Antioxidants Activity of the Kecombrang Flower (*Etlingera elatior*) Extract by using 1,1-diphenyl-2-picrilhidrazyl (DPPH) Method

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Abstract: Kecombrang (*Etlingera elatior*) is a zingiberaceae plant, which has long been known as one of the vegetables and used by the community as a nutritious food to preserve food because of the active substances contained in it, such as saponins, flavanoids, and polyphenols. In this research, antioxidants activity of kecombrang flower extract was measured by using 1,1-diphenyl-2-picrilhidrazil (DPPH) method. The kecombrang flower extract has several secondary metabolite compounds, such as alkaloid, terpenoid, steroid, phenolic, flavonoid, tanin with very small antioxidant activity, which is showed by inhibition percentage. As many as 100 ppm concentration of the kecombrang flower extract, ie, methanol extract 3.21%, ethyl acetate extract 5.08 %, and n-hexane extract 30.29%. So it can be concluded that the antioxidant activity of flower kecombrang (*Etlingera elatior*) is less active.

1 INTRODUCTION

Human health is very dependent on the environment nowadays. Besides, the environment which is full of pollutants, will be able to poison the body either through air or food. One of the causes of environmental pollution is pesticide residue. It has been reported that pesticide residues can cause the diseases because of the presence of radical compounds that can oxidize cells (Zakaria et al, 1996). Excessive cell oxidation process will lead to many diseases, such as cancer, diabetes, heart disease (Fajriah et al, 2007) atherosclerosis, cataracts and premature aging (Langseth et al, 2000). The body itself produces antioxidants that can reduce the negative effects of free radical reactions. As long as the balance between free radicals and endogenous antioxidants is maintained, the adverse effects of free radicals can be neutralized (Subarnas et al, 2001).

Various diseases caused by radical compounds are growing, this makes the research continue to be done as an effort to be able to find substances as drugs that can play an active role in preventing and overcoming radical compounds in the body reported that there are two types of drugs that can be used as an alternative treatment of synthetic drugs and traditional medicine in the world of drugs. Besides, choosing a drug should consider as the side effects of drug performance. Drugs from natural ingredients have relatively fewer side effects compared to synthetic drugs (Utami et al, 2008). Generally, Indonesian prefer to take the natural-made medicines in the effort to prevent and treat an attack of disease by drinking water extract from certain plants or by attaching the extract to the sick body part (Mohd Jaafar et al, 2008).

Free radical compounds can be overcome by a free radical prophylactic called antioxidants. The antioxidant is a component capable of inhibiting nucleic acid, lipid oxidation by initiation or propagation of chain oxidation reactions. Antioxidants can protect the body from various degenerative diseases in accordance with the main function of antioxidants namely, neutralizing free radical compounds (Winarsi et al, 2007), reducing agents, free radical damping and metal pro-oxidant complex (Poerawinata M, et al, 2007). The isolated antioxidant compounds contained in high plants are ß-carotene, vitamin C, vitamin E, flavonoids, curcuminoids and polyphenol compounds (Alamendah's et al, 2013).

Based on the above explanation, this study aims to conduct the research on kecombrang plant, which is

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believed to have antioxidant activity as concluded by Tan et al.. Regarding to the activity of chewing as an antioxidant, testing of antioxidant kecombrang can be done by using of the DPPH method. The DPPH method is low-cost and easy-to-prepare method where the DPPH acted as free radical will react with antioxidant compound so that the color of test solution changes from purple to yellow (Pratama et al, 2015).

2 MATERIALS AND METHODS

2.1 Materials

The instruments used are UV-Vis Spectrophotometer, vacuum rotary evaporator, separator funnel, sonicator, incubator, analytical scales, dilution bottle, vial bottle. The ingredients used are flower samples of coconut, methanol solvent, Ethyl acetate solvent, n-Hexane solvent, DPPH powder, and methanol p.a.

2.2 Method

2.2.1 Sample Preparation

The sample was obtained from one of the areas in Deli Serdang with a weight of 2 Kg, which then dried and graded into small size and weighed the sample weight.

