

2.1 Temulawak

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The effect of basic ingredients form, cold storage and precipitate separation on bioactive components and antioxidant activity of *Curcuma xanthorrhiza* Roxb^{1,*}Septiana, A.T., ¹Erminawati and ²Winarsi, H.¹Department of Food Technology, Faculty of Agriculture, Universitas Jenderal Soedirman, Purwokerto, 53123, Central Java, Indonesia²Department of Nutrition Science, Faculty of Health Sciences, Universitas Jenderal Soedirman, Purwokerto, 53123, Central Java, Indonesia

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Curcuma xanthorrhiza Roxb water extract is like *temulawak* drinks, are easily precipitated when stored in cold temperatures (refrigerator). This study aimed to determine the effect of basic ingredients form, cold storage and precipitate separation of water extract on the bioactive components and antioxidant activity of the *C. xanthorrhiza* water extract. The bioactive components qualitatively observed were tannins, flavonoids, glycosides, terpenoids, and saponins. Furthermore, curcumin, flavonoids, total phenolic, and antioxidant activity were quantitatively analyzed. Antioxidant activity was carried out by testing the capacity of DPPH radical scavenging. The capacity of free radical scavenging, total phenol, flavonoids and curcumin from water extract of *C. xanthorrhiza* from fresh rhizome without cold storage and without separation of the precipitate was the largest compared to other treatments, were 76.39%, 1.68 mg/mL, 2.47 mg/mL and 0.71 mg/mL, respectively. Separation of the precipitate from *C. xanthorrhiza* water extract reduces curcumin, flavonoid contents, total phenols and DPPH radical scavenging capacity.

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1. Introduction

Curcuma xanthorrhiza Roxb (commonly known as *temulawak* in Indonesia) is an important medicinal plant in Indonesia and several other countries. This rhizome is often used as an ingredient for medicines and more than 50 traditional medicinal recipes contain *C. xanthorrhiza* (Dharma, 1985). As a medicinal plant, *C. xanthorrhiza* has a variety of benefits ranging from hepatoprotection, anti-inflammatory, anticancer, antidiabetic, antimicrobial, cholera prevention, and ⁵hyperlipidemic potential (Hwang *et al.*, 2000). Traditionally, this plant is used as an ingredient in health supplements known as "jamu" or to cure certain health problems including hepatitis, liver complaints, diabetes, rheumatism, anticancer, hypertension, and heart disorders (Salleh *et al.*, 2016).

The benefits of *C. xanthorrhiza* may be due to its antioxidant activity. According to Gordon (1990), an antioxidant is a substance with the ability to prolong or prevent the lipid oxidation process. *C. xanthorrhiza* and tumeric acetone extract from sliced rhizomes dried using a dryer cabinet for 14 hrs have a higher inhibitory activity of peroxide and malonaldehyde formations from

linoleic acid than ginger (Septiana, Mustaufik, Dwiyaniti *et al.*, 2006). Antioxidant activity of boiling dried *C. xanthorrhiza* is greater than that of dry turmeric (Samsudin and Panigoro, 2013). *C. xanthorrhiza* extract is thought to be used as an anti-atherosclerosis or inhibitor of blood clots because it has inhibitory activity of LDL oxidation and accumulation of macrophage cholesterol (Septiana, Dwiyaniti, Muchtadi *et al.*, 2006). Bioactive components of *C. xanthorrhiza* such as curcuminoids (Menon and Sudheer, 2007) and xanthorrhizol (Jantan *et al.*, 2012) can function as antioxidants. *C. xanthorrhiza* also contains bioactive components in the form of alkaloids, saponins, quinones and triterpenoids (Panigoro *et al.*, 2013).

Jamu or fresh *C. xanthorrhiza* drink is commonly prepared by extracting it in water, as water is the safest solvent. The weakness of extraction by water is that curcuminoids produced from *C. xanthorrhiza* extraction by water are less than that by ethanol (Santoso, 2012). Extraction of active components by water causes the starch to be extracted. Starch can be easily precipitated by a variety of treatments such as drying treatment on *C. xanthorrhiza* rhizome, and cold storage treatment of *C. xanthorrhiza* water extract. Removal of starch

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precipitated by these treatments is thought to have an effect on the amount of extracted bioactive components.

