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Research Article

Geographic Differences in DPPH Antioxidant Activity of Curcuma aeruginosa Rhizome Essential Oil from Selected Growing Areas in Java, Indonesia

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ABSTRACT

Curcuma aeruginosa (black turmeric), an aromatic medicinal plant used in Indonesian traditional medicine, exhibits chemical composition variations that influence its pharmacological effects depending on the rhizome's growth location. This study investigated whether the antioxidant activity of *Curcuma aeruginosa* essential oil varies among rhizomes collected from three growing areas in Java (Batu, Wonogiri, and Majalengka). Essential oils obtained by water–steam distillation were evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay. All samples exhibited concentration dependent radical scavenging activity, with strong logarithmic regression models. The Wonogiri oil showed the highest antioxidant activity (IC_{50} = 3.09 mg/mL), followed by Batu (12.45 mg/mL) and Majalengka (16.78 mg/mL). These results demonstrate geographic differences in the antioxidant potential of Curcuma aeruginosa essential oil. Further GC–MS (Gas Chromatography Mass Spectrometry) analysis is required to identify the volatile compounds responsible for these regional differences.

Keywords: *Curcuma aeruginosa*, Essential oil, Antioxidant Activity, DPPH Assay, Growing Areas.

1. INTRODUCTION

Curcuma aeruginosa Roxb. is a rhizomatous plant in the Zingiberaceae family that is an important component of traditional Indonesian medicine [1], [2]. It is recognized for its diverse array of secondary metabolites and has been explored for its pharmacological potential in various areas [3], [4], [5], [6]. The rhizome functions as the primary storage part for terpenoids, phenolics, and other constituents that underpin its biological activity and enduring application in traditional natural products [7], [8]. Java is Indonesia's main medicinal plant production region [9], particularly East Java, Central Java, and West Java, where *Curcuma aeruginosa* is widely grown and used. Variations in extraction yield and bioactivity among accessions from these regions have been documented, highlighting the influence of

geographical origin on phytochemical potential [10], [11], [12]. The phytochemical profile of *Curcuma* rhizomes is affected by environmental parameters such soil type, rainfall, climate, and altitude, which vary different on the island of Java [8], [13].

Investigations into the antioxidant effects of *Curcuma aeruginosa* are still few, with the majority of existing studies concentrating on crude rhizome extracts rather than essential oils. Studies that have utilized the DPPH method to look at extracts show a wide range of antioxidant strength. Antioxidant activity is expressed as the IC_{50} value, representing the concentration required to inhibit 50% of free radicals [14]. Nurcholis et al. (2017) showed IC_{50} values ranging from 89.81 to 505.65 ug/ml across Indonesian accessions [6], whereas Yurasbe et al. (2023) discovered similarly low activity in extract material from Peninsular Malaysia, with an IC_{50} of 142.51 mg/ml [15]. These findings align with the overall patterns observed in various *Curcuma* species, wherein environmental variations influence fluctuations in phenolic compound levels and antioxidant activity derived from extracts [8], [16], [17].

Research on a *Curcuma* species further shows that regional diversity might affect essential oil properties. Essential oils of *Curcuma caesia* obtained from several places in Eastern India shown notable variations in antioxidant efficacy, suggesting that the composition of volatile compounds might be influenced by cultivation circumstances [18]. The same study found that leaf oils were better at scavenging DPPH and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) than rhizome oils, which shows that different parts of the plant work differently. Changes in the profiles of essential oils from *Curcuma* species in Nepal have also been linked to the region, which supports the idea that volatile compounds often respond to environmental factors in ways that are different from polar extract fractions [5], [19].

Even with these findings, there is still not much knowledge regarding the essential oils of *Curcuma aeruginosa*. Most published research focuses on crude extracts. Although isolated compound like curcumenotone have significant antioxidant activity [20], [21], the antioxidant activities of entire essential oils have yet to be examined across various growing regions [8]. Given the established environmental sensitivity of terpenoid biosynthesis in Zingiberaceae plants, a regional comparison is necessary to elucidate how geographical factors may affect essential oil bioactivity in this species [22], [23].