2.2.2 Maceration

Samples that have been scaled to a small size, are inserted into glass bottles and methanol solvent added until the sample portion is completely submerged. Subsequent samples in glass bottles were left submerged for 2 x 24 h. After 2 x 24 h the sample is filtered and then the filtrate of the sample is floated in a clean glass bottle.

2.2.3 Vacuum Rotary Evaporator

The sample filtrate has been accommodated, then inserted into the rotavapor flask according to the pumpkin container, and subsequently in the rotary vapor until the filtrate becomes thickened thick and then allowed to evaporate until the viscous filtrate, then repeated rotary evaporator by incorporating other filtrate.

2.2.4 Separation of Extract with Separating Funnel

The viscous filtrate was taken slightly and diluted with some mL of methanol slowly, then inserted into the separating funnel and added with several mL of n-hexane solvent, subsequently separated funnel for a few seconds until the second solvent was evenly mixed and then placed the separating funnel in the stem neck stative until both solvents are perfectly separated. Further separated layers of methanol with a layer of n-hexane and each of them accommodated in glass bottles.

2.2.5 Vacuum Rotary Evaporator Methanol Extract and N-hexane Extract

Each filtrate of methanol extract and filtrate of n-hexane extracts were regenerated until each filtrate changed to blackish, and then allowed to dry until a thickened extract was formed.

2.2.6 Separation of Methanol Condensed Extract with Ethyl Acetate Solvent

The methanol condensed extract was extracted by adding the ethyl acetate solvent until the viscous portion of the methanol extract was insoluble in the ethyl acetate solvent.

2.2.7 Calculation of Yield Percentage

Each extract that has been produced then calculated the yield by using the following formula:

$$\%\ Yield = \frac{W\ extract}{W\ sample} x100\% \qquad (1)$$

2.3 Characterization

2.3.1 Phytochemical Screening

The three extracts have been obtained, phytochemical screening such as the identification of alkaloids, terpenoids, steroids, phenolics, flavonoids, saponins, quinones, and tannins. The alkaloid test was performed by reacting the extract with a Dragendroff reagent and producing a brown red and orange (Robinson et al, 1995). The terpenoid and the steroid tests are performed by reacting the extract with Lieberman-Burchard reagent and producing a pink or purple color (Harborne et al, 1987). Flavonoid test was done by dissolving the extract in water and then added Mg powder and the added concentrated HCl and shaken strong, positive test by red, orange, or purple (Robinson et al, 1995). The saponin test is

done by dissolving the extract in water and then shaking strongly for a few seconds and will cause a stable foam, then added 1% HCl if the foam arises with a height of 1-3 cm and persist for 15 minutes indicating the presence of saponins (Harborne et al, 1987). Phenolic test is performed by reacting the extract with 1% iron and 1% chloride reagent and positive when it gives strong green, red, violet, blue and black (Erwinsyah et al, 2016). Tannin test is done by dissolving the extract in water and then the added a few drops of iron reagent (III) chloride 1% positive result when giving rise to dark blue, green (LIPI, 2017). The quinone test is performed by diluting the extract into water and added a few drops of positive 1N NaOH by causing a red color (LIPI, 2017).

2.3.2 The Antioxidant Activity Test

The antioxidant activity is determined by free radical damping method using the DPPH. Weighed the DPPH powder as much as 2.4 mg and dissolved with methanol as much as 15 mL and then placed in a dark bottle. As many as 0.4 mM the DPPH solution was pierced 1 mL and inserted in a 5 mL scale reaction tube, then the added methanol pro analysis to the boundary marker, and the tube was covered with aluminum foil then homogenized.

Weighed each viscous the extract of 5 mg was used an analytical scale, then dissolved into 10 mL the methanol pro analysis, the solution is the parent liquor. Then the parent solution was piped into the test tube with a volume of 5 mL of 1000 µL to obtain a concentration of 100 ppm.

A total of 3 mg of the vitamin C was weighed and then dissolved with the methanol p.a up to 10 mL. Furthermore, the 150, 117, 83, 50, and 17 µL pipes were inserted into a tube of scale covered with aluminum foil and then added 1 mL of 1 mM the DPPH solution and added the methanol pa to 5 mL boundary marker on the scale tube to obtain concentration 1, 3 , 5, 7, 9 ppm.

