

Original Article

## Analysis of Total Flavonoid and Antioxidant Activity of Black Turmeric (*Curcuma caesia*)

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Received: 3 August 2023; Revised: 30 September 2023; Accepted: 12 October 2023; Published: 21 December 2023

**Abstract:** Black turmeric (*Curcuma caesia*) has a higher concentration of phytochemical compounds compared to other turmeric species. This means it's an incredibly potent natural antioxidant. Experts believe that black turmeric could have some amazing health benefits, such as protecting against oxidative stress and reducing inflammation. Black turmeric was extracted using the maceration method with a methanol solvent. After extracting black turmeric using the maceration method and methanol solvent, the resulting extracts were tested qualitatively for flavonoids, phenolics, and tannins. Then proceed with the quantitative test to determine the total levels of flavonoids and test the antioxidant activity using the ABTS method. The results of this study obtained a yield of 13.26%, positive qualitative test results for flavonoids, phenolics, and tannins, and total flavonoid levels of  $10.326 \pm 0.074$  mgEQ/g extract with an IC<sub>50</sub> of  $88.581 \pm 3.376$  ppm. Based on the results of the antioxidant activity, black turmeric methanol extract has strong antioxidant activity.

**Keywords:** *Curcuma caesia*; Flavonoid; Antioxidant; ABTS

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### 1. INTRODUCTION

Free radicals can be prevented with a compound called an antioxidant. Antioxidants can bind unpaired electrons to radical compounds so that cell damage and cell mutations in the body can be stopped [1]. Indonesia exhibits a notable level of plant diversity. People use different parts of plants to make modern medicine, which they use to both keep themselves healthy and treat various diseases. Some parts of the plant are known to have active chemicals that affect its antioxidant activity, like flavonoids and phenolic compounds. One example of a spice that has benefits as a natural antioxidant is turmeric. The potential benefits of utilizing black turmeric have not been fully realized by society. Black turmeric is the type of turmeric proven to act as a natural antioxidant. This statement is also supported by the research of Jyoti et al., (2012) that black turmeric has the potential as an antioxidant that contains more phytochemical compounds than other *Curcuma* species. Black turmeric (*Curcuma caesia*) contains flavonoids, alkaloids, phenols, resins, phytosterols, terpenoids, carbohydrates, tannins, glycosides, saponins, quinones, amino acids, curcuminoids, and essential oils. Black turmeric is often used because it can act as an antioxidant, antifungal, antibacterial, analgesic, anti-tumor, and hepatoprotective [3], [4]. In research Udayani et al., (2022),

black turmeric rhizome extract from Kintamani has the highest amounts of flavonoid phytochemical compounds. The flavonoid level of an 80% ethanol extract of black turmeric is 2775.65 mg/100g. The levels of flavonoids that are found in plants are susceptible to being influenced by a variety of factors, including the environment in which they are grown [6]. Flavonoids are secondary metabolites that contain lots of –OH groups, so they can react with free radicals as reducing agents and free radical scavengers [1], [6], [7]. Nayak and Bhatnagar (2018) study shows that black turmeric methanol extract has the most antioxidant activity. One of the various ways that may be used to determine free radical activity is an in vitro method that uses the ABTS. Methanol has a polarity index of 5.1 and is a universal solvent. Because of this, polar molecules such as ABTS are suitable for use in methanol extraction. ABTS tests antioxidant activity using the compound 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) as a free radical generator. ABTS is a substrate of the peroxidase enzyme, which can be oxidized by peroxide ( $H_2O_2$ ) into radical cations. The idea behind this method is to look at how antioxidant compounds can stabilize free radicals by giving them protons. This is shown by the color going from blue-green to clear as the amount of ABTS radical cation decreases [10]. The IC<sub>50</sub> value indicates the magnitude of the antioxidant activity. The smaller the IC<sub>50</sub> value, the stronger the antioxidant is at counteracting free radicals, or it can be said to have stronger antioxidant activity [11]. The ABTS method is very light-sensitive when forming ABTS radicals and requires 12–16 hours of incubation in the dark [10]. The ABTS method was chosen because it has a higher sensitivity than DPPH and can be used to analyze antioxidants in food [9]. Therefore, this research aimed to review the chemical content of black turmeric, which has the potential to be an antioxidant compound.

