Research Article
Open-Access Journal (ISSN: 2828-8734)
https://jurnal.umi.ac.id/index.php/PharmRep

Comparative Study of Extraction Methods on the Antioxidant Activity of *Curcuma caesia* Roxb. Leaf Extract Using the DPPH Assay

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Received: 19 November 2025 / Accepted: 11 February 2026 / Published: 31 October 2026

ABSTRACT: Antioxidants are compounds capable of inhibiting or delaying the oxidation of other molecules, thereby protecting biological systems from oxidative damage caused by free radicals. Natural antioxidants derived from plants have gained increasing attention due to their safety and potential health benefits. One such plant is black turmeric (*Curcuma caesia* Roxb.), which is widely recognized for its medicinal properties and rich content of bioactive secondary metabolites. The leaves of *C. caesia* contain various phytochemical constituents, including flavonoids, alkaloids, and phenolic compounds, which are known to exhibit significant antioxidant activity. This study aimed to evaluate the antioxidant activity of ethanol extract of *C. caesia* leaves using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method and to compare the effectiveness of two extraction techniques, namely maceration and water bath-assisted maceration, using 96% ethanol as the solvent. The extracts obtained from both methods were analyzed for their ability to scavenge DPPH radicals, and the antioxidant activity was expressed in terms of IC_{50} values. The results showed that the ethanol extract obtained through the maceration method exhibited an IC_{50} value of 19.7921 µg/mL, while the water bath-assisted maceration method yielded a slightly lower IC_{50} value of 19.5868 µg/mL. Both values fall within the range of 10–50 µg/mL, indicating strong antioxidant activity. The slight difference in IC_{50} values suggests that the water bath-assisted maceration method may enhance extraction efficiency, although both methods are effective in obtaining bioactive compounds with high antioxidant potential. In conclusion, *C. caesia* leaf ethanol extract demonstrates strong antioxidant activity, and both extraction methods are suitable for isolating antioxidant compounds, with water bath-assisted maceration showing marginally better performance. These findings support the potential use of black turmeric leaves as a natural source of antioxidants for pharmaceutical and nutraceutical applications.

KEYWORDS: Extraction; phytochemical; radical; oxidation; spectrophotometry.

1. INTRODUCTION

Black turmeric (*Curcuma caesia* Roxb.) is a medicinal plant with considerable potential in the health field due to its rich content of bioactive compounds. It has been reported to exhibit various pharmacological activities, including antibacterial properties, making it a valuable component in herbal medicine (Desire et al., 2016). The plant contains a wide range of secondary metabolites such as curcuminoids, demethoxycurcumin, bisdemethoxycurcumin, essential oils (e.g., camphor), flavonoids, phenolic compounds, alkaloids, and various amino acids, which contribute to its strong biological activities, particularly its antioxidant potential (Fina & St. Maryam, 2019). Previous studies have also reported that ethanol extracts of *C. caesia* contain significant phenolic compounds (Baghel et al., 2013), while its leaves are rich in flavonoids known for their antioxidant properties (Suryanto & Katja, 2019).

The diverse phytochemical composition of black turmeric underlies its broad pharmacological effects, including antioxidant, antibacterial, antimutagenic, and cytotoxic activities. In addition, *C. caesia* has been reported to inhibit Nuclear Factor kappa B (NF-κB), suggesting its potential role in preventing inflammation-related and degenerative diseases (Udayani, 2022). Traditionally, black turmeric has also been used as a topical remedy for bruises and sprains (Utami, 2024). Despite these promising properties, studies focusing on the antioxidant activity of black turmeric leaves, particularly in relation to different extraction methods, remain limited. Variations in extraction techniques may significantly influence the yield and effectiveness of bioactive compounds, yet comparative evaluations using simple and efficient methods are still lacking.

To assess antioxidant activity, the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is widely used due to its simplicity, rapidity, sensitivity, and minimal sample requirement (Julizan, 2019; Ainunnisa *et al*., 2025). Therefore, this study aims to evaluate the antioxidant activity of ethanol extract of *Curcuma caesia* leaves using the DPPH method and to compare the effectiveness of maceration and water bath-assisted maceration extraction techniques in obtaining compounds with high antioxidant potential.

This study performing comparative assessment of extraction methods on black turmeric leaf extracts using 96% ethanol, which has not been widely reported, as well as the focus on leaf material rather than the more commonly studied

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rhizome. This approach provides new insights into optimizing extraction techniques to enhance antioxidant activity and expands the potential utilization of black turmeric leaves as a natural source of antioxidants.

2. EXPERIMENTAL SECTION

2.1. General

The instruments used in this study included standard laboratory glassware, micropipettes, volumetric pipettes, a UV–Vis spectrophotometer (Thermo Scientific GENESYS 10S), an analytical balance, and a rotary vacuum evaporator. The materials used consisted of 96% ethanol, quercetin as a standard compound, DPPH (2,2-diphenyl-1-picrylhydrazyl) reagent, and black turmeric (*Curcuma caesia* Roxb.) leaf extract.