This work aims to fill the gap by investigating the DPPH scavenging activity of *Curcuma aeruginosa* rhizome essential oils sourced from Batu, Wonogiri, and Majalengka. The oils were extracted via steam distillation, and the samples were assessed under uniform analytical circumstances. This study gives a first look at the way the antioxidant activity of essential oils varies by area in *Curcuma aeruginosa.*

2. EXPERIMENTAL SECTION

2.1. Plant Materials

Sampling locations were selected based on the availability of *Curcuma aeruginosa* plants and existing local cultivation practices in each area. Samples were collected from East Java (Gunungsari Plantation, Bumiaji, Batu; hereafter referred to as EJ), Central Java (Gadungan Plantation, Kismantoro, Wonogiri; CJ), and West Java (Cipendeuy Plantation, Bantarujeg, Majalengka; WJ). Fresh rhizomes of *Curcuma aeruginosa* (15 kg) from each location (EJ, CJ, and WJ) were sorted, washed under running water with gentle brushing to remove adhering soil, and drained. The rhizomes were sliced transversely into 2–4 mm sections and air-dried indoors for 14 days under ambient conditions.

(a) (b) (c)

Figure 1. *Curcuma aeruginosa* rhizome samples collected from different regions showing variations in external color: (a) Batu, exhibiting a lighter whitish-brown external color; (b) Wonogiri, showing the darkest yellowish-brown external color; and (c) Majalengka, with a yellowish-brown external color.

After drying, the material was manually sorted to remove moldy or insufficiently dried samples, insects, and residual sand that may have adhered during the drying process. Botanical identification was conducted at the Faculty of Mathematics and Natural Sciences,

Universitas Padjadjaran, Indonesia. The samples were identified as *Curcuma aeruginosa* Roxb. under identification numbers 30/HB/01/2023 (EJ), 31/HB/01/2023 (CJ), and 70/HB/01/2023 (WJ) (Indonesian common name: *Temu Hitam/ Ireng*).

2.2. Instrumentation

Essential oil production used steam distillation apparatus, while the antioxidant activity was measured using Mettler Toledo® analytical balance and an Agilent BioTek Epoch Microplate Spectrophotometer®.

2.3. Procedure

2.3.1. Essential Oil Distillation

Essential oil was obtained by steam distillation with water and carried out in triplicate for each regional sample. Dried rhizome powder (1,000 g) was placed on a perforated plate above boiling water in the distillation chamber. Distillation proceeded at 100°C for 3 h, allowing volatile compounds to co-distill with the steam. The condensate was collected in a separatory funnel, and the essential oil (separating as the upper phase) was withdrawn and dried over anhydrous sodium sulfate. Samples were stored in amber vials at 4°C until analysis.

2.3.2. Microplate Assay and Serial Dilution for Antioxidant Activity

Absorbance was recorded at 517 nm using a microplate reader. Homogenization of oil solutions was performed in a microcentrifuge, and all pipetting steps were conducted with calibrated micropipettes using standard 96-well microplates. Working solutions (50,000 ppm) were prepared by mixing 500 µL essential oil with 500 µL methanol, followed by brief centrifugation. Separate solutions were prepared for samples from EJ, CJ, and WJ.

Each regional essential oil sample was analyzed in triplicate. A stock solution of 50,000 ppm was prepared and used to generate a series of two-fold dilutions. For each replicate, an aliquot of the stock solution was transferred into the first row of a microplate, followed by methanol in the subsequent rows. Serial dilution was performed down the plate to obtain final sample concentrations of 50, 25, 12.5, 6.25, 3.125, 1.5625, and 0.78125 mg/ml. After dilution, an equal volume of 50 ppm DPPH solution was added to each test well.

Control wells included a reagent blank containing ethanol plus DPPH and a negative blank consisting of ethanol alone. Additional sample controls were prepared using the same concentrations of essential oil but without DPPH to correct for intrinsic absorbance. All plates

were prepared in the same format for each replicate. The mixtures were incubated for 30 minutes at room temperature in the dark before absorbance was recorded.

