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Antioxidant Potential of Temulawak (*Curcuma xanthorrhiza roxb*)

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Abstract: Temulawak (*Curcuma xanthorrhiza roxb*) is a medicinal plant of the *Zingiberaceae* family. It grows in Indonesia and is used as a raw material for Indonesian traditional medicine. Temulawak is known to have many benefits, including being a potential antioxidant. The active component that is responsible for antioxidant activity in temulawak is curcumin. This study aims to analyze the potential of temulawak rhizome as an antioxidant. The raw materials used were obtained from temulawak rhizome farmers in Purworejo, Central Java. Extraction was performed using the liquid-liquid extraction method developed by PT Javaplant. Proximate analysis was performed and the antioxidant properties and curcumin level were measured. The antioxidant properties were measured using the DPPH method, whereas curcumin levels were measured using HPLC. The data obtained were processed and analyzed using SPSS and presented in the descriptive form. The curcumin and yield contents of the sample were 27.19 and 1.02%, respectively. The antioxidant capacity of temulawak (IC_{50}) was 87.01 ppm. The Temulawak extract exhibited antioxidant activity that was classified as active and is therefore considered to be a good natural antioxidant.

Key words: Temulawak, *Curcuma xanthorrhiza*, curcumin level, antioxidant

INTRODUCTION

Background: Temulawak (*Curcuma xanthorrhiza roxb.*) is one of the *Zingiberaceae* family medicinal plants that is grown in Indonesia and used as a raw material in Indonesian traditional medicine (Sidik *et al*., 1992; Prana, 2008). Empirically, the temulawak plant is widely used as a single drug or in combination with other drugs. There are more than 50 traditional medical formulations that contain temulawak (Achmad *et al*., 2007). Temulawak has long been recognized as a medicinal plant, particularly among Javanese populations. Temulawak rhizome is the predominant material used to make traditional medicine.

Temulawak is known for its health benefits as well as its ability to maintain health and prevent disease. As a medicine or a traditional medicine material, Temulawak represents a beacon of hope for the development of Indonesian traditional medicine as a phytotherapy preparation and its utility and safety have been previously reported (Sidik *et al*., 1992).

As a medicine or a traditional medicine, temulawak will be a new hope for developing traditional medicine in Indonesia as a form of phytotherapy preparation for which its usage and safety have been previously reported (Sidik *et al*., 1992).

Efficacy tests of temulawak rhizome have been previously reported in empirical *in vitro* studies, pre-clinical trials on animal models and a human clinical trial (BPOM, 2004). Empirically, temulawak rhizome has been reported to exhibit several health benefits, particularly as a potential antioxidant (WHO, 1999). The active components responsible for the antioxidant activity in temulawak rhizomes are curcumin, bisdemethoxycurcumin and demethoxycurcumin (Masuda *et al*., 1992). Previous studies have shown that temulawak rhizome exhibits antioxidant activity. The study by Jitoe *et al*. (1992) indicates that the antioxidant activity of temulawak extract is greater than the activity of three types of curcuminoid that can be individually derived from temulawak. Thus, it is conceivable that another substance in addition to those curcuminoids might contribute the antioxidant activity found in temulawak extract. A study by Rao (1995) showed that the antioxidant activity in curcumin is higher than that of vitamin E and beta-carotene. This is because the free radical-scavenging antioxidant activity of curcumin cannot be separated from its structure. Curcumin structure consists of a phenolic hydroxyl group and a beta-diketone group. The phenolic hydroxyl group serves as free radical scavenger within the first phase of the antioxidant process. The chemical structure of curcumin contains with two phenolic groups; each curcumin molecule can scavenge two free radical molecules. The beta-diketone group serves as free radical scavenger during the next phase. Therefore, this study aims to describe antioxidant activity of curcumin in temulawak (*Curcuma xanthorrhiza roxb.*) extract.

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MATERIALS AND METHODS

The material used in this study was sliced and dried temulawak rhizome (*Curcuma xanthorrhiza roxb.*) that was harvested at nine months of age. The material was obtained from temulawak farmers in Purworejo, Central Java.