2.2. Sample preparation

Black turmeric (*Curcuma caesia* Roxb.) leaves were collected and cleaned to remove adhering impurities, followed by washing under running water. The leaves were then drained, cut into smaller pieces, and dried by air circulation in a shaded room without direct sunlight exposure until completely dry to obtain simplicia. The dried simplicia leaves were subsequently ground into powder using a blender (Sumakno et al., 2021).

2.3. Extraction

2.3.1. Maceration

The dried simplicia powder was weighed (20 g) and transferred into a maceration container, followed by the addition of 250 mL of 96% ethanol. The mixture was macerated for 3 days (3 × 24 hours) with occasional stirring to ensure optimal extraction. Remaceration was carried out repeatedly until the solvent became clear, indicating complete extraction of the bioactive compounds. The combined filtrates were then concentrated using a rotary vacuum evaporator to obtain a viscous (thick) extract (Handayani et al., 2019).

2.3.1. Waterbath extraction

Twenty grams of black turmeric (*Curcuma caesia* Roxb.) leaf simplicia powder were placed into a beaker and extracted with 250 mL of 96% ethanol. The extraction was carried out using the maceration method for 6 hours with intermittent stirring, consisting of agitation every 15 minutes for 1 minute each cycle. After the extraction process, the resulting filtrate was collected, and the solvent was removed using a rotary evaporator to obtain a concentrated (thick) extract (Pramesti, 2008).

2.4. Antioxidant activity

2.4.1. Preparation of DPPH

A DPPH solution was prepared by dissolving 10 mg of DPPH (2,2-diphenyl-1-picrylhydrazyl) powder in 50 mL of 96% ethanol, followed by homogenization to ensure complete dissolution. The prepared solution was then stored in a dark (amber) bottle to protect it from light exposure. A total of 4 mL of 50 µg/mL DPPH solution was mixed with 1 mL of 96% ethanol and then incubated for 30 minutes in a dark environment to prevent photodegradation. The absorbance of the solution was subsequently measured using a UV–Vis spectrophotometer in the visible range to determine the maximum wavelength (λmax), which was found to be 516 nm (Fawwaz *et al*., 2023).

2.4.2. Preparation of quercetin

A total of 10 mg of quercetin was dissolved in 96% ethanol in a 10 mL volumetric flask to obtain a stock solution with a concentration of 1000 µg/mL. The stock solution was then diluted to prepare a 200 µg/mL intermediate solution. Subsequently, a series of standard solutions with concentrations of 1, 2, 3, 4, 5, and 6 µg/mL were prepared by pipetting 25, 50, 75, 100, 125, and 150 µL of the intermediate solution, respectively, and diluting each to a final volume of 5 mL with 96% ethanol. For the antioxidant assay, 1 mL of each standard solution was mixed with 1 mL of 50 µg/mL DPPH solution. The mixtures were then homogenized and incubated in the dark for 30 minutes. After incubation, the absorbance was measured at a wavelength of 516 nm using a UV–Vis spectrophotometer.

2.4.3. Antioxidant activity test of extract

A total of 10 mg of black turmeric (*Curcuma caesia* Roxb.) leaf ethanol extract was dissolved in 96% ethanol in a 10 mL volumetric flask to obtain a stock solution with a concentration of 1000 µg/mL. The stock solution was then diluted to prepare a 100 µg/mL working solution. Subsequently, a series of sample solutions with concentrations of 10, 12, 14, 16, 18, and 20 µg/mL were prepared by pipetting 500, 600, 700, 800, 900, and 1000 µL of the working solution, respectively, and diluting each to a final volume of 5 mL with 96% ethanol. For the antioxidant assay, 1 mL of each sample solution was mixed with 4 mL of 50 µg/mL DPPH solution. The mixtures were homogenized and incubated in the dark for 30 minutes. After incubation, the absorbance was measured at a wavelength of 516 nm using a UV–Vis spectrophotometer.

3. RESULTS AND DISCUSSION

The extraction results showed that the percentage yield of the ethanol extract obtained by conventional maceration was 6.3%, while the yield from water bath-assisted maceration was 5.55%. The extraction was carried out using 96% ethanol as the solvent. The yield calculation was performed to estimate the amount of secondary metabolites extracted into the solvent; however, it does not provide information regarding the specific types of compounds present in the extract, as presented in **Table 1**.

The difference in yield between the two extraction methods may be attributed to variations in extraction conditions, particularly temperature and extraction dynamics. Conventional maceration, which involves a longer extraction time at room temperature, allows for more extensive solvent penetration into plant tissues and gradual diffusion of soluble compounds, potentially resulting in a higher yield. In contrast, water bath-assisted maceration involves elevated temperatures that can enhance mass transfer and accelerate extraction; however, prolonged exposure to heat may also lead to the degradation of thermolabile compounds, thereby reducing the overall yield. Additionally, higher temperatures may promote the co-extraction of unwanted compounds or alter solvent–solute interactions, which can affect extraction efficiency.

