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Effect of the Extraction Solvent Polarity and the Ratio of Feed and Solvent on the Phytochemical Content and Antioxidant Activity of Red Betel Leaves (*Piper crocatum*)

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Abstract. Red betel leaf (*Piper crocatum*) is a plant growing in tropical areas and previously known as an ornamental plant, but then later used as medicinal plant. Previous researches found that red betel leaves contain bioactive compounds such as alkaloids, flavonoids, tannins, and many more that have potential to be used as antioxidant. The extractions were carried out using variations of solvent types (ethanol, water, and ethyl acetate) and feed to solvent (F:S) ratios in g/ml (F:S=1:20, F:S=1:25, F:S=1:30). The best conditions from this research were the usage of ethanol as the solvent. In addition to its ability to extract the compounds potential as antioxidant and F:S ratio of 1:30, it could give highest yield of extract. Variation of solvent type and variation of F:S have significant effect on the value of antioxidant activity (IC_{50}) of the red betel leaf sample.

Keywords: antioxidant, phytochemicals, red betel, solvent

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1. Introduction

Indonesia is a tropical country that is rich in plant species diversity. One of the plants that can live in Indonesia is red betel leaf (*piper crocatum*). Red betel leaf has the characteristics of branching vines and heart shaped leaves in a silver-red color. In addition to the leaf color, the difference between red betel and green betel is that the red betel leaf will release mucus and have a more fragrant aroma when it is crushed. According to several previous studies, red betel leaf has chemical contents potential as antioxidants such as flavonoids, tannins, alkaloids, saponins, steroids, and essential oils [1], [2], [3].

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Free radicals are reactive compounds so that when contained in the body in a large amount can cause damage to proteins, oxidize fats, and damage the DNA tissue [4]. This condition will trigger the emergence of various diseases such as coronary heart disease, diabetes mellitus, and cancer [5]. Antioxidants are compounds that can supplement electron deficiencies in free radical compounds. Antioxidants are divided into two types: synthetic antioxidants and natural antioxidants. Natural antioxidants are antioxidants derived from natural ingredients. Unlike synthetic antioxidants, natural antioxidants are not toxic and have no carcinogenic effects.

The potential of red betel leaf as a source of natural antioxidants motivates the research on red betel leaf. The optimum extraction time is sought to know the length of extraction time on red betel leaf samples used to represent all the extraction time in this research. There are three kinds of solvent used in this research: ethanol, water, and ethyl acetate representing the polarity level. The purpose of this research is to determine the effect of polarity of solvent extraction and the ratio of feed and solvent on phytochemical content and antioxidant activity of red betel leaf (*piper crocatum*).

2. Method

2.1. Pretreatment

Red betel leaves were washed and dried in an oven at 50 °C within 20 hours [6]. The water content was checked using a moisture analyzer. The dry red betel leaves were then blended and sieved to obtain red betel leaf powder of -20 + 30 mesh in size which was then used as the sample on the experiment.

2.2. Determination of Extraction Time

Determination of extraction time was conducted to determine the time that can represent the length of extraction time of the entire run of the experiment. Ethyl acetate solvent was used with the ratio of feed: solvent (F: S) = 1: 20 and the extraction process was carried out in batch. The extract sample was taken every 30 minutes in duplo.

2.3. Phytochemical Analysis and Antioxidant Activity

In this research, variations of types of solvents (ethanol, water and ethyl acetate) and variations of F:S (1:20, 1:25 and 1:30) were analyzed. The extraction process was carried out at the temperature room with a stirring speed of 200 rpm during the extraction time. In the preliminary experiment, phytochemical analysis and antioxidant activity analysis using DPPH method were performed. From the result of the calculation of IC_{50} value analysis of antioxidant activity [7], the best variation of type of solvent and F: S was determined. The phytochemical analyzes performed included alkaloids test, flavonoid test, tannin test, steroid and triterpenoid test and polyphenol test [8].

3. Results and Discussion

3.1. Pretreatment

First of all, the red betel leaves were washed and then were dried in an oven at 50°C within 20 hours. The drying temperature was not set too high to prevent possible damages to antioxidant compounds contained in the red betel leaf (Gupta et al., 2012; Chan et al., 2009). Drying was done to prevent damages to antioxidant compounds. Checking moisture content was done by using a moisture analyzer. The moisture content of the betel leaves was derived from the initial moisture content of 80.7% to 11.2%. After being dried, the red betel leaves that had turned to brownish were then blended and filtered with a sieve of -20 + 30 mesh. The result of this process can be seen in Figure 1. The red betel leaf powder collected in the desired sieve size was then used for further experiments as the sample. The purpose of reducing the sample size to such powder size was to facilitate the contact between the material and the solvent so that the extraction process would run well.

