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Turmeric Extraction (*Curcuma Longa L)* using *Reflux* Method and The Extract Characterization

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Abstract

Turmeric (Curcuma longa L) is one of the many spices that grow on the Asian continent, especially Southeast and South Asia, which can be utilized to its full potential, especially the antioxidant compounds contained in curcuminoids. This study aims to determine the effect of different forms of turmeric, powder and fresh, on the extraction using the reflux extraction method and antioxidant activity using the DPPH method (2,2-Diphenyl-1-PicrylHydrazyl). The reflux extraction process was carried out using distilled water with three variations of sample forms, turmeric powder, fresh turmeric (grated turmeric and pieces of turmeric) with a solute/solvent ratio (w/v) (1:5). The viscous extract in the form of a paste was obtained after the distillation process and solvent evaporation. The best extracts and essential oils obtained were extracts from powdered turmeric samples with yields of 8.28 (% w/w) and essential oils of 0.44 g, which were clearer than the other two samples. The analysis showed that the sample of turmeric with the highest antioxidant activity was a sample of freshly grated turmeric with an IC50 value of 114.7 ppm with a moderate level of antioxidant activity. The cut turmeric sample has an IC50 value of 158.3 ppm, which is included in the weak antioxidant activity. The powdered turmeric sample has an IC50 value of 134.1 ppm with moderate strength.

INTRODUCTION

In big cities, air pollution is a problem that always increases yearly (Abidin & Hasibuan, 2019). The oxidative burden of the body if living in a big city will be much higher than in places with lower pollution levels, such as rural areas. An environment with a relatively high pollution level triggers the emergence of free radicals. Free radicals are molecules that have unpaired electrons in their outer orbitals, so they are reactive and unstable (Sopiah et al., 2019). These radicals tend to have a chain reaction which, if they occur in the body, can cause ongoing and continuous damage. Free radicals produced by cigarette smoke, radiation, pollutants, and other radical-triggering substances can cause chronic diseases such as heart attacks, cancer, and cataracts to decreased kidney function (Fakriah et al., 2019). Antioxidant compounds are needed to prevent free radicals (Sopiah et al., 2019).

Antioxidants are inhibitors of the oxidation process, even at relatively small concentrations. If free radicals do not bind to antioxidants, the oxidation reaction will continue to cause cell damage (Andarina & Djauhari, 2017). One of the plants that contain antioxidants is turmeric.

Turmeric is one of the spices that are commonly found in Indonesia. Turmeric or turmeric rhizome contains curcumin and its derivatives by 3-15% (curcumin 71.5%, demethoxycurcumin 19.4% and bisdemethoxycurcumin 9.1%). The following chemical content is phenylpropene and other

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phenolic components such as terpenes, monoterpenes, sesquiterpenes, diterpenes, triterpenes, alkaloids, steroids, and fatty acids (Suprihatin et al., 2020). Curcumin is insoluble in ether, soluble in oil, brownish red in alkali, and light yellow in acid (Rezki et al., 2015). The level of antioxidants in turmeric is strongly influenced by the solvent used. The more polar the solvent, the higher the antioxidant content attracted by turmeric (Pratiwi & Wardaniati, 2019).

The uptake of curcumin in a plant allows the removal of essential nutrients and biological activity during processing. One of the most effective methods is extraction (Sa'adah, 2019).

Turmeric can be extracted using several methods, namely the soxhlet extraction method, maceration, reflux extraction and hydrodistillation. The reflux method is more efficient and requires a shorter time (Yurleni, 2018). Turmeric can be extracted using several methods, namely the soxhlet extraction method, maceration, reflux extraction and hydrodistillation. The reflux method is more efficient and requires a shorter time (Yurleni, 2018). Extraction by heating or reflux is a solvent-based extraction for a certain time with a limited and relatively constant amount of solvent (Susanty & Bachmid, 2016). In addition, extraction using water and acetone solvents is more suitable using the reflux method than maceration and soxhletation because the reflux method has higher yields of water solvent extract and acetone (Putra et al., 2014). Aquades is considered a solvent because it is a non-toxic stable solvent that tends to be safer, environmentally friendly, and cheaper in terms of cost (Sa'adah & Nurhasnawati, 2017). The extract's yield can be higher because the distilled water binds more of the extracted material due to the large number of hydrogen bonds formed (Masyitoh et al., 2016).