C. xanthorrhiza as an ingredient of herbal medicines or fresh drinks can be dried using conventional method in the sun before use. This method is less controlled, leading to poorer product quality. Drying treatment in a controlled batch dryer can improve the overall product quality. In general, the drying time of *C. xanthorrhiza* is shorter when the temperature increases between 40°C to 60°C (Sapei et al., 2017). Making herbal medicine and temulawak drink can use the main ingredients of dry rhizome in the form of *C. xanthorrhiza* powder or fresh *C. xanthorrhiza*. The drying process is thought to affect compound contents and antioxidant activity.

Moreover, active components of *C. xanthorrhiza* water extract is affected by temperature and storage duration of the extract. Storing *C. xanthorrhiza* water extract and temulawak drink at cold temperatures in the refrigerator can increase the amount of precipitated starch. During cold storage, the starch granules retrogradation occurs. Cooled gelatinized starch can generate starch recrystallization to form insoluble retrograded starch (precipitate) (Raigond et al., 2015). Separation of precipitated starch in *C. xanthorrhiza* water extract can affect activity and the amount of bioactive components. This study was conducted to determine the effect of main ingredients form, cold storage and precipitate separation on compounds contents and antioxidant activity of *C. xanthorrhiza* water extract.

2. Materials and methods

2.1 Materials and tools

The main ingredient used in this study was *C. xanthorrhiza* rhizome from farmers in Purbalingga, Central Java, while the chemicals used were methanol, ethanol, standard curcumin, chloroform, H₂SO₄, FeCl₂, HCl, Na₂CO₃, folin-ciocalteau, acetic anhydride, and DPPH or 1,1-diphenyl-2-picrylhydrazyl (Merck, Germany).

The tools used were a scale (Ohaus, United States), erlenmeyer, beaker glass, test tubes (Pyrex, Germany), vortex, microwave (Electrolux), blender (Philips, Netherlands), refractometer (Krisbow, China), UV-Vis spectrophotometer, (Shimadzu 1240, Japan), and a cabinet dryer.

2.2 Production of *Cucurma xanthorrhiza* water extract

First, the *C. xanthorrhiza* rhizomes were washed and drained. The main ingredients used were fresh rhizomes and the powder of *C. xanthorrhiza*. *C. xanthorrhiza*

powder obtained from fresh rhizomes sliced 4 mm, dried at 57°C for 14 hrs using a cabinet dryer, crushed and filtered using a 60 mesh filter. This powder was dissolved in warm water, shaken and filtered. *C. xanthorrhiza* water extract from fresh rhizome was made by crushing the fresh rhizomes with warm water using a blender and filtering it with a filter cloth. The water extract obtained was given a storage treatment at refrigerator temperature for 12 hrs and precipitate separation. Each treatment was compared to the control treatment, namely *C. xanthorrhiza* water extract from fresh rhizomes without extract storage and without precipitate separation.

2.3 Phytochemical screening

Phytochemical screening such as terpenoids, glycosides, tannins, saponins and alkaloids (Roghini and Vijayalaksmi, 2018) and glycosides (Gul et al., 2017) were performed using the standard as follows.

2.3.1 Test for terpenoids

A volume of 1 mL of the extract was treated with 2 mL of chloroform and concentrated sulphuric acid. The formation of red-brown colour at the interface indicates the presence of terpenoids.

2.3.2 Test for tannins

A volume of 2 mL of extract was added to 0.5 mL of 2% ferric chloride. The formation of dark blue or greenish-black indicates the presence of tannins.

2.3.3 Test for saponins

A 2 mL of extract and 1 mL of distilled water was added together and shaken in a graduated cylinder for 15 min lengthwise. It resulted in the formation of 1 cm layer of foam that indicated the presence of saponins.

2.3.4 Test for alkaloids

To 2 mL extract, 2 mL of concentrated hydrochloric acid was added. Then few drops of Mayer's reagent were added. The presence of green color or white precipitate indicates the presence of alkaloids.

2.3.5 Tests for glycosides (Liebermann's Test)

The extract was added into 2 mL of acetic acid and 2 mL of chloroform. The mixture was then cooled and H₂SO₄ was added. The green colour showed the entity of aglycone, the steroidal part of glycosides.

