	201.74	< 0.001	2, 24	B > T > W*

Note: B = Blitar; T = Temanggung; W = Wonogiri. * All pairwise comparisons significant at $p < 0.05$ by Tukey HSD. df = degrees of freedom (between groups, within groups).

The higher curcuminoid content observed in Blitar samples may be influenced by several converging environmental factors. First, the lower altitude of Blitar (150–250 m asl) is associated with higher solar irradiance and UV-B exposure, which are known to stimulate phenylpropanoid biosynthesis the metabolic pathway responsible for curcuminoid production as part of the plant's photoprotective response. Second, the volcanic andosol soils of Blitar, enriched with organic matter (3.8–4.2%) and micronutrients from Kelud volcanic deposits, may provide superior soil fertility compared to the clay-loam lithosols of Wonogiri. Temanggung's intermediate content may reflect



the moderating influence of its higher altitude: despite nutrient-rich Sindoro-Sumbing volcanic andosol soils, lower mean temperatures may limit biosynthetic enzyme activity and curcuminoid flux.

All samples from all three sites substantially exceeded the FHI minimum ($\geq 1.0\%$ total curcuminoids), with actual values ranging from 3.37% to 4.63% (w/w, dry basis). These values are consistent with USP standards for standardized turmeric extract ($\geq 3.0\%$ curcumins) and are comparable to or exceed reported values for turmeric from other high-quality production regions in South and Southeast Asia (1.8–4.1% curcumin). This confirms that Java constitutes a premium production environment for high-curcuminoid turmeric.

4. Implications for National Standardization and Traceability

The significant geographic variation in curcuminoid content documented here (up to 32% relative difference in total curcuminoid between Blitar and Wonogiri) has important practical implications for standardization of turmeric-based herbal preparations. The current FHI threshold of $\geq 1.0\%$ total curcuminoids, while appropriate as a minimum floor, fails to differentiate between raw materials of substantially different potency. Manufacturers of fixed-dose phytopharmaceuticals requiring consistent curcuminoid input would benefit from region-specific quality specifications and a tiered raw material classification system.

The distinct HPLC curcuminoid fingerprint profiles generated for each site offer a basis for geographical origin determination of Indonesian turmeric. Chemometric analysis of HPLC fingerprints using principal component analysis (PCA) or linear discriminant analysis (LDA), combined with essential oil GC-MS profiling or stable isotope ratio analysis, could enable robust authentication and anti-adulteration verification. Integration of these standardization data into a national herbal raw material database (linked to BPOM) would support comprehensive traceability from cultivation site to finished product, aligned with Indonesia's *Saintifikasi Jamu* strategy and international GACP guidelines for medicinal plants.

DISCUSSION

The results of this study indicate that turmeric crude drug from Temanggung, Blitar, and Wonogiri had distinct but acceptable quality characteristics. Organoleptic examination showed clear differences in color intensity among the samples, with Blitar exhibiting the most intense orange-yellow color, Temanggung showing a deep yellow-orange color, and Wonogiri appearing paler. This color variation is consistent with differences in curcuminoid content, since curcuminoids are the main pigments responsible for the yellow to orange coloration of turmeric (Priyadarsini, 2014). At the same time, all samples retained the characteristic pungent-aromatic odor and slightly bitter, spicy taste of turmeric, confirming their botanical identity as *Curcuma longa* L.

Microscopic examination further supported the authenticity of the samples. The observation of oval starch grains with a central hilum, parenchyma cells containing yellow-brown oleoresin droplets, spiral and reticulate vascular bundles, thin-walled parenchymatous cells, and prismatic

calcium oxalate crystals are typical diagnostic features of turmeric rhizome powder (Suhendi et al., 2019). The absence of foreign organic matter or adulterants indicates that all samples were properly processed and free from significant contamination. These microscopic findings are important because they confirm species identity at the crude drug level before chemical standardization is carried out.

The physicochemical parameters also showed that all three samples met the FHI requirements (BPOM RI, 2020). Moisture content ranged from 7.85% to 9.04%, which is below the maximum limit of 10%, indicating adequate drying and low risk of microbial growth during storage. Total ash values ranged from 5.97% to 6.72%, still within the acceptable limit of 8%, suggesting that inorganic contamination such as soil and sand was not excessive. Acid-insoluble ash values were very low, ranging from 0.74% to 0.91%, which confirms that the rhizomes had been sufficiently cleaned before processing. In addition, both water-soluble and ethanol-soluble extractable matters were within the FHI standard, indicating good quality of the crude drug and an adequate amount of extractable constituents.

Among the physicochemical parameters, ethanol-soluble extractable matter showed the same trend as total curcuminoid content, namely Blitar > Temanggung > Wonogiri. This suggests that ethanol-soluble extractable matter may serve as a useful preliminary indicator of curcuminoid richness in routine quality control. Because curcuminoids are relatively more soluble in ethanol than in water, the ethanol-soluble fraction is logically associated with higher levels of these bioactive compounds (Wichitnithad et al., 2009). Therefore, the similarity in ranking between ethanol-soluble extractive value and total curcuminoid content strengthens the usefulness of this parameter as a rapid screening tool for raw material quality.