Turmeric has a high potential to be a source of natural antioxidants. However, to produce maximum curcumin compounds, research still needs to examine the effect of turmeric form on antioxidant activity. This study aims to determine the effect of variations in the shape of turmeric rhizome as a solute in the extraction of curcumin from the reflux process using distilled water as a solvent. Variations in the curcumin extraction process are carried out with variations in turmeric, which are powdered, grated, and pieces.

MATERIALS AND METHODS

Materials

Turmeric (*Curcuma Longan L.*) with three types of sample treatments namely turmeric rhizome (1) powder, (2) grated, and (3) pieces were obtained from Sampangan market, Semarang, Indonesia. Aquadest, water, 2,2-*diphenyl*-1-*picrylhidrazyl* (DPPH) and methanol pro analysis (99,8%) was obtained from CV. Indrasari, Karangkidul, Central Semarang, Indonesia.

Methods

Sample Preparation

Dry turmeric powder has been sifted and weighed as much as 50 grams. Meanwhile, fresh turmeric was peeled, cleaned, and then grated using a grater and cut to a size of ± 3-5 mm using a knife and a cutting board. Grated turmeric pieces was then weighed as much as 50 grams.

Extraction Process

The process of making turmeric extract using the reflux method. Samples was weighed for 50 grams and 250 mL of distilled water were put into neck flask 2. Next, neck flask two was paired with reflux extraction tools. The reflux process was carried out for 150 minutes at 100°C. After the process was complete and cooled, filtering was carried out to separate the filtrate and residue. The filtrate resulting from the reflux process was then distilled until the remaining filtrate in the distillation flask was ± 25 mL. The filtrate was then evaporated using an oven at a temperature of 100°C to evaporate the remaining solvent. Then after a constant filtrate mass was produced during evaporation, a thick extract was produced in the form of a paste.

Antioxidant Activity Test

Antioxidant activity test using DPPH (*2,2-diphenyl-1-picrylhydrazil*), whose absorbance was measured using a UV-Vis spectrophotometer.

Data Analysis

The absorbance value data obtained from the analysis of the UV-Vis spectrophotometer were used to calculate the inhibition value (% antioxidant activity). Then the resulting data were analyzed using the GraphPad Prism 8 program.

RESULTS AND DISCUSSION

Effect of Variation in Form on Extract Results

The observations found that the appearance was in the form of a slightly reddish brown paste (viscous extract), soluble in alcohol. The curcumin obtained can be seen in Figure 1**.** The results of the qualitative analysis research are not much different from the journals reported by (Popuri & Pagala, 2013) and also (Rezki et al., 2015). Of the three samples studied, the brown color in sample 1, the dry powder sample, was more concentrated than the other two. In comparison, the colors of samples 2 and 3, namely samples of fresh turmeric in grated form and pieces of both, have almost no difference in color.

Figure 1. Concentrated extract of turmeric sample (1) powder (2) grated (3) pieces.

Yield is the percentage ratio between the extract portion and the total weight of the initial sample used (Sari et al., 2020). Data on the % yield of turmeric extract in various forms of powder and fresh (grated and pieces) with distilled water can be seen in Table 1.

Table 1. Data on % yield of turmeric extract (*Curcuma Longa L*).

Sample shape	Initial sample weight (g)	Extract weight (g)	Yield (% w/w)
Powder	50	4.14	8.28
Grater	50	4.08	8.16
Piece	50	3.97	7.94
Average yield value			8.13

The average yield value of the extraction results was 8.13% (w/w). This value is influenced by several factors, one of which is the particle size of the sample (Wijaya et al., 2018). The size of the sample affects the amount of yield. The smaller the sample surface area will further expand the contact and increase the solvent interaction, the more yields will be produced (Sineke et al., 2016).

The Effect of Variations in Forms on the Yield of Essential Oils

From the observations made, it was found that the essential oil was distilled by distillation. From the results obtained that the good essential oil was from a variety of powder forms because the volatile oil produced was very clear, and the second best result was produced through turmeric rhizome material with grated treatment because the more small surface area of the material affects the yield of the essential oil itself. The results of these essential oils can be seen in Figure 2 and Table 2.

Figure 2. The results of the turmeric essential oil sample (1) powder (2) grated (3) pieces.

Table 2. Data on essential oil yields of turmeric extract (*Curcuma Longa L*).