Ethanol extraction of temulawak rhizome: This study used a liquid-liquid extraction of ethanol extract of temulawak in a hexane solvent developed by PT Javaplant. Up to 2.5 kg of dried temulawak powder was extracted using 70% ethanol and water and a maceration and percolation method at a 1:10 ratio for 2 hours at ±60°C inside an extraction flask with overhead stirring. After the extraction process, the extract was filtered using filter paper. The residue was re-extracted using similar extraction process followed by ethanol extraction using a liquid-liquid solvent hexane with stirring on a scale 7. Hexane exhibits non-polar characteristics, whereas curcumin exhibits polar characteristics. Thus, the polar compounds (such as essential oils) were drawn into the hexane phase, whereas the curcumin compound was expected to remain within the ethanol phase. The next step was the evaporation process at approximately 60°C, followed by sterilization at approximately 140°C for 2 sec and drying using a dry vacuum system.

Antioxidant test: Antioxidant activity was assessed using DPPH (1,1-diphenyl-2-picrylhydrazyl) based on Gaulejac *et al.* in Kiay *et al.* (2011). First, 0.5 mL of extract of methanol and water (dry and wet, respectively) were added to 2 mL of DPPH and vortexed for 2 min. The solution changed color from purple to yellow, indicating the free radical scavenging efficiency. Furthermore, at 5 min prior to the 30 min incubation, the absorbance was measured at a wavelength of 517 nm using a UV-VIS spectrophotometer. Activity of free radical scavengers was calculated as a percentage reduction of DPPH color using the equation:

$$\left[\begin{matrix}\text{Activity of free-radical}\\ \text{scavengers (\%)}\end{matrix}\right] = \frac{\left[\begin{matrix}\text{1-absorbance of}\\ \text{samples+control}\end{matrix}\right]}{\text{Absorbance of control}} \times 100\%$$

Determination of curcumin levels: Curcumin levels in temulawak were analyzed using high performance liquid chromatography (HPLC) (Jayaprakasha *et al.*, 2002). The HPLC injection was prepared as curcuminoid standard solutions in methanol at several concentrations as follows: 0.25, 0.5, 0.75 and 1 ppm. Next, all of the standard solutions were filtered using a 0.2 µm filter. A calibration curve was made by connecting curcuminoid concentration standard values with the outside area. Samples were prepared from a condensed extract of temulawak. Thirty milligrams of temulawak extract were dissolved in 10 mL of methanol. Furthermore, the stock solution was diluted multiple times to generate a curve that was appropriately fitted with standard series. Next, all of the samples were filtered using a 0.2 µm filter and the samples were ready for injection. Elution was performed by gradient phase with a water flow of 1 mL/min, at room temperature and an injection volume of 20 mL. The UV-VIS detector used in this study was set at a wavelength of 425 nm. The mobile phase consisted of a mixture of acetonitrile, 2% acetic acid and methanol. The column used was a C18 with a length of 300 x 4.6 mm.

Water levels: Water levels were determined by the oven drying method at 105°C to obtain permanent weight. Porcelain grails were dried in an oven at 105°C for 3 h and then placed in a desiccator for 1 h. After weighing, 2.0-2.5 g of sample was added into a grail and placed into the oven at 105°C for 3 h, followed by incubation in the desiccator for an hour. Grails and samples were weighed together to determine the water levels. This stepwise analysis was repeated four times per sample.

Analysis of ash levels: The ash levels were determined by removing organic materials through incineration at high temperatures between 600-650°C in a heat-resistant-furnace. Porcelain grails were dried in an oven at 105°C for 3 h, followed by desiccation for 1 h. After weighing, 2.0-2.5 g of sample was added into the grail. The grails and samples were dried in an electric furnace at 650°C for 18-24 h. The samples that were rendered into ashes were placed in a desiccator for 1 h. Grails and samples were weighed together to determine the ash levels. This stepwise analysis was repeated for four times per sample.

Analysis of protein levels: Protein content was determined using the Kjeldahl method. Nitrogen content was converted into ammonium sulfate by destruction at high temperature using potassium sulfate and mercury oxide as catalysts. Next, the resulting ammonium sulfate was steam distilled after the addition of natrium chloride to convert ammonium sulfate into ammonium. The ammonium was then bound by boric acid as a container for distillation. Total nitrogen level, which was obtained through titration, was multiplied by a factor of 6.24 to determine the protein levels.

Analysis of fat level: Fat levels were determined using a modified Soxhlet-Weibull method. Next, 2.0-2.5 g of sample was added into a 400-mL glass beaker and hydrolyzed with chloride acid to release the fat for further extraction by diethyl ether. Diethyl ether was evaporated in an oven at 105°C. After being chilled, final fat levels were determined by weighing the fat residue. This analysis process was repeated four times.