## 2. MATERIALS AND METHODS

### 2.1. Material

Black turmeric rhizome was obtained from Sinduadi, Mlati District, Sleman Regency, Yogyakarta. Methanol p.a, ABTS reagent (sigma), sodium acetate (sigma), aquadest,  $FeCl_3$  (sigma),  $AlCl_3$  (sigma), quercetin (sigma), and potassium persulfate (sigma).

#### 2.1.1. Preparation of Black Turmeric Methanol Extract

At the Plant Systematics Laboratory, Faculty of Biology, Gadjah Mada University (UGM), the black turmeric rhizome obtained was determined. The maceration technique was used to extract black turmeric *Simplicia* with methanol 96%. Methanol and simplicial powder (1 : 25) were combined in a container and left at room temperature for 3–4 days, shaking every 12 hours.

### 2.2. Phytochemical Screening

#### 2.2.1. Flavonoids

Take 1 mL of black turmeric extract and react with 1 mL of  $AlCl_3$  1%, then observe the color that forms when the solution turns yellow, indicating the presence of flavonoids in black turmeric methanol extract.

#### 2.2.2. Phenolic

Take 1 mL of black turmeric extract and react with 2 drops of 5%  $FeCl_3$ . If a black-green color is formed, it indicates that the black turmeric extract is positive for phenolic content.

#### 2.2.3. Tannins

Take 1 mL of black turmeric extract and react with 2-3 drops of 1%  $FeCl_3$ . If there is a change in color to a strong blue or green, it indicates that the black turmeric extract positively contains tannins.

### 2.3. Determination of Total Flavonoid Content

Calculation of Total Flavonoid Content (TFC) is carried out with the following formula [12]:

$$TFC \text{ (mg QE/g extract)} = \frac{\text{sample concentration (mg/mL)} \times \text{volume (mL)}}{\text{extract weight (gr)}} \times FP$$

#### 2.3.1. Maximum Wavelength and Operating Time

By reacting, 60 ppm 0.5 mL quercetin, 0.1 mL 10% AlCl<sub>3</sub>, 1 M 1 mL CH<sub>3</sub>COONa, and 2.8 mL distilled water were read at λ 400-500 nm using a UV-Vis spectrophotometer for maximum wavelength. Also, the Operating time is read at the maximum wavelength obtained at 1-minute intervals for 60 minutes.

#### 2.3.2. Standard Curve

Quercetin 1000 ppm was diluted to 20, 30, 40, 60, 70, and 80 ppm concentrations. At each concentration taken, 0.5 mL was added, 0.1 mL 10% AlCl<sub>3</sub>, 1 M CH<sub>3</sub>COONa 1 mL, and 2.8 mL distilled water were incubated during OT and read at the maximum wavelength.

#### 2.3.3. Sample Preparation (1000 ppm)

100.0 mg of the sample was weighed and put in a 100.0 mL measuring flask. Add methanol up to the mark. Samples taken 0.5 mL were added 0.1 mL 10% AlCl<sub>3</sub>, 1 M CH<sub>3</sub>COONa 1 mL, and 2.8 mL distilled water were incubated during OT and read at the maximum wavelength. Repeat three times.

### 2.4. Antioxidant Activity

Calculation of antioxidant is carried out with the following formula:

$$\% \text{ inhibition} = \frac{(\text{control absorbance} - \text{sample absorbance})}{\text{control absorbance}} \times 100\%$$

Following this, the results of the calculations are input into the equation for linear regression. IC<sub>50</sub> number determined by calculating the percentage of inhibition at 50% [13].

#### 2.4.1. Maximum Wavelength and Operating Time

Take 1 mL of a 0.007 M ABTS solution and 0.002 M K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> solution, and read the result between 600 and 800 nm. In addition, the operating time was established by adding 1 mL of 1 ppm quercetin to 2 mL of a mixture consisting of 0.007 M ABTS and 0.002 M K<sub>2</sub>S<sub>2</sub>O<sub>8</sub> and measuring the mixture at maximum at 1 minute intervals for 30 minutes.