Despite the slightly lower yield obtained from the water bath-assisted method, this technique may still be advantageous due to its shorter extraction time and potential selectivity toward specific bioactive compounds. Therefore, differences in yield do not necessarily reflect lower extract quality, and further phytochemical analysis is required to determine the composition and bioactivity of the extracts obtained from each method.

Table 1. The yield of black turmeric leaf extract (*Curcuma caesia* Roxb.)

Extraction Type	Sample Weight(g)	Total Solvent (mL)	Extract Weight (g)	Extract Yield (%)
Maceration	20	1000	1.26	6.3
Waterbath extraction	20	250	1.11	5.55

The parameter commonly used to evaluate antioxidant activity is the inhibitory concentration (IC_{50}), which represents the concentration of an antioxidant required to inhibit 50% of DPPH free radicals. In other words, IC_{50} reflects the ability of a compound to reduce radical activity by half, and lower IC_{50} values indicate stronger antioxidant activity.

Quercetin, a flavonoid compound, is widely recognized as a potent antioxidant due to its ability to scavenge reactive oxygen species (ROS), such as superoxide anions (O_2^-) and peroxynitrite ($ONOO^-$). These reactive species are known to cause oxidative damage to cells and tissues, contributing to the development of various diseases. Therefore, quercetin has significant potential in the pharmaceutical field, exhibiting activities such as antioxidant, anti-obesity, anti-carcinogenic, antibacterial, and anti-inflammatory effects (Barikah et al., 2024).

In this study, quercetin was used as a reference standard to compare the antioxidant activity of black turmeric (*Curcuma caesia* Roxb.) leaf extract. The comparative results of antioxidant activity between quercetin and the extract are presented in **Tables 2**, **3**, and **4**.

Table 2. Quercetin comparative antioxidant activity

Concentration (µg/mL)	Absorbance	Inhibition (%)	IC_{50} (µg/mL)
1	0.344	25.7019	3.2743
2	0.322	30.4535	
3	0.230	50.3239	
4	0.167	63.93089	
5	0.149	67.8186	
6	0.109	76.4579	
DPPH Control (50 µg/mL)	0.463		

Table 3. Antioxidant activity of black turmeric leaf extract by maceration

Concentration (µg/mL)	Absorbance	Inhibition (%)	IC_{50} (µg/mL)
10	0.527	3.8321	
12	0.458	16.4233	

14	0.426	22.2628	19.7921
16	0.353	35.5839	
18	0.328	40.1459	
20	0.270	50.7299	
DPPH Control (50 µg/mL)	0.548		

Table 4. Antioxidant activity of black turmeric leaf extract by waterbath maceration

Concentration (µg/mL)	Absorbance	Inhibition (%)	IC_{50} (µg/mL)
10	0.569	3.0664	
12	0.537	8.5179	
14	0.477	18.7393	19.5868
16	0.427	27.2572	
18	0.323	44.9744	
20	0.276	52.9812	
DPPH Control (50 µg/mL)	0.587		

The results showed that the IC_{50} value of the quercetin standard was 3.2743 µg/mL, indicating very strong antioxidant activity, as it falls well below the threshold of IC_{50} < 50 µg/mL. This finding confirms that quercetin is a highly potent antioxidant and is therefore appropriate as a reference compound in this study. In comparison, the IC_{50} value of the black turmeric (*Curcuma caesia* Roxb.) leaf extract obtained through conventional maceration was 19.7921 µg/mL, while the extract obtained through water bath-assisted maceration exhibited an IC_{50} value of 19.5864 µg/mL. Both values are within the range of IC_{50} < 50 µg/mL, indicating strong antioxidant activity.

Although the antioxidant activity of both extracts is lower than that of quercetin, this is expected because quercetin is a pure compound, whereas the plant extracts are complex mixtures of various phytochemicals. The slightly lower IC_{50} value observed in the water bath-assisted maceration extract suggests that this method may enhance the extraction of antioxidant compounds more efficiently compared to conventional maceration. However, the difference between the two methods is relatively small, indicating that both extraction techniques are effective in producing extracts with strong antioxidant potential. Overall, these findings demonstrate that black turmeric leaf extract possesses significant antioxidant activity and has potential as a natural source of antioxidants, although its potency remains lower than that of the standard compound.

4. CONCLUSION

Based on the results of this study, it can be concluded that variations in the extraction method influence the IC_{50} values obtained. The ethanol extract of black turmeric (*Curcuma caesia* Roxb.) leaves produced using water bath-assisted maceration exhibited an IC_{50} value of 19.5868 µg/mL, which was slightly lower than that obtained from conventional maceration (19.7921 µg/mL). This indicates that the water bath-assisted maceration method provides marginally better extraction efficiency, resulting in stronger antioxidant activity.

Acknowledgments: The authors are grateful to The Head of Pharmaceutical Chemistry Laboratory, Universitas Muslim Indonesia. The authors are thankful to The Dean of Faculty of Pharmacy Universitas Muslim Indonesia for the space to do this research.

Author contributions: All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

Conflict of interest: The authors declared no conflict of interest.

Ethical Approval: Not applicable

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