Analysis of carbohydrates levels: Carbohydrate levels were determined using the following formula:

$$\begin{bmatrix}\text{Percentage of}\\ \text{Carbohydrate levels}\end{bmatrix} = \begin{bmatrix}(100\%-(\text{ash content}+\text{protein}\\ \text{content}+\text{fat content})\end{bmatrix}$$

Data analysis: The data obtained were processed and analyzed using SPSS and presented in descriptive form.

RESULTS AND DISCUSSION

Temulawak composition: The temulawak rhizomes used in this study were prepared by washing, slicing and drying in the sun. Proximate analysis of the dried temulawak was conducted to determine nutrient content, such as carbohydrate, protein, fat and ash. Table 1 shows that the water level within the temulawak sample is 9.80%. Traditionally, temulawak is dried by sunlight for 3-5 days. The purpose of this drying process is to reduce damage caused by microbes, extend the shelf life and render the resulting material less fragile. One of the main quality parameters of temulawak is its water level (RSNI, 2006), as microorganisms can easily grow in temulawak rhizomes if the water level is in excess of 10%. This can affect the enzymatic reactions that facilitate decomposition. A study conducted by Cahyono *et al.* (2011) compared water levels of temulawak when it was dried using an oven at 60°C and/or a 30-watt lamp. The study showed that oven-/lamp-dried temulawak exhibited more favorable water levels compared to sunlight-dried temulawak. The water levels of temulawak that was dried using an oven at 60°C and a lamp for five days were 4.06 and 6.38%, respectively.

Table 1 shows that based on proximate analysis, the predominant component found in temulawak is starch. Temulawak starch is a white-yellowish powder that contains trace amounts of curcuminoid. Curcuminoids are ovate-to-oblong molecules, with one square end, a hilum position that is not in the central position and a non-concentrated lamella. The unique structure of temulawak molecules can be used to identify temulawak rhizome simplisia. Starch levels of temulawak depend on the location of origin. The higher the elevation of origin, the lower the starch level (Sidik *et al*., 1992). Proximate analysis indicated that starch levels are the predominant component found in temulawak. Thus, it can be developed as a raw material in the food industry and as tablets material in the pharmaceutical industry (Eni, 2006).

Ash levels in dried temulawak (3.29%) represent a parameter to reflect mineral (inorganic materials) composition. Inorganic materials found in temulawak include calcium, potassium, phosphorus, iron and magnesium. Among the *Zingiberaceae* family, temulawak contain less ash than ginger or turmeric. This is consistent with a study by Yusron *et al.* (2009), which found that ash levels of 5.28% compared to our finding of 3.67% in the first strain of temulawak. These findings indicate that temulawak contains the lowest mineral levels of members of the *Zingiberaceae* family. The level of curcumin in the dried temulawak sample was 2.02%. Curcumin levels in this study were lower than those found in a study by Aan (2004). The level of curcumin found by Aan was 2.43%, whereas Afif (2006) stated that the level of curcumin in temulawak is 2.98%. However, Aries (2012) mentioned that the level of curcumin in temulawak is only 1.45%. Those differences are attributed to the harvest age, geographical location of cultivation, varieties of temulawak, types of rhizome and different analysis methods employed.

Temulawak extract yield: The temulawak yield obtained using the ethanol liquid-liquid extraction with hexane solvent method developed by PT Javaplant was 1.02%. Although the method used was similar, the yield obtained in this study was less than yield obtained by Afif (2006), who obtained the following: 1.29-1.47% (with a ratio of material:solvent of 1:1); 1.36-1.65% (with a ratio of material:solvent of 1:2); 1.42-1.70% (with a ratio of material:solvent of 1:3). Studies by Ria (1989) and Yusro (2004) reported yields of 15.70-19.19 and 11.22%, respectively, using the maceration method. In contrast, a study by Suwiah (1991) reported temulawak yields of 21.87-66.74% using the reflux method. According to Afif (2006) those differences are attributed to method, the amount of solvent, the extraction time, the powder size and the temperature. The age of the harvest also affects the amount of temulawak yield. Rosiyani (2010) reported temulawak yields between 9.092-10.605%.

The extracted yields of differentially aged temulawak rhizomes are significantly different. Yields obtained from 8- and 9-month-old temulawak rhizomes are significantly different from 7-month-old rhizomes. The yield reflects the number of components that are extracted during maceration. Differences in extracted yield obtained from temulawak rhizomes are affected by the amount of active compounds present in the rhizomes. This suggests that 8-month-old temulawak rhizomes contain more active compounds than 8- and 9-month-old rhizomes. However, the yield cannot be used as a measurement of curcuminoid or xanthorrhizol levels not only because of the extracted secondary metabolites but also because of the total metabolites extracted during the extraction process.