The test solution with a concentration of 100 ppm was incubated with 37ºC for 30 minutes. Subsequent absorption of the solution was measured at a maximum absorption wavelength of 517 nm by using a visible light spectrophotometer.

Formal absorption, positive control and absorbance of the test solution measured on the UV-Vis spectrophotometer and recorded by entering the absorption results percentage inhibitor in the following formula:

$$\%\ inhibition = \frac{A\ blank - A\ sample}{A\ blank} x100\% \qquad (2)$$

3 RESULTS AND DISCUSSION

3.1 Results

The results of research conducted at Bioproses Laboratory Politeknik Teknologi Kimia Industri Medan can be seen in Table 1; 2; 3; and 4 below:

Table 1: Value of Randemen Percentage of Kecombrang Flower (*Etlingera elatior*) Extract.

Extract	Weight Extract (g)	Yield (%)
n-hexane	10.21	0.51
Ethyl acetate	2.41	0.12
Methanol	1.45	0.07

Table 2: Phytochemical Screening Results of Kecombrang Flower (*Etlingera elatior*) Extract.

	Methanol Extract	Ethyl Acetate Extract	*n*-hexane Extract
Alkaloid	-	+	+
Terpenoid	+	-	+
Steroid	+	-	-
Phenolic	+	+	-
Flavonoid	-	+	-
Saponin	-	-	-
Quinon	-	-	-
Tannin	+	+	+

Table 3: Value of Inhibitor percentage of the Kecombrang Flower Extract (*Etlingera elatior*) at 100 ppm Concentration.

Extract	Concentration	Absorbance	% inhibition
n-hexane	100	0.672	30.29
Ethyl Acetate	100	0.915	5.08
Methanol	100	0.933	3.21
Blank	100	0.964	-

Table 4: Antioxidant Activity Test Results of Vitamin C.

Concentration (ppm)	Absorbance	% inihibition	IC_{50} (ppm)
1	0.896	7.05	4.51
3	0.725	24.79	
5	0.406	57.8	
7	0.066	93.15	
9	0.024	97.51	

3.2 Discussions

3.2.1 Extraction

The samples from flowering plants were cut into small pieces and dried, then the dried samples were macerated using organic solvent, methanol solvent for 2 x 24 h with 4 repeats of maceration until the solvent became translucent until the secondary metabolite was no longer soluble in the methanol solvent. Maceration is a technique of immersion to the material to be extracted. Samples that are small in size are immersed in organic solvents for some time and then filtered and the result is a filtrate (Sitorus et al, 2010). The purpose of maceration so that the secondary metabolite compounds contained in the sample can be dissolved in the solvent so that it can be obtained filtrate sample flowers kecombrang. Maceration uses organic solvents, the function of organic solvents to penetrate the cell wall of the sample and into the cell cavity resulting in the secondary metabolite compounds contained in the cell to dissolve. The dissolution of secondary metabolite compounds is due to the difference in concentration between the secondary metabolite compounds and the organic solvent resulting in diffusion. After the diffusion process takes place, the sample filtrate is accommodated into a glass for the rotary evaporator process.

In the rotary evaporator, the filtrate concentration process of the kecombrang flower sample is based on the vapor pressure of the solvent influenced by the temperature. The concentration process uses a vacuum pump with a cooling water stream so that the solvent present in the apparatus will decrease the vapor pressure. When the solvent vapor pressure drop is equal to the atmospheric pressure the solvent will boil, so that the solvent present in the sample filtrate will be able to evaporate faster at a temperature below its boiling point. This process is done until the extract obtained thick flowers kecombrang.

The obtained flower extract of the resulting kecombrang was then extracted with a n-hexane solvent in order for the non-polar secondary metabolite compounds to be separated by a polar one. The extract of thick flower extract of kecombrang was done by using separating funnel tool so that separation of secondary metabolite compounds with different polarity properties will be seen clearly with the formation of 2 layers where the top layer is n-hexane solvent while the bottom layer is methanol solvent. Each flower extract that will be extracted in a separating funnel is dissolved first with a methanol solvent so that the extract becomes more dilute and at the time of added n-hexane solvent will be easier separation process. After separation by using separating funnel, both layers of flower extract of kecombrang are accommodated in glass bottles'. Furthermore, the two flower extracts concentrated by using a rotary evaporator. The concentrated methanol extract of the kecombrang flower was further extracted with ethyl acetate solvent by adding ethyl acetate solvent in the methanol concentrated extract until the methanol extract of the flower kecombrang was insoluble in the ethyl acetate solvent, then the ethyl acetate extract of the flower kecombrang was left alone the solvent evaporated. The result of extract process was obtained three extracts from kecombrang interest that is methanol extract, ethyl acetate extract, and n-hexane extract then calculated% yield, phytochemical screening, calculation of DPPH damping resistor.