2.4 Determination of curcumin content

Determination of curcumin content of *C. xanthorrhiza* water extract used curcumin as a standard

(Delfiya et al., 2014). The standard curve was made by making a series of curcumin dilutions in ethanol with various concentrations (0.2, 0.4, 0.6, 0.8 and 1.0 µg/mL). Curcumin from various *C. xanthorrhiza* water extracts and standardized curcumin were analyzed by inserting each sample into a spectrophotometer cuvette and measuring its absorbance at a wavelength of 425 nm. The absorbance vs concentration of the standardized curcumin curve was made by connecting a line at these points and a correlation equation was made. Curcumin concentrations were calculated using the absorbance vs concentration of standardized curcumin charts. Testing on water extract was carried out in the same fashion as the standard based on the concentration of standardized curcumin.

2.5 Determination of total phenolic content

The total phenolic compound of tamarind turmeric herbal drink determined by Folin-Ciocalteu methods (Hammerschmidt and Pratt, 1978) and gallic acid as the standard.

2.6 Determination of flavonoid content

Total flavonoid content is expressed in Quercetin Equivalent (QE) was measured using the colorimetric method at 415 nm (Pourmorad et al., 2006).

2.7 Determination of DPPH scavenging capacity

DPPH scavenging capacity was carried out by following the procedure of Sheikh et al. (2009). A 2 mL water extract sample (40000 ppm) was added into 2 mL of 0.16 mM DPPH solution in methanol. The mixture was shaken for 1 min, left for 30 mins in a dark place and the absorbance (Abs.) was measured at a wavelength of 517 nm. DPPH radical scavenging ability was calculated by the following equation:

2.8 Statistical analysis

DPPH radical scavenging capacity and content of curcumin, flavonoid and total phenolic contents were analysed by using Analysis of variance (ANOVA). If there was a significant effect, the analysis was continued with Duncan's Multiple Range Test (DMRT) at a 5% significance content.

3. Result and discussion

The results of the qualitative analysis indicated that *C. xanthorrhiza* water extracts both from fresh and dry *C. xanthorrhiza*, with or without 14 hrs of cold storage, and with or without precipitate separation contained terpenoids, phenolics, saponins, alkaloids, and glycosides (Table 1). This result confirmed with a study

reported by Panigoro et al. (2013), whereby the bioactive components of *C. xanthorrhiza* are in the form of alkaloids, saponins, quinones and triterpenoids.

Table 1. Results of phytochemical screening analysis

Treatment	Terpenoid	Phenolic	Saponin	Alkaloid	Glycoside
B1R1M1	++	+++	+	+	+++
B2R1M1	+	+	-	+	+
B1R1M2	++	++	-	+	+++
B2R1M2	+	+	-	+	+
B1R2M1	+	+++	+	+	+++
B2R2M1	-	+	-	+	+
B1R2M2	++	++	+	+	+++
B2R2M2	-	+	-	+	+

The form of main ingredients = fresh (B1) and dry or powder rhizome (B2), cold storage = without (R1) and with (R2) storage, precipitate separation = without (M1) and with (M2) precipitate separation

The analysis result of the effect of the main ingredients is presented in Table 2. Curcumin, flavonoid, total phenolic contents and DPPH radical scavenging capacity of water extract of dry *C. xanthorrhiza* were lower than that of fresh *C. xanthorrhiza*. Bioactive components in the form of curcumin, flavonoids and phenolic compounds in *C. xanthorrhiza* were sensitive to drying temperature at 57°C. In this case, heating may have caused the bioactive components to undergo a reaction, resulting in a decrease in their content. In line with this result, Panigoro et al. (2013) reported that curcumin content in fresh *C. xanthorrhiza* rhizomes were much higher than curcumin content in boiled dry *C. xanthorrhiza*.

The flavonoid content of *C. xanthorrhiza* water extract from fresh rhizomes was greater than that of dry *C. xanthorrhiza*. This is because flavonoids are active compounds that are sensitive to temperature. Drying or heating may cause the flavonoids to undergo a reaction, resulting in a decrease in their content. Therefore, the drying process of *C. xanthorrhiza* lowers flavonoid contents with air drying at room temperature (24°C) in herbs that contained more flavonoids than oven drying herbs at 40°C (Rababah et al, 2015). In this research, *C. xanthorrhiza* rhizomes were dried at a temperature of 57°C for 14 hrs in a cabinet dryer.