2.3.3. Data Analysis

Corrected sample absorbance was obtained by subtracting the absorbance of the corresponding sample negative control. Corrected blank values were calculated similarly. Antioxidant activity was expressed as percent inhibition:

$$\%\ \text{Inhibition} = \left(\frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}}\right) \times 100\% \quad (1)$$

The relationship between inhibition (y) and concentration (x) followed a logarithmic model below and IC_{50} values were determined by substituting y=50 into the regression equation.

$$Y = a\ln(x) + b \quad (2)$$

3. RESULTS AND DISCUSSION

The essential oils distilled from *Curcuma aeruginosa* rhizomes collected in Batu, Wonogiri, and Majalengka exhibited measurable radical-scavenging activity across all tested concentrations. The essential oils distilled from *Curcuma aeruginosa* rhizomes collected in Batu, Wonogiri, and Majalengka exhibited measurable DPPH radical-scavenging activity across all tested concentrations. As shown in Table 1, increasing concentrations of essential oil resulted in a gradual decrease in absorbance values, indicating enhanced radical inhibition. Samples exhibiting the lowest absorbance values corresponded to the highest radical-scavenging capacity. This effect is attributed to the reduction of DPPH radicals to the non-radical DPPH-H form, accompanied by a visible color change from deep violet to pale yellow [24], [25], [26]. These concentration-dependent responses were subsequently used to calculate IC_{50} values for each essential oil sample.

Table 1. Concentration and Absorbance of *Curcuma aeruginosa* Essential Oil Grown in Batu, Wonogiri, and Majalengka.

Sample	Concentration (mg/ml)	Absorbance			
		R1	R2	R3	Ave±stdev
Batu (EJ)	3.125	0.1657	0.2497	0.2477	0.2210±0.0479
	6.25	0.1250	0.2117	0.2107	0.1824±0.0498
	12.5	0.0793	0.1733	0.1653	0.1393±0.0521
	25	0.0507	0.1257	0.1200	0.0988±0.0417
	50	0.0373	0.0850	0.0793	0.0672±0.0261
Wonogiri (CJ)	0.78	0.2127	0.2700	0,2440	0.2422±0.0287
	1.56	0.1693	0.2313	0.1967	0.1991±0.0311
	3.125	0.1320	0.1810	0.1570	0.1567±0.0245

Sample	Concentration (mg/ml)	Absorbance			
		R1	R2	R3	Ave±stdev
	6.25	0.0860	0.1367	0.1217	0.1148±0.0266
	12.5	0.0587	0.1003	0.0847	0.0812±0.0210
Majalengka (WJ)	3.125	0.2697	0.2697	0.2697	0.2548±0.0224
	6.25	0.2390	0.2390	0.2390	0.2181±0.2980
	12.5	0.1970	0.1970	0.1970	0.1742±0.0325
	25	0.1540	0.1540	0.1540	0.1308±0.0320
	50	0.1103	0.1103	0.1103	0.0869±0.0266

R : Replicate
Ave±stdev : absorbance average±deviation standard

As demonstrated in Table 1, the essential oils from all regions exhibited a concentration-dependent decrease in DPPH absorbance, indicating progressive radical-scavenging activity. This trend enabled reliable logarithmic regression analysis, which gave excellent model fitting for all datasets ($R^2 > 0.99$), as shown in Table 2. This allowed for accurate estimation of IC_{50} values. Notably, the antioxidant activity of *Curcuma aeruginosa* essential oil varied throughout the three areas of Java. CJ samples had the highest radical-scavenging capacity (IC_{50} = 3.09 mg/mL), followed by EJ (12.45 mg/mL) and WJ (16.78 mg/mL). This regional variation in antioxidant activity is consistent with previous research indicating that environmental conditions such as temperature, rainfall, soil characteristics, altitude, and ecological stress influence essential oil yield and terpenoid composition in Curcuma species [5], [19], [23].

Table 2. Antioxidant Activity of *Curcuma aeruginosa* Essential Oil Grown in Batu, Wonogiri, and Majalengka.