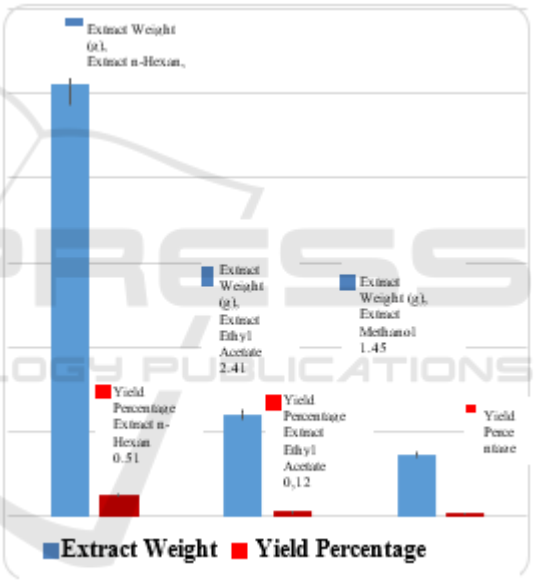

Figure 1: Value of Randemen Percentage of Kecombrang Flower (*Etlingera elatior*) Extract.

The graph above illustrates the percentage of yield of kecombrang flower (*Etlingera elatior*) extract. The extract was done yield percentage calculation with the aim to know how many extracts will be obtained from so many samples was used, so to conduct further research will be easy to predict how many samples will be extracted to get the extract according to requirement. Randemen percentage were extracted methanol 1.45 g with percentage of 0.07%, ethyl acetate extract 2.41 g with randemen percentage 0.12% and n-hexane extract of 10.21 g with 0.5% yield percentage.

3.2.2 The Phytochemical Screening

The three extracts that have been calculated rendemen percentage phytochemical screening. The purpose of phytochemical screening is to know the type of secondary metabolite compounds contained in the sample. The method was used because easier and simpler to do it.

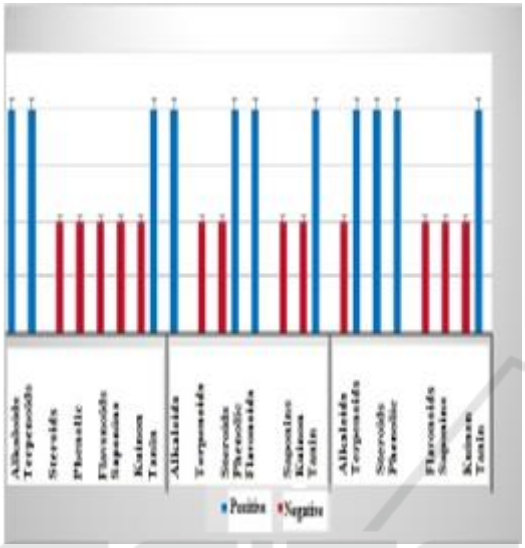

Figure 2: Phytochemical Screening Results of the Extract.