As with curcumin and flavonoids, the total phenolic content in the *C. xanthorrhiza* water extract from fresh rhizome was higher than that of the dry rhizome. This is because total phenolic compounds were degraded due to heating. The drying process of turmeric rhizome

Table 2. Curcumin, flavonoid, total phenolic contents and DPPH radical scavenging capacity of *C. xanthorrhiza* water extract

Treatment	Curcumin (mg/mL)	Flavonoid (mg/mL)	Total Phenol (mg/mL)	DPPH radical
Main ingredients (B)				
Fresh	0.59±0.17 ^a	1.92±0.77 ^a	1.76±0.17 ^a	70.15±7.42 ^a
Dry	0.08±0.03 ^b	0.31±0.21 ^b	0.59±0.25 ^b	32.13±14.44 ^b
Cold storage (R)				
Without storage	0.38±0.31 ^a	1.27±1.01 ^a	1.13±0.61	57.49±19.22
With storage	0.29±0.27 ^b	0.96±0.97 ^b	1.22±0.71	44.79±24.06
Precipitate separation (M)				
Precipitate + filtrate	0.39±0.32 ^a	1.48±1.06 ^a	1.34±0.67 ^a	55.69±19.58 ^a
Filtrate	0.27±0.26 ^b	0.76±0.78 ^b	1.01±0.61 ^b	46.60±24.63 ^b

Values are expressed as mean±standard deviation. Values with the same superscript within the column are not significantly different ($p<0.05$).

(Prathapan *et al.*, 2009), *Ficus carica* leaves (Jahangiri *et al.*, 2011) reduces the total phenolic contents.

In line with the result that drying of *C. xanthorrhiza* at 57°C resulted in the decrease in curcumin, flavonoid and phenolic contents, its DPPH radical scavenging capacity also decreased. DPPH radical scavenging capacity can be used to determine the antioxidant activity of *C. xanthorrhiza* water extract. The antioxidant activity of *C. xanthorrhiza* water extract from dry rhizome (32%) was less than that of the fresh rhizome (70.15%). Long drying time causes decreased antioxidant activity. The result of this study is in line with the studies on the effect of heat treatment on turmeric rhizomes (Prathapan *et al.*, 2009), and drying treatment on *Ficus carica* leaves (Jahangiri *et al.*, 2014) indicating that the treatments can reduce antioxidant activity in scavenging DPPH radicals. This antioxidant activity has a positive correlation with the contents of phenolic compounds, similar to that produced by Akinola *et al.* (2014).

Table 2 shows the result that cold storage on *C. xanthorrhiza* water extract reduces curcumin and flavonoid contents. The result contradicts the study conducted by Lu *et al.* (2019) that storage at cool temperatures (4°C) for 1 day has no effect on flavonoid contents in "cara cara" juice, and flavonoid contents in broccoli (Kałużewicz *et al.*, 2012). A decrease in curcumin and flavonoid contents during cold storage is thought to be caused by the precipitation of these compounds during cold storage.

In addition, Table 2 also shows that precipitate separation in water extract of *C. xanthorrhiza* can reduce the contents of curcumin, flavonoids, and phenolics. This causes precipitate separation to reduce DPPH radical scavenging capacity. Precipitate separation in *C. xanthorrhiza* water extract from fresh *C. xanthorrhiza* stored in cold temperature reduced contents of curcumin (Figure 1), flavonoids (Figure 2) and total phenolics (Figure 3) compared to that of fresh *C. xanthorrhiza*

without cooling. Curcumin (Kumavat *et al.*, 2013), flavonoid (Kumar and Pandey, 2013) and phenolic compounds (Soares *et al.*, 2020) are insoluble in water, extraction by water causes curcumin, flavonoid, and phenolic compounds to easily precipitate during cold temperature storage. Curcumin can be extracted by water because curcumin is thought to be covered in starch or water-soluble compounds such as sugar to form droplets. A study by Sahne *et al.* (2016) revealed that curcumin contents from extracted turmeric by amylase (4.1%) was greater than curcumin contents extracted using microwave-assisted extraction (3.7%) or using ultrasonic (3.92%). It appears that curcumin in turmeric and *C. xanthorrhiza* is protected or covered in starch that the extraction of curcumin can be carried out by amylase. As with curcumin, it is suspected that flavonoid and phenolic compounds other than curcumin are insoluble in water because they are covered in water-soluble compounds to enable these bioactive compounds to be extracted by water.

