

Antioxidant Activity of Black Turmeric Rhizome (*Curcuma caesia* Roxb.) Extract Using DPPH Radical Scavenging Assay

Andi Muflihunna*, Rahmawati, Nurul Rahmah*

Faculty of Pharmacy, Universitas Muslim Indonesia, Makassar, Indonesia

* Corresponding Author. E-mail: 15020210200@umi.ac.id; andi.muflihunna@umi.ac.id

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ABSTRACT: Free radicals are highly reactive molecules that contribute to the development of various diseases, particularly degenerative disorders such as cancer, cardiovascular diseases, and aging-related conditions. Antioxidants play a crucial role in neutralizing free radicals and preventing oxidative damage. Black turmeric (*Curcuma caesia* Roxb.) is a medicinal plant widely used in traditional medicine and is known for its rich content of bioactive compounds, including alkaloids, flavonoids, phenolics, curcuminoids, terpenoids, tannins, and proteins, which contribute to its therapeutic potential. This study aimed to evaluate the antioxidant activity of ethanol extracts of black turmeric rhizomes using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method, with a comparison of different extraction techniques. The extracts were obtained using maceration and water bath-assisted maceration methods with 96% ethanol as the solvent. Antioxidant activity was expressed in terms of the half-maximal inhibitory concentration (IC₅₀). The results demonstrated that the extraction method significantly influenced antioxidant activity. The maceration method yielded an IC₅₀ value of 9.2503 µg/mL, indicating very strong antioxidant activity (<10 µg/mL). In contrast, the water bath-assisted maceration method produced an IC₅₀ value of 12.9274 µg/mL, which falls within the strong antioxidant category (10–50 µg/mL). In conclusion, black turmeric (*Curcuma caesia* Roxb.) rhizome extract exhibits potent antioxidant activity, with the maceration method providing superior results compared to water bath-assisted maceration. These findings suggest that black turmeric has significant potential as a natural antioxidant source and that extraction methods play a critical role in optimizing its bioactive properties.

KEYWORDS: Extraction; flavonoid; oxidative; phytochemical; turmeric

1. INTRODUCTION

Black turmeric (*Curcuma caesia* Roxb.) is a medicinal plant widely recognized for its potential applications in the health sector due to its rich content of bioactive compounds (Nuraeni et al., 2023). As a member of the Zingiberaceae family, black turmeric has long been utilized in traditional medicine for its therapeutic properties. Its pharmacological potential is primarily attributed to the presence of various secondary metabolites, including curcuminoids (curcumin, desmethoxycurcumin, and bisdesmethoxycurcumin), essential oils (such as camphor), flavonoids, phenolic compounds, alkaloids, terpenoids, tannins, and amino acids. Among these compounds, curcumin is particularly well known for its potent antioxidant activity, as well as its anti-inflammatory and anticarcinogenic effects (Baghel et al., 2013).

The rhizome of black turmeric is especially rich in these bioactive constituents. Quantitative studies have reported that flavonoid content in black turmeric rhizomes can reach 2775.65 mg/100 g, while tannin and alkaloid contents are approximately 2714.75 mg/100 g and 1470.59 mg/100 g, respectively (Udayani et al., 2022). These high concentrations of phytochemicals contribute significantly to the biological activities of black turmeric, particularly its antioxidant capacity. Antioxidants play an essential role in protecting the body from oxidative stress by neutralizing free radicals, which are known to contribute to cellular damage and the development of various chronic diseases.

The antioxidant activity of black turmeric extract can be evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, a widely used method based on redox reactions. In this method, antioxidant compounds donate hydrogen atoms or electrons to stabilize DPPH radicals, resulting in the formation of non-radical species. This reaction is visually indicated by a color change from purple to yellow, which can be quantitatively measured using a UV-Visible spectrophotometer (Kristanti et al., 2019; Kartini et al., 2023). The DPPH method is favored due to its simplicity, rapid analysis, cost-effectiveness, and minimal reagent requirements compared to other antioxidant assays, which are often more complex, time-consuming, and expensive (Kesuma, 2015).

In addition to the analytical method, the extraction technique plays a crucial role in determining the yield and activity of bioactive compounds. Different extraction methods can influence the efficiency of compound recovery and, consequently, the antioxidant potential of the extract. Therefore, selecting an appropriate extraction method is essential for optimizing the bioactivity of plant extracts.

Based on these considerations, this study was conducted to evaluate the antioxidant activity of ethanol extracts of black turmeric (*Curcuma caesia* Roxb.) rhizomes using the DPPH radical scavenging method, with a comparison of different extraction techniques. This research is expected to provide further insight into the potential of black turmeric as a natural antioxidant source and the influence of extraction methods on its bioactive properties.