Sample shape	Initial sample weight (g)	Oil weight (g)
Powder	50	0.44
Grater	50	0.29
Piece	50	0.20

Based on the results in Table 2, it is known that the highest essential oil yield was sample 1, namely turmeric powder. The highest essential oil yield was sample 3, namely the cut sample. The result indicates that the smaller the sample surface area, the more volatile oil is produced (Sineke et al., 2016).

Antioxidant Analysis

Quantitative testing using a UV-Vis spectrophotometer with a wavelength of 517 nm. The test was carried out by dividing the sample solution into five concentration variations, namely

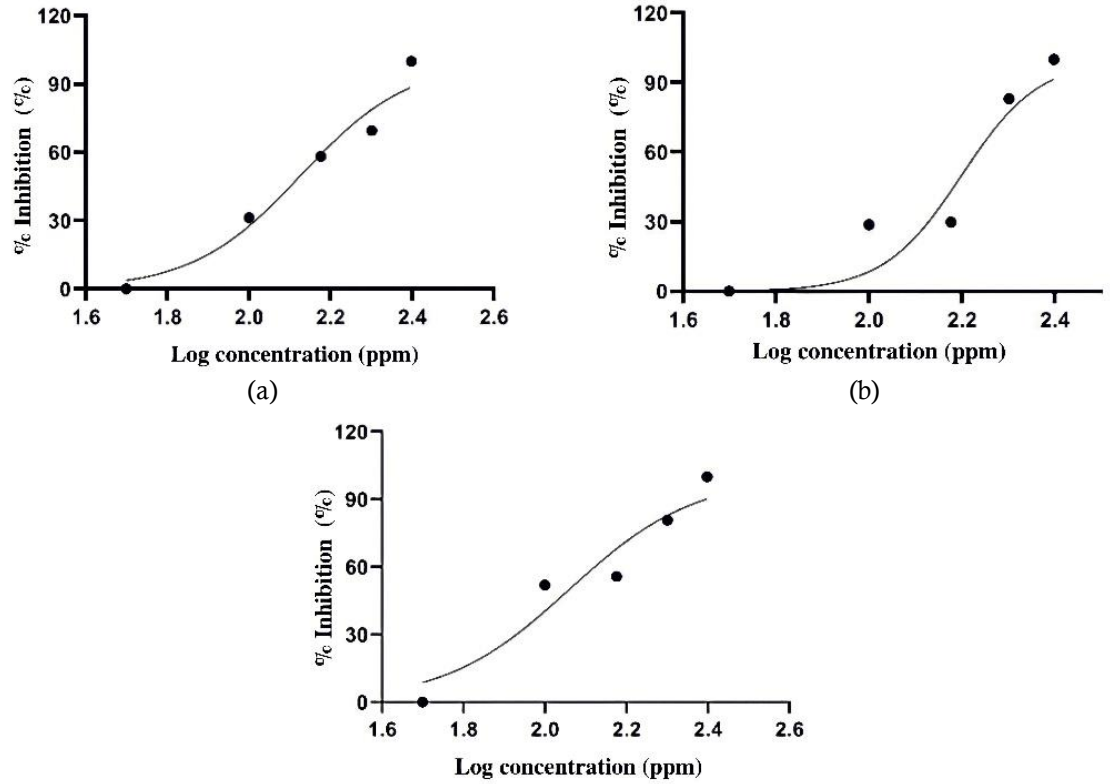

Figure 3. The curve of the relationship of % antioxidant activity to the concentration.

50 ppm, 100 ppm, 150 ppm, 200 ppm, and 250 ppm. The test solution added with DPPH solution was incubated for 30 minutes at room temperature to optimize binding between antioxidant compounds in capturing unpaired electrons from free radicals. Research (Irianti et al., 2016) stated that DPPH has a stable absorbance at 30 to 40 minutes. After incubation, a color change from purple to yellowish was caused by antioxidants reacting with DPPH, stabilizing free radicals and reducing DPPH (Erviana et al., 2016). The color change of the turmeric extract solution from purple to yellow indicates that the turmeric extract has antioxidant potential. The DPPH method was chosen because it has several advantages, including being simple, easy, fast, sensitive, Figure 3. The curve of the relationship of % antioxidant activity to the concentration and requires a small number of samples (Erviana et al., 2016).

