



The Effectiveness of Adding Red Fruit Oil (*Pandanus conoideus Lamk.*) into Ethanol Extract of Temulawak rhizome (*Curcuma xanthorrhiza Roxb.*) as Antioxidant

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Abstract

Curcuma xanthorrhiza Roxb (temulawak) is a native Indonesian plant; ethnobotanically, it can facilitate breast milk, body fresheners, stomach lozenges, and seizure medications. Temulawak contains essential oils, curcuminoids, and xanthorrhizol. Curcuminoids and xanthorrhizol have potential as antioxidants. Antioxidants in the body are useful for preventing oxidation reactions caused by free radicals, both from body metabolism and external factors. Curcuminoids are difficult to dissolve in water and have a very low bioavailability. Combining curcuminoids with oil can increase the absorption of curcuminoids into the systemic system. The low solubility and bioavailability can be overcome by combining temulawak rhizome extract and red fruit oil. *Pandanus conoideus Lamk* (red fruit), ethnobotanically, effectively prevents blindness, intestinal worms, skin diseases, and increasing stamina. Red fruit contains vitamin C, vitamin E, flavonoids, and β -carotene, potentially as antioxidants. Each sample of *Curcuma* and red fruit, as well as a mixture of *Curcuma* and red fruit samples, were tested for their antioxidant activity using the DPPH method (1,1-diphenyl-2-picrylhydrazyl). The ethanol extract of temulawak rhizome has intense antioxidant activity because it has an IC_{50} of 55.21 ppm. Red fruit oil has feeble antioxidant activity because it has an IC_{50} of 2604.77 ppm. The 1:1 ratio between red fruit oil and the ethanol extract of temulawak rhizome has weak antioxidant activity because it has an IC_{50} of only 1568.24 ppm. Meanwhile, the 1:10 ratio of red fruit oil and ethanol extract of temulawak rhizome has robust antioxidant activity because it has an IC_{50} of 19.85 ppm. It is concluded that the addition of oil to the ethanol extract of *Curcuma* can increase antioxidant activity.

1. Introduction

Degenerative diseases are caused by the antioxidants present in the body are unable to neutralize the increased concentration of free radicals. Free radicals become toxic to the body, which further damages body cells' function and can cause degenerative diseases [1]. Antioxidants are needed to prevent oxidative stress. Oxidative stress is an imbalance between the number of free radicals present and the number of antioxidants in the body. Various natural ingredients native to Indonesia contain a lot of antioxidants with different active ingredients. The use of

native Indonesian natural ingredients as antioxidants is needed to improve public health quality [2].

Temulawak is empirically widely used as a traditional medicine in single or mixed forms to treat various diseases. Several studies state that *Curcuma* has anti-tumor, anti-cancer, and hepatoprotective activity [3, 4]. Curcuminoids and xanthorrhizol in temulawak rhizome have potential as antioxidants [5, 6].

The clinical application of curcuminoids is minimal because of their poor water solubility and low bioavailability [7]. Efforts should be made to overcome this problem, including combining curcuminoid extracts

into the solid fat nanoparticle carrier system [8]. According to the WO patent 2013/176555, combining curcuminoids with oil can increase curcuminoid absorption into the systemic system. Research on curcumin combined with shellfish oil in a ratio of 1: 1 to 1:20 has been carried out [9].

The red fruit is empirically used as traditional medicine, natural dyes, and a source of food ingredients by the Papuan people. Red fruit juice extracted from fruit flesh has been used by the community to treat degenerative diseases [10]. Red fruit is a natural ingredient that can function as an antioxidant because red fruit contains vitamin C, vitamin E, flavonoids, β -carotene, and other compounds. Red fruit also contains high doses of unsaturated fatty acids (omega-9 and omega-3), easily digested and absorbed, thereby accelerating the metabolic process in overcoming various degenerative diseases [11].

One of the ways to determine the antioxidant activity is the DPPH (1,1-diphenyl-2-picrylhydrazyl) capture method. DPPH is a stable radical that can react with other radicals, form a stable compound, or react with hydrogen atoms (which come from an antioxidant) to form reduced DPPH (DPPH-H). This method is an easy and accurate radical scavenging method with reliability to measure the antioxidant capacity and has a simple technique [12]. DPPH radicals' ability to be reduced or stabilized by antioxidants was measured using a decrease in absorbance at a wavelength of 517 nm.

Based on the above background, in this study, an attempt was made to replace the combination of curcuminoids and shellfish oil or solid fat by combining curcuminoids in the ethanol extract of temulawak rhizome red fruit oil, and its activity as an antioxidant was measured.