2. EXPERIMENTAL SECTION

2.1. Tools and Materials

The equipment used in this study included standard laboratory glassware, porcelain crucibles, micropipettes, a UV–Vis spectrophotometer (Thermo Scientific GENESYS 10S), an analytical balance, a vortex mixer (IKA® VORTEX 3), a water bath, and a rotary vacuum evaporator. The materials used comprised 96% ethanol, quercetin, DPPH powder (2,2-diphenyl-1-picrylhydrazyl), and black turmeric (*Curcuma caesia* Roxb.) rhizome extract.

2.2. Extraction

2.2.1. Conventional Maceration

Black turmeric rhizomes are extracted by the Maceration method using 96% ethanol solvent with a powder weight of 20 grams. The extraction method is carried out by soaking the sample with a filter solution for 3 times 24 hours at room temperature protected from light and occasionally shaken. Then the extraction results are filtered and the filtrate is taken and the residue is remacerated 3 times. After the extraction process produces a filtrate which is then evaporated the solvent using *Rotary Evaporator* and *Squirt* until a thick extract is obtained (Rahmah, Sukardi and Dila, 2019).

2.2.2. Maceration by Waterbath

A total of 20 g of black turmeric (*Curcuma caesia* Roxb.) rhizome simplisia powder was placed into a 1000 mL Erlenmeyer flask and extracted with 250 mL of 96% ethanol. The extraction was performed using the maceration method at a controlled temperature of 50°C for 6 hours. During the extraction process, the mixture was agitated every 15 minutes for 1 minute using a vortex mixer, following a modified method based on previous study. After completion of the extraction, the resulting filtrate was collected and the solvent was removed using a rotary evaporator, followed by further evaporation in a water bath to obtain a concentrated viscous extract (Pramesti, 2008).

2.4. Determination of Antioxidant Activity

2.4.1. Preparation of DPPH solution

A total of 10 mg of DPPH (2,2-diphenyl-1-picrylhydrazyl) powder was dissolved in 50 mL of 96% ethanol and homogenized to obtain a stock solution. Subsequently, 12.5 mL of this solution was diluted to 50 mL with 96% ethanol to prepare a working solution. The solution was homogenized and stored in an amber (dark) bottle to protect it from light (Fawwaz *et al.*, 2021).

2.4.2. Determination of Maximum Wavelength (λ_{max})

An aliquot of 4 mL of 50 $\mu\text{g/mL}$ DPPH solution was mixed with 1 mL of 96% ethanol and incubated in the dark for 30 minutes. The absorbance was then measured using a UV–Vis spectrophotometer within a wavelength range of 400–800 nm. The maximum absorption wavelength (λ_{max}) was determined to be 517 nm.

2.4.3. Preparation of Quercetin Standard Solution

A total of 10 mg of quercetin was dissolved in 96% ethanol and diluted to 10 mL in a volumetric flask to obtain a 1000 $\mu\text{g/mL}$ stock solution. The stock solution was then diluted to obtain a 200 $\mu\text{g/mL}$ intermediate solution. From this solution, a series of standard solutions with concentrations of 1, 2, 3, 4, 5, and 6 $\mu\text{g/mL}$ were prepared by pipetting 25, 50, 75, 100, 125, and 150 μL , respectively, and diluting each to a final volume of 5 mL with 96% ethanol. For antioxidant testing, 4 mL of 50 $\mu\text{g/mL}$ DPPH solution was mixed with 1 mL of each quercetin standard solution. The mixtures were homogenized and incubated in the dark for 30 minutes, after which the absorbance was measured at 517 nm (Fawwaz *et al.*, 2024).

2.4.4. Antioxidant Activity Test of Black Turmeric Extract

A total of 10 mg of black turmeric (*Curcuma caesia* Roxb.) rhizome ethanol extract was dissolved in 96% ethanol and diluted to 10 mL to obtain a 1000 $\mu\text{g/mL}$ stock solution. This stock solution was further diluted to obtain a 100 $\mu\text{g/mL}$ working solution. Subsequently, test solutions with concentrations of 4, 6, 8, 10, 12, and 14 $\mu\text{g/mL}$ were prepared by pipetting 200, 300, 400, 500, 600, and 700 μL of the stock solution, respectively, and diluting each to a final volume of 5 mL with 96% ethanol. For the assay, 4 mL of 50 $\mu\text{g/mL}$ DPPH solution was mixed with 1 mL of each sample solution. The mixtures were homogenized and incubated in the dark for 30 minutes. The absorbance was then measured at 517 nm using a UV–Vis spectrophotometer (Fawwaz *et al.*, 2023).

3. RESULTS AND DISCUSSION

Results of extraction of black turmeric rhizome samples (*Turmeric caesia* Roxb.) with a variety of extraction methods (maceration and maceration *Waterbath*) As shown in **Table 1**. The purpose of the percentage yield calculation is to find out the number of compounds that are attracted in a certain solvent but cannot determine the type of compound carried (Ukieyanna, 2012).