Figure 1. Red Betel Leaf Powder

3.2. Determination of Extraction Time

Ethyl acetate as a solvent with the ratio of F: S = 1: 20 was expected to represent the extraction time of all variations performed. The extraction process was carried out in batches and the extractor circuit used during the experiment can be seen in Figure 2.

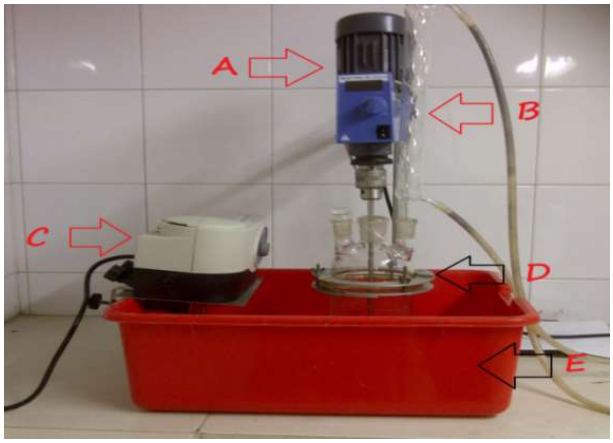

Figure 2. Batch Extractor Circuit on the Experiment

Description: A: Mixer Motor, B: Condenser, C: Thermostat, D: Extractor, E: Waterbath

At this stage, the extract sampling was done every 30 minutes in duplo. The sample that has been taken was then dripped with 2 ml of DPPH and was taken into and left in the oven with 37 °C temperature for 30 minutes. The DPPH solution used has a concentration of 0.1 mM prepared by dissolving 3.943 mg of black DPPH powder with 100 ml of purple methanol p.a. DPPH solution. After 30 minutes, the %T of the sample was then measured using a spectrophotometer at $\lambda = 517$ nm with methanol as the blank form. Once the measured %T value did not show any changes, the extraction process was stopped. The average absorbance data obtained at each t (time) is passed on the absorbance graph versus the operating time seen in Figure 3.

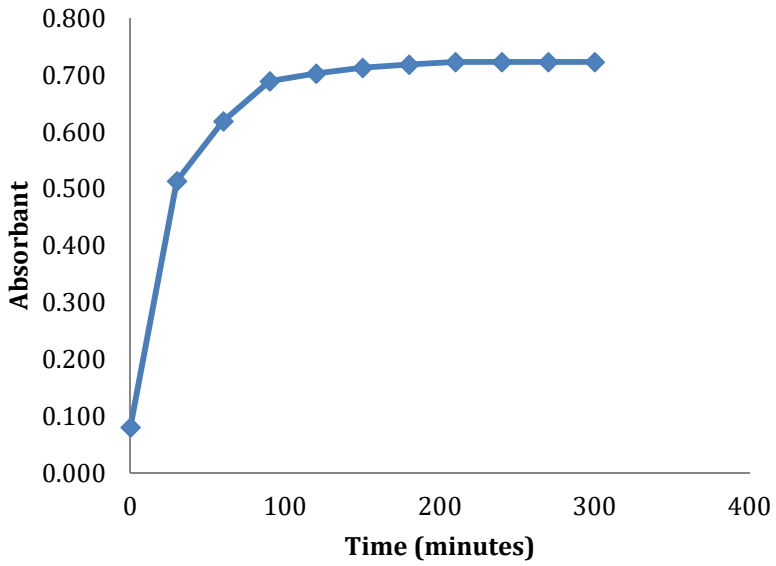

Figure 3. Determination of Extraction Time

In Figure 3, it is visible that from the 0th minute to the 120th minute there is a considerable change in the absorbance value. Meanwhile, from the 210th minute to the 300th minute, there is no more change in the absorbance value. Therefore, it can be concluded that the length of the extraction

time is 210 minutes (3.5 hours) taking the first time of the constant absorbance value after the first three constant data.

3.3. Phytochemical Analysis

The extraction results were filtered using a vacuum filter to separate the dregs from the red betel leaf extract. The red betel leaf extract was separated from its solvent using a vacuum rotary evaporator to obtain the sample of pasta as can be seen in Figure 4.

Figure 4. The Sample of Evaporator Results

The phytochemical analysis was performed to test for the presence of an organic compound in the sample of red betel leaf extracted with solvent variations including ethanol, water and ethyl acetate. From the phytochemical analysis, the red betel leaf contained alkaloids, flavonoids, tannins, polyphenols and steroids as can be seen in Table 1. However, there was a different result when using ethyl acetate as the solvent because the red betel leaf using ethyl acetate alkaloids and tannins as the solvent could not be extracted as ethanol and water could.