Sample	Concentration (mg/ml)	Inhibition (%)	Equation	IC_{50} (mg/ml)
Batu (EJ)	3.125	22.18	y = 19.875ln(x) – 137.41	12.45
	6.25	35.76	r = 0.998	
	12.5	50.94		
	25	65.21		
	50	76.33		
Wonogiri (CJ)	0.78	24.07	y = 18.377ln(x) - 97.661	3.09
	1.56	37.58	r = 0.998	
	3.125	50.89		
	6.25	64.02		
	12.5	74.54		
Majalengka (WJ)	3.125	17.81	y = 19.691ln(x) – 141.55	16.78
	6.25	29.64	r = 0.9994	
	12.5	43.80		
	25	57.81		
	50	71.97		

Curcuma aeruginosa grows in three distinct locations at differing altitudes. EJ and WJ samples were gathered in highland areas, whereas CJ came from a lowland area at an altitude of 0-10 m above sea level [27], that are typically associated with higher ambient temperatures [28]. Elevated temperatures have been shown to enhance the production of reactive oxygen species (ROS) in plant tissues [29], [30], causing oxidative stress. In response, plants produce secondary metabolites with antioxidant capabilities to protect themselves from free radical damage [31]. In this regard, essential oils from CJ indicate that this region may encourage the accumulation of oxygenated monoterpenes and sesquiterpenes, groups of compounds known to contribute significantly to the antioxidant activity of *Curcuma* essential oils [5], [19], [32]. Sesquiterpenes, in instance, have been identified as the primary constituents of *Curcuma aeruginosa* rhizomes [2].

Studies on *Curcuma caesia* have shown that essential oils high in eucalyptol, camphor, and other oxygenated volatile compounds have much higher DPPH and ABTS radical-scavenging properties [18], [33]. Similar tendencies have been observed in other *Curcuma* species, where agroecological variables cause shifts in volatile profiles and concomitant variations in antioxidant effectiveness [19]. Although GC-MS profiling was not performed in this work, the findings indicate that the lowland environmental circumstances of CJ may favor the manufacture of more antioxidant-active volatile compounds.

Findings from this study align with broader trends reported across the *Curcuma* genus, in which essential-oil antioxidant activity has been consistently associated with shifts in volatile composition driven by environmental variability [5], [34]. Specifically, Dosoky and Setzer (2018) reported that variations in altitude, soil conditions, and temperature regimes influence the relative abundance of monoterpenes and sesquiterpenes across multiple *Curcuma* species, resulting in measurable differences in radical-scavenging activity [5]. Similarly, Destryana et al. (2024) highlighted that environmental and agroecological factors modulate essential-oil composition in Zingiberaceae rhizomes, thereby affecting antioxidant performance as evaluated by DPPH and related assays [22].

Comparable patterns have been documented in *Curcuma xanthorrhiza* essential oils collected from distinct regions of West Java, Indonesia, where site specific environmental profiles corresponded to significant variation in antioxidant strength [8]. These species-level observations reinforce the likelihood that geographic factors also influenced the volatile

composition of *Curcuma aeruginosa* in the present study. Although specific environmental parameters were not directly quantified, the cited literature provides mechanistic support for the observed regional differences.

Beyond regional differences, variations in antioxidant performance among *Curcuma* essential oils are also shaped by the inherent chemical behavior of volatile constituents. Oxygenated monoterpenes and sesquiterpenes, compounds frequently reported in *Curcuma* species, display distinct radical scavenging mechanisms that depend on structural features such as electron-donating groups and steric accessibility [5], [20]. The ability of these compounds to neutralize DPPH radicals is also influenced by their solubility and interaction dynamics within the assay medium, making essential-oil activity more sensitive to shifts in oil composition than polar extracts. Observations from *Curcuma xanthorrhiza* and other *Curcuma* species demonstrate that even modest changes in the relative abundance of key volatiles can result in noticeable differences in antioxidant behavior [8], [18]. These mechanistic considerations help explain why the CJ oil, despite being analyzed under identical conditions, achieved markedly lower IC_{50} values than oils from the two highland regions.

4. CONCLUSION

The essential oil from Wonogiri, Central Java showed greater antioxidant activity than those from Batu, East Java and Majalengka, West Java; suggesting that environmental conditions interactions shape the volatile antioxidant profile of the rhizome.

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AUTHOR CONTRIBUTIONS

ANEMH led and coordinated the research team and was responsible for the study's conception, design, validation, and manuscript writing. NKN contributed to the study's conception and assisted with material preparation, data collection, and data analysis.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest to report regarding the present study.

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