The results of antioxidant measurements using a UV-Vis spectrophotometer were obtained in the form of absorbance values from the control and sample, which were then used to determine the percent antioxidant activity (% inhibition) (Pratiwi & Wardaniati, 2019). The wavelength used in the UV-VIS spectrophotometer is 517 nm because DPPH provides strong absorption at a wavelength of 517 nm with a dark violet color which provides information on the reactivity of the compound tested with a stable radical (Katrin, 2015). The following equation determines the % antioxidant activity: (Kuntorini & Astuti, 2012).

$$\text{antioxidant activity} = \frac{A_0 - A_1}{A0} \times 100 \quad (1)$$

Where, A_0 is absorbance of control solution and A_1 is Absorbance of sample solution

The absorbance and % inhibition values in the sample can be seen in Table 3. Table 3 shows that the greater the extract concentration, the greater the value of % antioxidant activity(Mariani et al., 2018). The highest % activity value was obtained from sample 3, namely sliced turmeric with a concentration variation of 250 ppm

According to Handayani et al., (2014), percent (%) antioxidant activity indicates the ability of an antioxidant compound to inhibit free radicals, namely the number of hydrogen atoms of an antioxidant compound that captures DPPH radicals so that they are reduced to more stable DPPH-H.

Table 3. Data on absorbance and % inhibition

Concentration	Absorbance			% Inhibition		
(ppm)	1	2	3	1	2	3
50	0.375	0.379	0.354	52.711	52.327	55.471
100	0.339	0.352	0.327	57.250	55.723	58.867
150	0.308	0.350	0.326	61.160	55.974	58.993
200	0.295	0.337	0.276	62.799	57.610	65.283
250	0.260	0.327	0.260	67.213	58.867	67.295
Control	0.793	0.795	0.795	-	-	-

The value of IC50 (50% Inhibitor Concentration) determines the radical scavenging activity. This value describes the concentration of the test compound that can capture radicals by 50% (Erviana et al., 2016). The smaller the IC50 value, the higher the antioxidant activity (Sumarlan et al., 2018). The IC50 value using a non-linear regression equation using *GraphPad Prism 8 software.* IC50 value data is shown in Table 4.

Table 4. The IC_{50} value of the turmeric extract sample.

Sample	IC50 value(ppm)
Tumeric powder	134.1
Grated tumeric	114.7
Cut tumeric	158.3

Based on the results obtained in Table 4, it is known that the lowest IC50 value is sample 2, grated turmeric, which shows that its antioxidant activity is higher than the other two samples (Yuliani et al., 2016). The correlation between the IC50 value and the strength of antioxidant activity is presented in Table 5.

Table 5. Strength Level of Antioxidant Activity (Tristantini et al., 2016).

Antioxidant Properties	IC50 value (ppm)
Very strong	<50
Strong	50-100
Medium	100-150
Weak	150-200

Table 4 and Tabel 5 show that the best results were obtained in samples of grated turmeric with an IC50 value of 114.7 in the range of 100-150, indicating that the antioxidant activity is moderate (Yuliani et al., 2016). Likewise, turmeric powder samples have weak antioxidant activity with an IC50 value of 134.1. At the same time, the cut sample has the largest IC50 value, 158.3, in the range of 150-200, so it is included in the weak category (Yuliani et al., 2016). This difference in IC50 value can be caused by the number of antioxidants in the extract. This phenomenon can occur due to antioxidant damage in the extract, which is influenced by the length of contact time between the active substance and the solvent, whose temperature increases due to prolonged heating (Tristantini et al., 2016).

The results of antioxidant activity obtained are not much different from the statement (Pratiwi & Wardaniati, 2019), namely the IC50 value of dried powdered turmeric samples is lower than the IC50 value of fresh-cut turmeric samples, which indicates that the antioxidant activity of powdered turmeric samples is higher. However, this study also produced the best IC50 value in grated turmeric samples, where the surface area was much smaller than the cut samples.

CONCLUSION

The difference in variations in the form of simplicity and fresh turmeric has an effect, although not too large, on the results of the reflux method turmeric extract with distilled water, namely the highest yield in the powder sample, which is 8.28% (w/w) and the weight of the oil produced is 0.44 g. The antioxidant activity tested by the DPPH method was also influenced by differences in dried powder and fresh (grated and pieces) with the best IC50 value, namely the grated fresh turmeric sample of 114.7 ppm with a medium strength category.

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