2. Methodology

2.1. Materials and Equipment

The equipment used was a set of glassware, a collection of maceration tools, a blender, analytical balance, Rotary evaporator R-100, Sonicator LBS2-10, Centrifuge 5424 R, Spectrophotometer UV-Vis M200 PRO, LC-MS Q-TOF MS. The materials used were red fruit (*Pandanus conoideus* Lamk.), temulawak (*Curcuma xanthorrhiza* Roxb.), 96% ethanol, methanol, Milli-Q Water, DPPH (1,1-diphenyl-2-picrylhydrazyl).

2.2. Sample Preparation

4 kg of red fruit samples (*Pandanus conoideus* Lamk.) came from Wamena, Papua. Fresh red fruit is cleaned and separated from the stump. 6 kg of *Curcuma xanthorrhiza* Roxb. Samples were obtained from medicinal plants and spices, Lembang, Bandung. The temulawak rhizome is cleaned, chopped, dried, and mashed. Plant determination was carried out at the SITH Herbarium Laboratory, Bandung Institute of Technology.

2.3. Extraction by Maceration Method [13]

Temulawak and the prepared red fruit were then extracted by maceration using 96% ethanol solvent for 24

hours at room temperature and a closed container. The extract was filtered using filter paper. The resulting residue was macerated again with the same solvent. The liquid ethanol extract was evaporated until all the solvents evaporated to obtain the ethanol extract of red fruit oil and concentrated temulawak rhizome ethanol extract. The ethanol extract of concentrated red fruit oil was decanted to produce ethanol extract of red fruit and red fruit oil.

2.4. Liquid chromatography-mass spectrometry (LC-MS) [14]

The ethanol extract samples of temulawak rhizome and red fruit oil dissolved with methanol after the sample dissolved and then filtered using a 0.45-micron millipore. The sample filtrate was injected into the vial and measured by LC-MS.

2.5. Antioxidant Activity Test [15]

1 mg of DPPH was dissolved with 6.25 mL of methanol (4×10^{-4} M DPPH solution). 0.1 gram of the sample was dissolved in methanol to the 10 mL limit mark. The samples were ionized until dissolved and centrifuged. Then the supernatant was taken (a 10,000-ppm solution was obtained). The antioxidant activity test of the temulawak rhizome ethanol extract, red fruit oil, then a mixture of red fruit oil and temulawak rhizome ethanol extract with a ratio of 1: 1 and 1:10. The solution was then incubated for 30 minutes, then measured at a wavelength of 517 nm using a methanol blank. The absorbance value of each concentration variation was recorded, and the IC_{50} value was calculated.

3. Results and Discussion

3.1. Sample Preparation

Temulawak samples are converted into dry powder to remove water and avoid oxidation or hydrolysis that causes microorganisms' growth (fungi). The refinement of the sample aims to obtain the same surface area, thereby increasing secondary metabolites' extraction in the sample. Fresh red fruit samples are not dried in the sun to avoid oxidation or hydrolysis that causes microorganisms' growth (fungi). Red fruit has a moist physical characteristic because it contains oil.

3.2. Extraction by Maceration Method

Maceration aims to attract the compounds contained in each of these samples. During the maceration process, stirring is carried out so that the compounds contained are quickly extracted and homogeneous. The maceration method can avoid the destruction of thermolabile compounds [16]. Ethanol is considered a solvent because it is more selective. Molds and germs are difficult to grow in ethanol 20% and above. Also, ethanol is non-toxic, neutral, has good absorption, and inhibits enzyme action. The right maceration time results in optimal compound extraction, but the maceration time is too short, so that not all dissolved compounds in the solvent are used [17]. The evaporation principle is a pressure drop in the round bottom flask and the spinning of the round bottom flask so that the solvent can evaporate more quickly below its boiling point. The result of ethanol extraction from

concentrated temulawak rhizome was 2.88% yield, and ethanol extract from concentrated red fruit oil was 2.38% yield.

3.3. Liquid chromatography-mass spectrometry (LC-MS)

LC-MS is an analytical technique that combines physical separation capabilities with the specificity of mass spectrometric detection of a chemical against the presence of other substances (in complex mixtures).