#### 2.4.2. Antioxidant Activity of Quercetin

Standard 100 ppm quercetin was diluted to 0.25; 0.5; 1; 2 and 3 ppm. At each concentration, 1 mL was taken and reacted with 2 mL of a mixture of 0.007 M ABTS and 0.002 M K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>, then incubated for 13 minutes and read at λ 745 nm with a UV-Vis spectrophotometer.

#### 2.4.3. Antioxidant Activity of Black Turmeric Extract

From a 1000 ppm, methanol extract of black turmeric was diluted to concentrations of 25, 50, 75, 100, and 125 ppm. At each concentration, 1 mL was taken and reacted with 2 mL of a mixture of 0.007 M ABTS and 0.002 M K<sub>2</sub>S<sub>2</sub>O<sub>8</sub>, then incubated for 13 minutes and read at the maximum wavelength with a UV-Vis spectrophotometer.

### 3. RESULTS AND DISCUSSION

#### 3.1. Black turmeric extract

The determination was carried out at the Plant Systematics Laboratory, Faculty of Biology UGM with botanical identity 0298/S.Tb./IV/2023, the results of the determination showed that the plant used was *Curcuma caesia*. Black turmeric rhizome extraction using methanol solvent and yield values obtained as shown in Table 1.

**Table 1.** The Yield of Black Turmeric Methanol Extract

Sample	Sample weight	Extract weight	yield (%)
Black Turmeric	250 g	33.1403 g	13.26%

Maceration is an extraction method used in this study to extract black turmeric extract flavonoid compounds. The principle of maceration extraction is the extraction of the active ingredient by submerging the simplicia powder in an appropriate solvent [14]. According to Dewi et al. (2022); Savitri et al. (2017), methanol is the solvent that produces the highest yield. The categorization of flavonoids determines their solubility. Solvent ethyl acetate can be used to extract the alkylated flavonoid aglycone group. As for hydroxylated aglycones and flavonoid glycosides, they can be dissolved in water, acetone, or alcohol. In this study, the flavonoid compound quercetin was utilized [17],[18]. Since quercetin is a hydroxylated aglycone with hydroxyl groups at C-7, C-4, and nearby C-3 or C-5, it can be considered to be soluble in methanol. Factors that can affect the yield value are extraction time, temperature, type of solvent, number of samples, and unnecessary sample preparation [19].

#### 3.2. Phytochemical Screening

The phytochemical test of black turmeric methanol extract for flavonoid, phenolic, and tannin compounds can be seen in Table 2.

**Table 2.** Phytochemical Test of Flavonoids, Phenolics, and Tannins

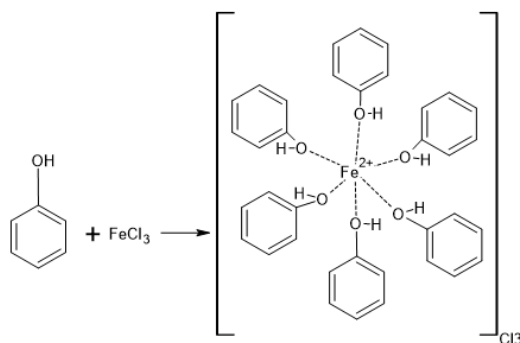
Secondary metabolite	Reagent	Discoloration	Results
Flavonoids	Sample + AlCl <sub>3</sub>	Yellow	+
Phenolic	Sample + FeCl <sub>3</sub> 5%	Blackish green	+
Tannin	Sample + FeCl <sub>3</sub> 1%	Greenish brown	+

##### 3.2.1. Flavonoids

Test the content of flavonoids by reacting the sample with an AlCl<sub>3</sub> reagent. The sample, which was originally bright purple after being reacted with AlCl<sub>3</sub> reagent, showed a change in bright yellow color. AlCl<sub>3</sub> reacts with the ketone group on C4 and the -OH group on C3 or C5 in flavones and flavonol molecules to produce a persistent yellow complex chemical [20]. Flavonoid compounds have the potential as antioxidants because they have hydroxyl groups attached to the carbon of the aromatic ring to capture free radicals.