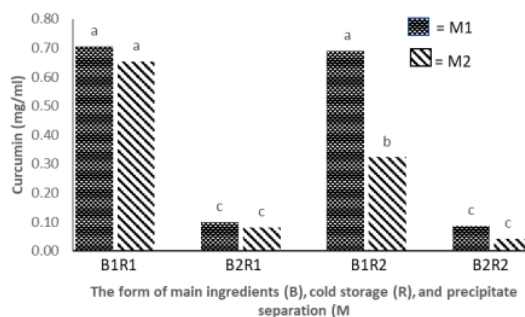


Figure 1. Effect of the form of main ingredients (B), cold storage (R) and precipitate separation (M) on curcumin content of *C. xanthorrhiza* water extract. The form of main ingredients = fresh (B1) and dry or powder rhizome (B2), cold storage = without (R1) and with (R2) storage, precipitate separation = without (M1) and with (M2) precipitate separation. Bars with the same alphabet notations for each treatment are not significantly different ($p<0.05$).

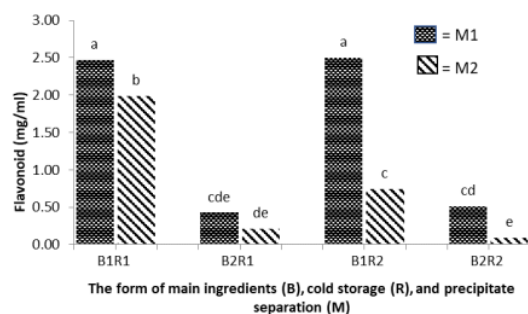


Figure 2. Effect of the form of main ingredients (B), cold storage (R) and precipitate separation (M) on flavonoid content in *C. xanthorrhiza* water extract. The form of main ingredients = fresh (B1) and dry or powder rhizome (B2), cold storage = without (R1) and with (R2) storage, precipitate separation = without (M1) and with (M2) precipitate separation. Bars with the same alphabet notations for each treatment are not significantly different ($p < 0.05$).

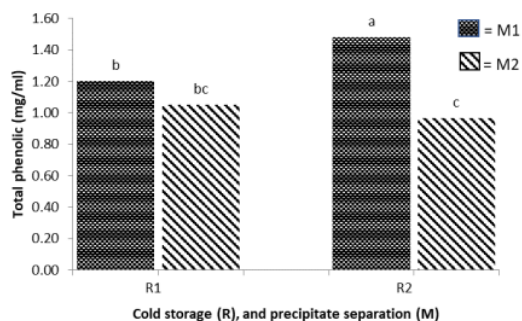


Figure 3. Effect of cold storage (R) and precipitate separation (M) on total phenolic content of *C. xanthorrhiza* water extract. Cold storage = without (R1) and with (R2) storage, precipitate separation = without (M1) and with (M2) precipitate separation. Bars with the same alphabet notations for each treatment are not significantly different ($p < 0.05$).

The total phenolic, curcumin, and flavonoid compounds have potential as an antioxidant. The correlation analysis between total phenolics, curcumin, flavonoids and antioxidant activity aims to determine the significance of the relationship between these compounds and antioxidant activity. The relationship between total phenolic, curcumin, and flavonoid contents and antioxidant activity by scavenging the DPPH had a correlation coefficient (R^2) of 0.90, 0.91, and 0.91 respectively. The resulting value was positive. Thus, the relationship between total phenolic, curcumin, and flavonoid contents on antioxidant activity was directly proportional. Based on the value of correlation coefficient (R^2) of more than 0.8, total phenolic, curcumin (as part of phenolic compounds) and flavonoid compounds had a significant correlation or these bioactive compounds had a significant effect on

antioxidant activity based on DPPH radical scavenging capacity.

4. Conclusion

C. xanthorrhiza water extract contained terpenoids, phenolics, saponins, alkaloids, and glycosides. Curcumin, flavonoid, total phenolic contents and DPPH radical scavenging capacity of *C. xanthorrhiza* extract water from fresh *C. xanthorrhiza* were higher than that of *C. xanthorrhiza* powder. Precipitate separation in *C. xanthorrhiza* water extract reduced curcumin, flavonoid, total phenolic contents and DPPH radical scavenging capacity. Precipitates of *C. xanthorrhiza* water extract should not be separated, both from the drying and cooling processes.

Conflict of interest

The authors declare no conflict of interest.

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