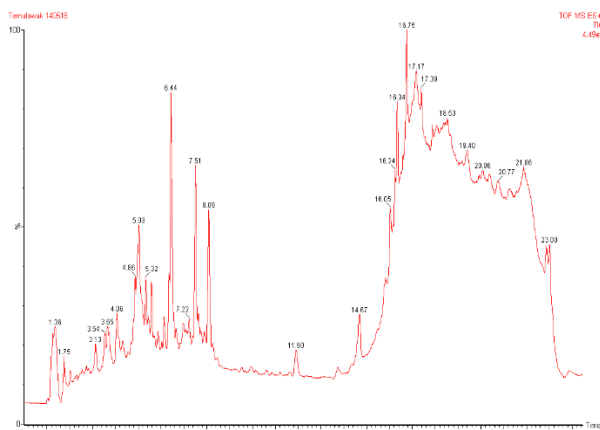


Figure 1. LC-MS chromatogram. Ethanol extract of temulawak rhizome

The results of LC-MS ethanol extract of temulawak rhizome obtained curcumin at a retention time of 5.03 minutes, xanthorrhizol at a retention time of 7.51 minutes, and germacrone at a retention time of 8.09 minutes.

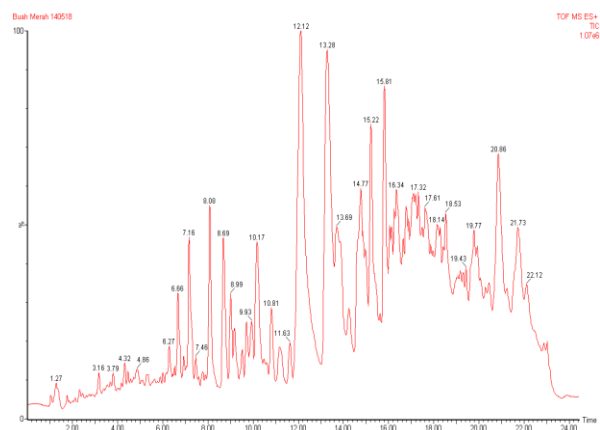


Figure 2. LC-MS Chromatogram of Red Fruit Oil.

From the LC-MS results of the first red fruit oil, it was found that oleic acid at a retention time of 8.08 minutes, linoleic acid at a retention time of 8.69 minutes, and decanoic acid at a retention time of 4.32.

3.4. Antioxidant Activity Test

DPPH is a stable radical that can react with other radicals to form a stable compound or react with hydrogen atoms (which come from an antioxidant) to create reduced DPPH (DPPH-H). DPPH, which reached a steady state due to the role of the antioxidants tested, was measured for its absorbance at the maximum wavelength

of DPPH. The measured absorbance value has decreased compared to the blank, due to reduced antioxidants or reacting with radicals. DPPH solution is purple. The reduction process is characterized by a change or fading of the solution's color, from dark purple (free radical compounds) to slightly yellowish colors (free radical compounds that are reduced by antioxidants). The color fading results in a decrease in the absorbance value of visible light from the spectrophotometer. The lower the absorbance value, the higher the antioxidant activity.

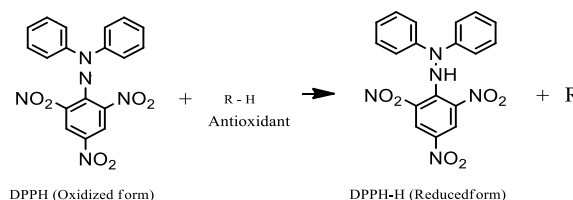


Figure 3. DPPH reaction with antioxidants

In this study, the antioxidant activity test was carried out on the ethanol extract of temulawak rhizome, red fruit oil, ethanol extract of temulawak rhizome, and red fruit oil at ratios of 1: 1 and 1:10.

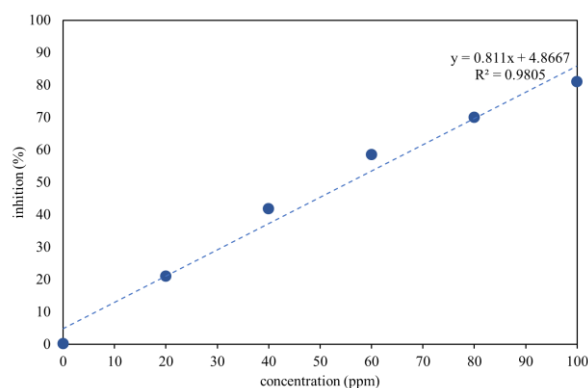


Figure 4. Antioxidant activity of the ethanol extract of temulawak rhizome.

According to Molyneux [15], the IC₅₀ value in the range of 50–100 ppm has intense antioxidant activity. The IC₅₀ obtained from the antioxidant test results of the ethanol extract of temulawak rhizome is 55.203 ppm, which means it has strong antioxidant activity.