##### 3.2.2. Phenolic

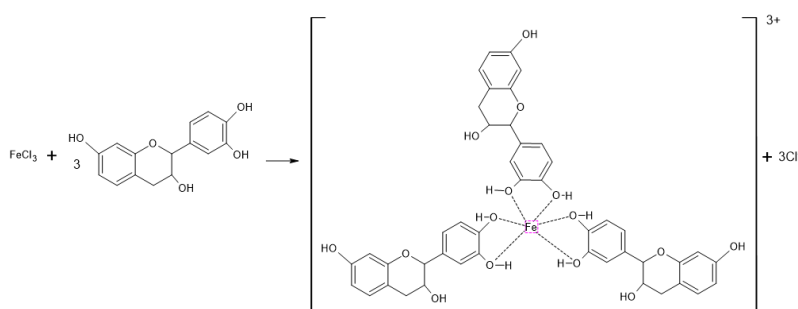
After reacting the sample with 5% FeCl<sub>3</sub>, a color change occurred, which was originally bright purple to a black-green color. This indicated that the positive sample contained phenolic. The formation of a blackish-green color in the extract after adding FeCl<sub>3</sub> forms a complex compound when FeCl<sub>3</sub> reacts with the hydroxyl groups present in phenolic compounds. The reaction between phenol and FeCl<sub>3</sub> is illustrated in Figure 1. Phenolic compounds have antioxidant activity due to conjugated ring structures and hydroxyl groups.



**Figure 1.** The reaction of Phenol with  $\text{FeCl}_3$

### 3.2.3. Tannin

When the sample was reacted, the color changed from bright purple to black-green. The formation of a blackish-green color in the extract after adding  $\text{FeCl}_3$  because tannins will react with  $\text{Fe}^{3+}$  ions and will form a complex compound tricyanoferritrikaliumferri (III) [21]. The complex reaction between tannin and  $\text{FeCl}_3$  can be seen in Figure 2.



**Figure 2.** The Reaction between Tannins and  $\text{FeCl}_3$  Forms The Compound Tricyanoferritrikaliumferri (III)

From the results of the qualitative data, it can be concluded that the methanol extract of black turmeric contains flavonoids, phenolic, and tannin compounds, which are known to have a role as antioxidants. Flavonoids are a class of secondary metabolites generated by plants that are classified as polyphenols. Flavonoids can scavenge free radicals and reduce the oxidation of lipids. The antioxidant activity of flavonoids and phenols is proportional to their levels, so the higher the levels, the more effective the antioxidants [22].

### 3.3. Determination of Total Flavonoid Levels

#### 3.3.1. Maximum Wavelength and Operating Time

The measurement of flavonoid concentrations is done using quercetin as the benchmark. The maximum wavelength was achieved by utilizing quercetin at a concentration of 60 ppm. This was followed by a reaction with 10%  $\text{AlCl}_3$  to produce a bathochromic effect. Finally, 1 M sodium acetate was added in order to stabilize the complex formation between  $\text{AlCl}_3$  and flavonoids. The maximum wavelength from this investigation, 433 nm, is identical to the maximum wavelength from earlier studies, 433.5 nm [23]. The purpose of searching for the maximum wavelength is because, at the maximum wavelength, there is the greatest change in absorbance for each concentration unit so that maximum sensitivity will be obtained and errors will be minimized. Determination of operating time

to see the absorbance time of the compound in a stable state to minimize errors [24]. Operating time is obtained starting from 9 minutes to 21 minutes.

### 3.3.2. Standard Curve and Total Flavonoid of Black Turmeric Extract

Determination of the standard curve is carried out to determine the relationship between the concentration of the solution and the absorbance value so that the concentration of the sample can be known. From the absorbance data, a linear regression value was obtained  $y = 0.0101x + 0.0072$ ;  $r = 0.9911$ . The r-value, close to 1, indicates a linear calibration curve and a relationship between quercetin and the absorbance value. A graph of the comparison of quercetin standard concentrations with absorbance values can be seen in Figure 3.