Table 1. Phytochemical Analysis Results

Test	Types of Solvent		
	Ethanol	**Water**	**Ethyl Acetate**
Alkaloid	+	+	-
Flavonoid	+	+	+
Tannin	+	+	-
Polyphenol	+	+	+
Steroid	+	+	+
Triterpenoid	-	-	-

Notes: + : Positive, - : Negative

In the antioxidant activity analysis, the sample with antioxidant activity would change its color from purple (after being dripped with DPPH) to yellow. The experiment result showed that the larger the concentration of the sample was the larger the antioxidant activity would be. The result of IC50 value calculation in the preliminary experiment can be seen in Figure 5. The IC50 value shows the antioxidant ability to counteract free radicals by 50%. The smaller the IC50 value is the

greater its antioxidant activity. From the results of IC_{50} value analysis, it can be concluded that ethanol is the best solvent to extract antioxidant compounds in the red betel leaf sample compared to the other two solvents (ethyl acetate and water). The ethanol as a solvent is also said to be more selective than water, resulting in more contents of active compounds in the extract yield. The results of this research also inform that the contents of compounds in red betel leaf which have the potential as antioxidants are mostly dissolved in polar solvents.

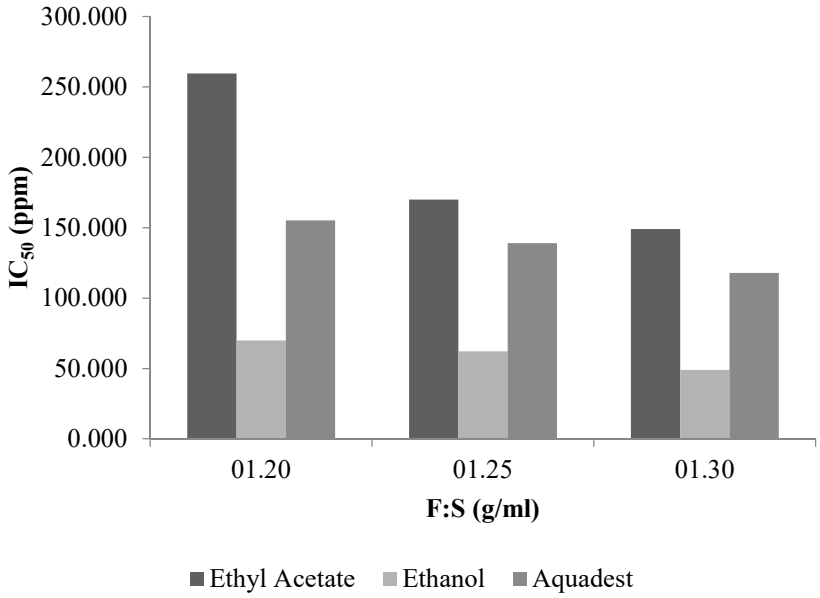

Figure 5. The Result of the IC_{50} Value Analysis of Antioxidant Activity

The best F:S variation is identified by looking at the yield of the dry weight of each sample. The results show that the sample extracted with F:S=1:30 obtained the highest yield of extract by 13.89%. The variant analysis was conducted to determine whether the variation of solvent types and the variation of F:S have an effect on the antioxidant activity of red betel leaf. From Table 2, it can be concluded that the variation of solvent types and the variation of F:S have an effect on the value of the antioxidant activity (IC_{50}). In addition, there is an interaction between the variation of solvent types and the variation of F:S which influences the value of the antioxidant activity (IC_{50}).

Table 2. ANOVA Table

Variation	Sums of Square	Degrees of Freedom	Mean Square	Fo	Note	Ftable	Conclusion
Treatment A	8,688.29	2	4,344.15	136.36	>	4.26	Effect
Treatment B	55,004.07	2	27,502.03	863.28	>	4.26	Effect
Interaction	4,599.50	4	1,149.88	36.09	>	3.63	Interaction
Error	286.72	9	31.86				
Total	68,578.58	17					
Subtotal	68,291.86						

Notes: A: Feed : Solvent (F:S), B: Types of Solvent

4. Conclusion

The polarity of extraction solvent has an effect on the red betel leaf extract results. The contents of compounds in red betel leaf with the potential as antioxidants are more dissolved in ethanol rather than in water and ethyl acetate. The red betel leaf sample extracted with the ratio of feed and solvent of 1:30 obtains the highest yield. The results of phytochemical analysis inform that red betel leaves contain alkaloids, flavonoids, tannins, polyphenols, and steroids. The variation of solvent types and the variation of F:S have a significant effect on the value of antioxidant activity (IC_{50}) of the red betel leaf sample.

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