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

Extraction Type	Sample Weight (g)	Total Solvent (mL)	Extract Weight (g)	Extract Yield (%)
Maceration	20	1000	0.68	3.40
Maceration <i>Waterbath</i>	20	250	0.57	2.85

In this test, the determination method used is the DPPH damping method using an instrument in the form of a UV-Vis spectrophotometer to measure its absorbance. The parameters in this test are determined by an IC value_{of 50}, where the sample used is able to reduce 50% of the DPPH solution as free radicals. Analysis of the flavonoid content of ethanol extract of kluwih fruit seeds (*Atrocarpus camansi*) was carried out using a UV-Vis spectrophotometer because the flavonoids contain conjugated aromatic compounds so that they show strong absorption bands in the ultraviolet light spectrum and visible light spectrum.

The results of the comparative analysis of quercetin, ethanol extract of black turmeric rhizome (*Curcuma caesia* Roxb.), the results of maceration extraction and *waterbath* maceration can be seen in **Tables 2, 3** and **4** below.

Table 2. Results of the analysis of quercetin-comparable antioxidant activity.

Quercetin			
Concentration (µg/mL)	Absorbance	Inhibition (%)	IC ₅₀ (µg/mL)
1	0.548	8.9700	4.7488
2	0.461	23.4219	
3	0.418	30.5647	
4	0.392	34.8837	
5	0.277	53.9867	
6	0.203	66.2790	
Control (DPPH 50 µg/mL)	0.602		

Table 3. Antioxidant activity of black turmeric (*Curcuma caesia* Roxb.) samples maceration extraction method.

Concentration (µg/mL)	Absorbance	Inhibition (%)	IC ₅₀ (µg/mL)
4	0.352	46.8278	9,2503
6	0.345	47.8858	
8	0.343	48.1873	
10	0.330	50.1511	
12	0.317	52.1148	
14	0.306	53.7764	
Control (DPPH 50 µg/mL)	0.662		

Table 4. Antioxidant activity of black turmeric (*Curcuma caesia* Roxb.) sample waterbath maceration extraction method.

Concentration (µg/mL)	Absorbance	Inhibition (%)	IC ₅₀ (µg/mL)
4	0.567	8.1037	12.9274
6	0.558	9.5623	
8	0.495	19.7730	
10	0.424	31.2803	
12	0.340	44.8946	
14	0.248	59.8055	
Control (DPPH 50 µg/mL)	0.617		

According to Phongpaichit *et al.* (2007) states that a compound is declared to be very strong antioxidant if the IC₅₀ value is less than 10 µg/mL, strong if the IC₅₀ value is between 10-50 µg/mL, while if the IC₅₀ value ranges from 50-100 µg/mL, weak if the IC₅₀ value is 100-250 µg/mL, and inactive if the IC₅₀ value is above 250 µg/mL.

For each extract, a concentration is used. The concentration is chosen to know at what concentration the sample can inhibit 50% of the free radicals of DPPH or commonly called the IC value₅₀, where the purpose of calculating the value of IC₅₀ that is, to determine the level of antioxidant activity of a sample. The comparator used in this study is quercetin, where this comparator is an aglicon compound of a routine molecule without glycosides and is the largest flavonol compound found in almost every type of plant (Jusuf, 2010).

The results obtained are based on measurements with UV-Vis spectrophotometers at a wavelength of 517 nm, i.e. IC₅₀ On the quercetin comparator, 4.7488 µg/mL was obtained, this shows that the antioxidant activity of quercetin is a very strong antioxidant (<10 µg/mL). For samples using the maceration extraction method, a value of 9.2503 µg/mL was obtained so that the IC value₅₀ it is declared very strong due to the IC₅₀ (<10 µg/mL). IC₅₀ It is the concentration of test compounds that are able to reduce free radicals by 50%. The smaller the IC₅₀ value, the higher the free radical suppression activity (Phongpaichit *et al.*, 2007).

4. CONCLUSION

Based on the results of this study, it can be concluded that variations in extraction methods significantly influence the antioxidant activity of black turmeric (*Curcuma caesia* Roxb.) rhizome extract, as reflected by the IC₅₀ values obtained. The extract produced using the maceration method exhibited a very strong antioxidant activity, with an IC₅₀ value of 9.2589 µg/mL (<10 µg/mL). In contrast, the extract obtained through the water bath-assisted maceration method showed a slightly lower antioxidant activity, with an IC₅₀ value of 12.9274 µg/mL, which falls within the strong antioxidant category (10–50 µg/mL). These findings indicate that the conventional maceration method is more effective in preserving or extracting antioxidant compounds from black turmeric rhizomes compared to the water bath-assisted method. The difference in antioxidant activity may be attributed to the influence of temperature and extraction conditions on the stability of bioactive compounds, particularly flavonoids and phenolic constituents. Therefore, the selection of an appropriate extraction method is crucial for optimizing the antioxidant potential of plant-based extracts.

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