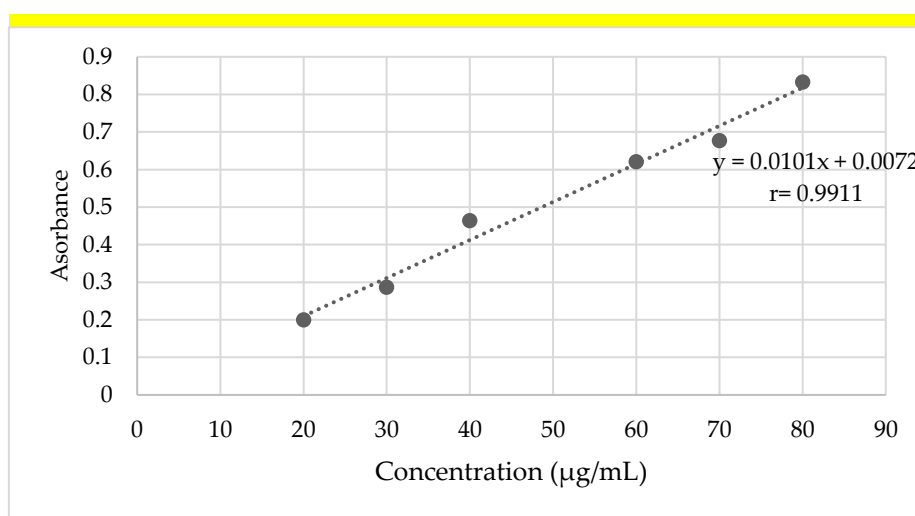


Figure 3. Graph of Quercetin Standard Curve between Absorbance and Concentration

Table 3 shows the levels of total flavonoids in the black turmeric methanol extract of  $10.326 \pm 0.074$  mgEQ/g extract.

Table 3. Total Flavonoid Content of Black Turmeric Extract

Replication	Sample Concentration	Absorbance	Total Flavonoid Content (mgEQ/g extract)	$\bar{x} \pm LE$
1		0.53	10.35	
2	1000	0.529	10.33	$10.326 \pm 0,074$
3		0.527	10.29	

In previous studies [25], the levels of flavonoids obtained were 2,709.88 mg/100g, greater than the research results. This is because at the drying stage, there is excessive heating which the researcher cannot control, and during the sifting process, a large sieve is used so that the contact between the solvent and the simplicity is less. Flavonoids are stable at 50°C. If the temperature is used > 50°C, there will be structural changes, and low levels of flavonoids will be obtained. The levels of flavonoids can be affected by growing location of the sample, such as temperature and CO<sub>2</sub>. The levels of flavonoid compounds are found to increase in direct proportion to both the temperature and the amount of CO<sub>2</sub> present [26].

### 3.4. Antioxidant Activity

Antioxidant activity test using the ABTS method. The parameters used in this method are the IC50 value or 50% Inhibition Concentration. IC50 is the concentration that can inhibit 50% of free radicals. In this test, quercetin was used as a comparison because quercetin is part of a flavonoid compound that can act as a strong antioxidant [25]. A mixture of 0.007 M ABTS solution with 0.002 M K2S2O8 acts as a free radical generator, and black turmeric extract is used as a sample which can act as an antioxidant. A mixture of ABTS and K2S2O8 forms a radical cationic compound that will get an electron pair by taking an H atom from an antioxidant compound [27]–[29].

#### 3.4.1. Maximum Wavelength and Operating Time

Determination of the maximum wavelength aims to determine the maximum absorption absorbed by the ABTS solution. The principle of measuring the antioxidant activity of the ABTS method is the reduction of ABTS+ free radicals so that the blue color of ABTS+ free radicals disappears. In this study, the maximum wavelength of the ABTS stock solution was 745 nm. There is no difference in the maximum wavelength with previous studies in journals, namely 745 nm [30]. The operating time obtained starts from 13 minutes to 17 minutes. At that minute, the reaction between ABTS and the sample was optimal as indicated by the stable absorbance value.