3.2.3 Kecombrang Flower (*Etlingera elatior*)

The graph above describes the results of phytochemical screening of the kecombrang flower extract (*Etlingera elatior*). Based on the Fig. 2. can be explained that the extract of n-hexane found the secondary metabolite content of alkaloids, terpenoids, and tannins. Ethyl acetate extract of secondary metabolite content in the form of alkaloids, phenolics, flavonoids, and tannins. While the extract methanol terpenoid content, steroids, fennolik and tannins. The results of screening of studies that have been performed with similar extracts from bogor areas indicate the presence of secondary metabolites from ethanol extracts of alkaloids, flavonoids, saponins, tannins, steroids and terpenoids, ethyl acetate extracts of alkaloids, flavonoids, steroid and terpenoidal saponins, and n-hexane extract not detected secondary metabolite content (Verawati et al, 2014). The content of secondary metabolite of kecombrang flower extract from Deli Serdang area with extract of kecombrang flower from Deli Serdang area has difference such as ethanol extract of kecombrang flower from Deli Serdang area detected alkaloid and falvonoid content and not found in methanol extract of kecombrang flower from Tanah Karo area. This difference in detectable secondary metabolite content may be caused by the precursor of the biosynthesis of secondary metabolite formation as well as the texture of the soil in which the flower derived origin (Verawati et al, 2014). (Milana et al, 2016) also reported that the formation of secondary metabolites is strongly influenced by soil nutrients having linear relationships such as nitrogen, potassium, organic matter and carbon.

3.2.4 Test of Antioxidant Activity by using the DPPH Method

The antioxidant activity test was performed to see the bioactivity of the kecombrang flower by looking at the percentage value of the sample inhibitory power. To determine the inhibitory power of the sample as antioxidant can be done by using the DPPH method. The antioxidant activity test used the DPPH method because it is easier to do and not expensive.

Testing of antioxidant activity using 0.4 mM DPPH solution made by weighing DPPH powder as much as 2.4 mg and dissolved into 15 mL methanol pa. Further weighed the extract to be tested as much as 5 mg and dissolved in a solution of methanol pa 10 mL and disonikasi so that the solution becomes homogeneous. Test solution was then prepared with concentration of 100 ppm by pipette 1 mL of DPPH solution into three scale test tubes wrapped with aluminum foil and added extract solution on each tube as much as 1 mL then added methanol pa to 5 mL scale and for blank DPPH solution in 5 mL test tube. The test tube was then detoxified for 30 minutes to speed up the reaction between samples acting as antioxidants with DPPH free radicals. After 30 minutes the test solution is ready for measurement by a UV-Vis spectrophotometer to determine the absorbance of each test solution.

Testing with UV-Vis spectrophotometer was performed at 517 nm wavelength because at that wavelength had the optimum absorbance ability for antioxidant activity test. Furthermore, the measurement of the absorbance of each test solution in duplicate.

For methanol extract test solution obtained absorbance 0.933, and for ethyl acetate extract obtained absorbance of 0.915, while extract n-Hexan obtained absorbance of 0.672, and for blank obtained absorbance 0.964.

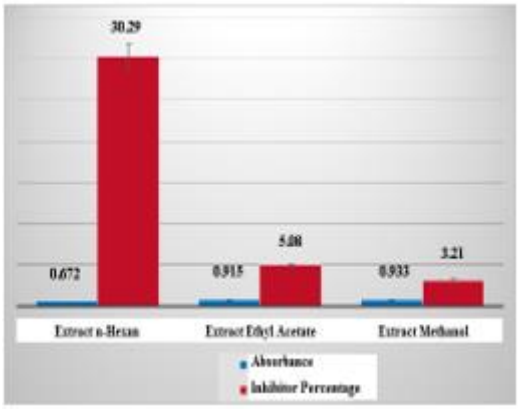

Figure 3: Value of Inhibiton Percentage of Kecombrang Flower Extract (*Etlingera elatior*) at 100 ppm Concentration.

After calculation can be obtained percentage of the inhibitory of each-tipa extract. The extract of n-hexane has an inhibitory percentage of 30.29%, ethyl acetate extract has 5% inhibition of 5.08% and methanol extract has an inhibitor percentage of 3.21%, so it can be said that the antioxidant activity of the three extracts are very small. (Anisa et al, 2014) says antioxidant activity can be expressed as IC50 (Inhibition Concentration fifty) if concentration to free radical clearance is 50%. Vitamin C test results have very good antioxidant activity and obtained IC50 4.51 ppm, while the results of calculation of the inhibitory of the three extracts are not up to 50%, Therefore the three extracts in the test beforehand in a concentration of 100 ppm in order to know whether the extract has activity 50 good percentage or not before IC50 measurement.