The HPLC method developed in this study showed excellent performance for simultaneous determination of curcumin, demethoxycurcumin, and bisdemethoxycurcumin. The optimized mobile phase, acetonitrile and 1% acetic acid in a 50:50 ratio, produced good peak symmetry, stable baseline, and sufficient resolution between the three analytes. The addition of acetic acid helped reduce ionization of the phenolic hydroxyl groups, thereby improving chromatographic behavior and minimizing tailing (Jayaprakasha et al., 2002). Detection at 425 nm was appropriate because it corresponds to the maximum UV absorbance of the conjugated system shared by curcuminoids, allowing sensitive and selective detection.

The elution order of bisdemethoxycurcumin, demethoxycurcumin, and curcumin followed their increasing lipophilicity. Bisdemethoxycurcumin, which has no methoxy group, is the most polar compound and therefore elutes first on the C18 column. Curcumin, with two methoxy groups, is more lipophilic and retained longer (Lechtenberg et al., 2004). The capacity factor, resolution values, and tailing factors all indicate that the chromatographic separation was adequate and the method was suitable for quantitative analysis. In practical terms, this means the method can reliably distinguish the three major curcuminoids in turmeric crude drug.

The validation results confirmed that the HPLC method was reliable, precise, and accurate. The coefficients of determination were very high, showing excellent linearity across the tested



concentration ranges. The low LOD and LOQ values indicate that the method is sensitive enough for low-content samples. Precision values were below 1%, which is far better than the accepted maximum limit of 2%, while recovery values remained within the acceptable range of 98%–102%. Robustness testing confirmed that the method was not significantly affected by small deliberate variations in mobile phase composition, flow rate, or column temperature. These results demonstrate that the method is fit for routine laboratory application and has sufficient analytical robustness for quality control of turmeric materials.

The quantification results revealed a clear geographical effect on curcuminoid content. Blitar consistently showed the highest levels of curcumin, demethoxycurcumin, bisdemethoxycurcumin, and total curcuminoids, followed by Temanggung, while Wonogiri showed the lowest values. Statistical analysis confirmed that all differences among sites were highly significant. This indicates that cultivation site has a strong influence on curcuminoid biosynthesis and accumulation in turmeric rhizomes. The consistent rank order across all analytes also suggests that the environmental factors affecting one curcuminoid likely affect the others in a similar way. These findings are consistent with previous reports on geographical variation in curcuminoid content across different agroclimatic zones in India (Bhatt et al., 2020) and Bangladesh (Zaman et al., 2020).

The higher curcuminoid content observed in Blitar samples may be influenced by favorable environmental conditions. Its lower altitude likely provides stronger solar irradiation and higher UV-B exposure, which can stimulate secondary metabolite synthesis as a form of plant defense (Kusbiantoro & Purwaningrum, 2018). In addition, volcanic andosol soil enriched with organic matter and micronutrients may support better plant growth and metabolite production. Temanggung showed intermediate values, which may reflect a balance between fertile volcanic soil and cooler temperatures at higher altitude. Meanwhile, the lower levels in Wonogiri may be associated with less favorable soil fertility and environmental conditions for curcuminoid accumulation. However, further studies with detailed soil and climate characterization are needed to confirm these relationships.

All samples still exceeded the FHI minimum requirement of 1.0% total curcuminoids, with actual values ranging from 3.39% to 4.63%. This shows that turmeric from all three cultivation centers is suitable as herbal raw material. However, the observed variation of approximately 32% between the highest and lowest total curcuminoid values indicates that the current minimum standard alone does not fully capture differences in potency. For industries producing standardized herbal medicines or phytopharmaceuticals, such variation is highly relevant because consistency of active compound content is critical for product quality and therapeutic reproducibility (Garg et al., 2018).

These findings have important implications for national standardization and traceability. The distinct HPLC profiles obtained from each cultivation center can serve as chemical fingerprints for geographical origin identification. When combined with chemometric analysis, these profiles may help differentiate turmeric based on origin and detect adulteration or mislabeling. In the long term, integration of such data into a national herbal raw material database would support stronger



traceability from cultivation site to finished product. This would contribute to better quality assurance systems for Indonesian herbal raw materials and support the development of higher-value turmeric-based products.

CONCLUSIONS

This study successfully developed and validated an HPLC method for simultaneous quantification of curcumin, demethoxycurcumin, and bisdemethoxycurcumin in turmeric crude drug, fully compliant with ICH Q2(R1). The method demonstrated excellent linearity, precision, accuracy, and robustness. All nine sample batches from the three Javanese cultivation centers satisfied FHI physicochemical standardization requirements. Total curcuminoid content differed significantly across sites ($p < 0.001$): Blitar ($4.48 \pm 0.09\%$) > Temanggung ($3.96 \pm 0.07\%$) > Wonogiri ($3.39 \pm 0.06\%$), all markedly exceeding the FHI minimum of $\geq 1.0\%$.

Geographic variation in curcuminoid content appears to be influenced by altitude, solar irradiance, soil composition, and agricultural management. These findings underscore the necessity of region-informed standardization frameworks for turmeric crude drug in Indonesia, and provide a scientifically rigorous dataset to support development of geographically differentiated national monographs. Blitar emerges as a particularly high-potential sourcing region for curcuminoid-rich turmeric raw material. Broader multi-site studies integrating metabolomics, genomics, and geo-referenced agroclimatic data are recommended to further elucidate the determinants of curcuminoid accumulation across Indonesia's diverse turmeric cultivation landscape.

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