#### 3.4.2. Antioxidant Activity of Quercetin and Sample

Quercetin was used in this experiment as a comparison because of its part in a flavonoid molecule that has the potential to be a strong antioxidant. The solution will change color from blue-green to progressively clear as the absorbance value is reduced, which is used to measure free radical scavenging activity. This shows that when the absorbance value falls, antioxidant activity rises. To measure the quercetin test solution, a concentration series of 0.25, 0.5, 1, 2, and 3 ppm was utilized. The mean LE IC50 value for quercetin was  $1.09 \pm 0.198$  ppm. This illustrates that to reduce ABTS free radicals by 50%, a reference solution with a concentration of  $1.09 \pm 0.198$  ppm is needed. Quercetin's IC50 value of 50 ppm indicates that it has extraordinarily strong antioxidant activity in comparison. [25]. Quercetin standard IC50 values can be seen in Table 4.

**Table 4.** % Free Radical Scavenging and IC50 Value of Quercetin

No	%Free Radical Scavenging					Linear regression	IC50 (ppm)
	0.25 ppm	0.5 ppm	1.0 ppm	2.0 ppm	3.0 ppm		
1	42.89	44.50	47.45	56.56	67.02	$y = 8.7956x + 39.815 ; r = 0.9955$	1.15
2	43.96	47.58	51.47	54.95	65.14	$y = 6.997x + 43.181 ; r = 0.9822$	0.97
3	42.62	47.18	50.13	54.02	63.27	$y = 8.7956x + 39.815 ; r = 0.9955$	1.15
Average $\pm$ LE							$1.09 \pm 0.198$

In the antioxidant activity test, the sample obtained an average  $\pm$  LE IC50 value of  $88.581 \pm 3.376$  ppm (Table 5). This means that the concentration needed by the sample to reduce 50% of ABTS free radicals is  $88.581 \pm 3.371$  ppm. Based on IC50 calculations, black turmeric methanol extract has strong antioxidant properties because it is <100 ppm. As a result, flavonoid-rich samples of black turmeric methanol extract may have antioxidant properties. The calculated r-values for quercetin ( $y = 8.795x + 39.815$  with a calculated r value of 0.9960) and black turmeric extract ( $y = 0.3232x + 21.933$  with r count = 0.9882 greater than r table = 0.8783 with a 95% confidence level) show that the data from the regression equation is correct.

**Table 5.** % Free Radical Scavenging and IC50 Value of Black Turmeric Extract

No	% Free Radical Scavenging					Linear regression	IC50 (ppm)
	25 ppm	50 ppm	75 ppm	100 ppm	125 ppm		
1	29.207	39.221	44.645	57.162	60.639	$y = 0.3232x + 21.933$ ; $r = 0.9882$	86.840
2	13.953	31.600	43.365	58.549	64.021	$y = 0.5083x + 4.1724$ ; $r = 0.9878$	90.158
3	14.147	33.425	43.134	59.362	64.909	$y = 0.5098x + 4.7573$ ; $r = 0.9857$	88.745
Average $\pm$ LE							88.581 $\pm$ 3.376

#### 4. CONCLUSION

This study revealed that black turmeric has the potential to be employed as a natural ingredient. Black turmeric is an effective antioxidant. This is due to black turmeric's ability to inhibit free radicals, as indicated by its high flavonoid content  $10.326 \pm 0.074$  mgEQ/g extract and strong antioxidant activity with an IC50 of  $88.581 \pm 3.376$  ppm. With the increasing popularity of natural products in today's herbal medicine, the antioxidant and cytotoxic activity of *Curcuma caesia* Roxb. must be determined. In further studies, it is necessary to carry out comparative research using various methodologies and solvents, as well as antibacterial testing.

**Acknowledgments:** The author wishes to express gratitude to Jenderal Achmad Yani University Yogyakarta for providing the facilities and infrastructure required for this research. Additionally, the author would like to thank everyone who helped with this project.

**Conflicts of interest:** Declare conflicts of interest or state "The authors declare no conflict of interest."

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