4 CONCLUSIONS

Based on the data of the research, it can be concluded that the sample extract of kecombrang flowers has some secondary metabolite compounds such as Alkaloid, Terpenoid, Steroid, Phenolic, Flavonoid, Tanin. In addition, the extract of kecombrang flower sample also has very small antioxidant activity at concentration of 100 ppm that is, methanol extract 3.21%, etil acetate extract 5.08%, and 30.29% n-hexane extract, so it can be concluded that the antioxidant activity of flower kecombrang (*Etlingera elatior*) less active.

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REFERENCES

Alamendah's Blog. 2009. Tingkat pencemaran Udara di Indonesia. https://alamendah.org/2009/09/23/tingkat-pencemaran-udara-di indonesia/. Tanggal akses 12 November 2017.

Erwinsyah, 2016. Skrining Fitokimia dan Uji Aktivitas Antioksidan Ekstrak Benalu yang Tumbuh pada Tanaman Benalu Bungur (Scurrula ferruginea (Jack) Danser) Dengan Metode DPPH (1.1-difenil-2-pikrilhidrazil). Universitas Mulawarman, Samarinda.

Fajriah S, Akhmad Darmawan, Andini Suridowo dan Nina Artanti. 2007. Isolasi Senyawa Antioksidan dari Ekstrak Etil Asetat Daun Benalu (Dendrophthoe petandra L) yang Tumbuh pada Inang Lobi-Lobi Kawasan Puspitek, Serpong: Pusat Penelitian Kimia Lembaga Ilmu Pengetahuan Indonesia.

Harborne, J. B. 1987. Metode Fitokimia Penuntun Cara Modera Menganalisis Tumbuhan. Terjemahan: Kosasih P, Soediro iwang, Bandung ITB.

Jackie et al. BMC Research Notes. 2011.Antioxidant effects of Etlingera elatior flower extract against lead acetate - induced perturbations in free radical scavenging enzymes and lipid peroxidation in rats. https://bmcresnotes.biomedcentral.com/articles/10.1186/1756-0500-4-67. Tanggal akses 12 November 2017.

Langseth, L. 2000. Antioxidants and Their Effect on Health. Essentials of Functional Foods. Aspen Publication, Gainthersbur

Lipi, 2014. Bioteknologi Untuk Kehidupan Lebih Baik. http//www.biotek.lipi.go.id. Tanggal akses 29 Novmber 2017.

Mohd Jaafar, Faridahanim et al. 2007. Analysis of Essential Oils of Leaves, Stems, Flowers, And Rhizomes Of Etlingera Elatior (JACK) R. M. Smith.The Malaysian Journal of Analytical Scienses. Universiti Teknologi MARA. Kuala Lumpur.

Naufalin Rifda and Herastuti Sri Rukmini. 2016. Antioxidant Activity and Physicochemical Properties of Nicolaia speciosa Flower extract.Agriculture and Agricultural Science Procedia 9. Jendral Soedirman University. Purwokerto.

Rifda, Naufalin. Antimicrobial Activity of Fruit Peel Kecombrang Formula (Nicolaia speciosa HORAN) As Natural Preservative. Program Studi Ilmu dan Teknologi Pangan. Universitas Jenderal Soedirman. Purwokerto

Poerawinata M. 2007 Uji Aktivitas pada Daun Pandan (Pandanus polycephalus). skripsi. Universitas Indonesia, Depok.

Pramata, Galih Putra. 2015. Identifikasi Senyawa Aktif Antioksidan dalam Tanaman Selada Air (Nasturtium officinale R. Br). Skripsi. Universitas Pancasila, Jakarta.

Robinson, T. 1995. Kandungan Organik Tingkat Tinggi. Bandung: Institut Teknologi Bandung.

Subarnas, A. 2001. Komponen Aktif Antioksidan dalam Bahan Alam, Seminar Nasional dan Lokakarya Pemahaman dan Konsep Radikal Bebas dan Peranan Antioksidan dalam Meningkatkan Kesehatan Menuju Indonesia Sehat 2010. Pusat Penelitian Padjadjaran: 28-40.

Utami N., dan Robbara. M. 2008. Identifikasi Senyawa Alkaloida dari Ekstrak Heksana daun Argratum conyzoides. Lin. Prosiding Hasil Penelitian Unila.