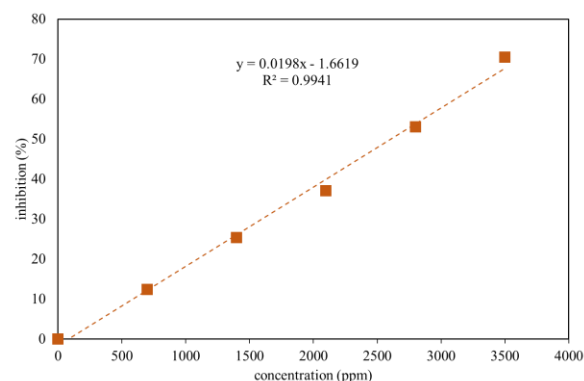


Figure 5. Antioxidant activity of red fruit oil

According to Molyneux [15], the IC₅₀ value >200 ppm has feeble antioxidant activity. The IC₅₀ obtained from the

antioxidant testing results for red fruit oil is 2611.38 ppm, which means it has weak antioxidant activity.

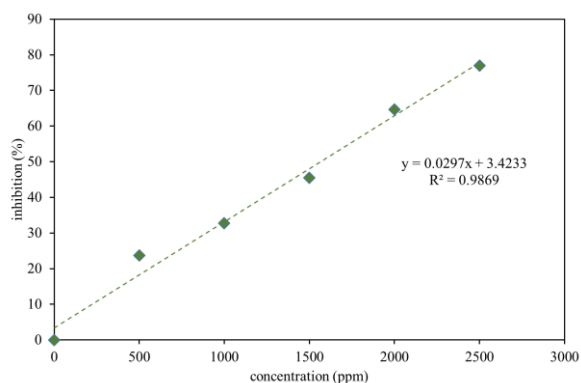


Figure 6. Antioxidant activity of a mixture of red fruit oil and ethanol extract from temulawak rhizome with a ratio of 1: 1

Judging from the IC_{50} results of the temulawak rhizome ethanol extract and red fruit oil, a comparison between the red fruit oil and the ethanol extract of the temulawak rhizome was made with a ratio of 1: 1. According to Molyneux [15], the IC_{50} value > 200 ppm has feeble antioxidant activity. The IC_{50} obtained from the antioxidant test results of a mixture of red fruit oil with ethanol extract of temulawak rhizome with a ratio of 1: 1 is 1565.62 ppm, which means it has feeble antioxidant activity.

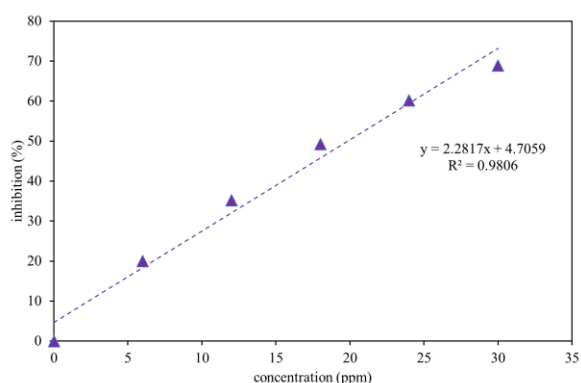


Figure 7. Antioxidant activity of a mixture of red fruit oil and ethanol extract from temulawak rhizome with a ratio of 1: 10

The antioxidant activity test with a ratio of 1: 1 is still very weak, so a ratio of 1:10 is carried out to see its effectiveness. According to Molyneux [15], IC_{50} values in the range <50 ppm have robust antioxidant activity. The IC_{50} obtained from the antioxidant test results of the mixture of red fruit oil and the ethanol extract of the temulawak rhizome with a ratio of 1:10 was 19.85 ppm, which means that it has a robust antioxidant activity. From the IC_{50} data ratio of 1: 1 and 1:10, the best effectiveness of adding red fruit oil to the ethanol extract of temulawak rhizome is a ratio of 1:10 because this ratio can reduce the IC_{50} value, which means increased antioxidant activity.

4. Conclusion

The IC_{50} obtained from the antioxidant testing results for the ethanol extract of temulawak rhizome, red fruit oil, a mixture of red fruit oil, and ethanol extract with a ratio of 1: 1 and 1:10 are 55.21 ppm, 2604.77 ppm, 1568.24 ppm, and 19.85 ppm respectively. The highest effectiveness of adding red fruit oil to the ethanol extract of temulawak rhizome is in the ratio of 1:10 because this ratio can reduce the IC_{50} value, which means increased antioxidant activity.

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