Curcumin levels in temulawak extract: The curcumin level obtained in this study was 27.19%. This result was lower than that previously reported by Afif (2006). Studies by Yusro (2004), Aan (2004) and Aries (2012) reported curcumin levels of 1.68, 13.65 and 0.70%, respectively. Among the various types of separation methods, liquid-liquid extraction is the optimal method, because this

separation can be performed both at the macro and micro levels. The liquid-liquid extraction method is principally based on the distribution of solvent with a specific ratio of two solvents that cannot be mixed (Khopkar, 1990). The difference of curcumin levels, in addition to extraction method, is also attributed to the age of the temulawak rhizomes at harvest. Rosiyani (2010) reported that the highest levels of curcumin obtained from temulawak were those from rhizomes harvested at 9 months of age rather than those harvested at 7 or 8 months of age.

Antioxidant activity of temulawak extract: Antioxidant activity can be determined by measuring the ability of temulawak extract to scavenge free radicals. Antioxidant compounds play important roles in the body's defenses against the adverse effects of free radicals. We measured antioxidant activity using the DPPH method. The DPPH method is based on the ability of antioxidants to inhibit free radicals by donating a hydrogen atom. Table 2 shows that IC_{50} value of temulawak extract is 87.01 ppm. The IC_{50} value indicates that temulawak extract can scavenge 50% of the free radical DPPH at a concentration of 87.01 ppm. The lower the IC_{50} value of a material, the higher its antioxidant activity. Thus only a small amount of sample is needed to quench 50% of the free radical DPPH. According to Jun *et al*. (2003), a substance can be classified as an active antioxidant if it has a IC_{50} value of 50-100 ppm. The antioxidant activity of temulawak is higher than that of vitamin C (Rachman *et al*., 2008). The antioxidant activity of temulawak is affected by its harvesting age. Rosiyani (2010) reported that 9-month-old temulawak rhizomes exhibit higher antioxidant activity than those harvested at 7 or 8 months of age. This is attributed to the curcuminoid levels found in older temulawak rhizomes. The higher the curcuminoid level in the temulawak extract, the higher the antioxidant capacity. However, a study by Kusuma (2012), which compared the antioxidant activity of turmeric and temulawak, showed that curcuminoid levels found in kunyit (74.57%) is higher than that found in temulawak (20.04 mg/g).

We measured antioxidant activity in this study using the DPPH method. According to Rosiyani (2010), the antioxidant activity of DPPH is influenced by the active components found in temulawak. Those active components act as an antioxidant that convert free radicals into a stable form by electron transfer. The reactive group in DPPH is a nitrogen group, which pairs with a hydrogen atom in the antioxidant to form a stable DPPH radical. The ability of antioxidants in temulawak extract to absorb DPPH radicals can be colorimetrically detected. The decreased color intensity occurs via a single electron transfer mechanism that causes the color to change from purple to yellow. The more electrons that are donated, the more the purple color will fade and the more the yellow-brownish color will appear, reflecting a high concentration of antioxidant. The antioxidant activity measured by the DPPH method is based on DPPH radical absorption by the antioxidant compounds found in temulawak rhizome extract. DPPH is a stable free radical in aqueous or methanol solution and exhibits significant absorption at a wavelength of 517 nm.

Table 1: Proximate analysis result for dried temulawak

Composition	Dry curcuma (%)
Water	9.80
Ash	3.29
Fat	2.84
Protein	3.30
Starch	48.59
Curcumin	2.02

Table 2: Antioxidant activity of temulawak extract

Samples	Ppm	Inhibition (%)	IC_{50}
Temulawak	0	0	87.01
	31.25	22.07	
	62.5	39.77	
	93.75	59.08	
	125	71.03	
	156.25	80.69	
Vitamin C	0	0	1.47
	1.00	28.46	
	1.50	50.00	
	2.00	70.74	
	2.50	93.09	
	3.00	98.67	

Conclusion: Temulawak extract has an antioxidant activity of 87.01 ppm, which can be categorized as a relatively active and favorable source of natural antioxidant. Temulawak extraction using the liquid-liquid method resulted in a curcumin level of 27.19% and yield of 1.02%.

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