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Antioxidant Activity and Total Phenol of Extract of Kecombrang Flower, Stem and Leaves with Different Types of Solutions

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ABSTRACT. Kecombrang (*Eriogonum elatior*) is one of the spices which is quite widespread in Indonesia and has many uses. Kecombrang extract has the potential as an antioxidant and natural antimicrobial to extend the shelf life of food products. Extraction was carried out by multilevel maceration method with different types of solvents. This study aims to determine the effect of extraction on the bioactive components of flowers, stems and leaves of kecombrang in different types of solvents and determine the antioxidant activity and total phenols of each type of kecombrang plant extract. The results showed that extraction with distilled water produced the highest total phenol, antioxidant activity and yield on kecombrang leaves. The total phenol extracts of *n*-hexane leaves, leaves of ethyl acetate, and leaves of kecombrang distilled water were 19.116, 10.276, and 45.008 (mg TAE g dw⁻¹) respectively. The antioxidant activity value of flowers, stems and leaves of distilled water solvent kecombrang are 69.754, 77.648, and 78.003 (%) respectively. The yield for flowers, stems and leaves with distilled water solvent is 15.9, 16.6 and 32.95 (%) respectively.

Keywords: antioxidant, extract, kecombrang, and total phenol.

INTRODUCTION

The diversity of biological sources in Indonesia, especially spices, has the potential as a source of natural antioxidants. Spices are generally used as seasonings by the community to make them delicious. This type of spice comes from certain plant parts such as roots, bark, stems, leaves, flowers, fruit, and seeds. Plants included in the spice category include cardamom and its family, cloves, coriander, pepper, nutmeg, cinnamon, cumin, and cardamom (Boga, 2014). Spices contain bioactive compounds and can produce extracts and essential oils that have various benefits, both in the food and non-food fields. Its benefits include, among others, as a preservative, and it is even used as an insecticide. Plant extract obtained from the extraction process. Extraction is the process of separating materials using certain solvents. After the extraction process, the solvent is separated from the sample by filtering (Mukhlison, 2014).

Extraction is the process of separating a substance using certain solvents. When the equilibrium between the concentration of the compound in the solvent and the concentration in plant cells has been reached, the extraction process can be stopped. After the extraction process, the solvent is separated from the sample by filtration. The initial extract is difficult to separate when using a single separation technique to isolate a single compound. Therefore, the initial extract needs to be

separated into fractions that have the same polarity and molecular size (Mukhlison, 2014).

Kecombrang (*Eriogonum elatior*) is one of the spice plants which has many uses. These herbs are used as food and are also used for medicine. Kecombrang flower exhibited rich antioxidants, anticancer and antimicrobial activities (Naufalin & Herastuti, 2016). The results of the research, stated that the active compounds contained in the kecombrang parts include alkaloids, flavonoids, steroids, saponins and essential oils (Naufalin, 2019; Naufalin & Herastuti, 2017; Latifasari, Naufalin, & Wicaksono, 2019; Herastuti, Naufalin, & Wicaksono, 2019). All parts of kecombrang plants such as flowers, stems, leaves, fruits and rhizomes have the potential as a source of antioxidants. Antioxidant compounds in kecombrang include phenolic, flavonoids, terpenes, saponins, tannins, steroids, alkaloids, and glycosides. Crude extract of kecombrang flower water has the potential as an antioxidant. Compounds that are thought to be antioxidants are compounds that contain phenol groups. GCMS (Gas Chromatography Mass Spectrometry) analysis results of kecombrang flower water extract showed that there are 6 main compound groups, namely alkenes, alkenes, alcohols, fatty acids and phenols. Three of the dominant ones are 1: dodecanol, 3-methyl-1-octao-2-buten-1-[2',4',5'-trihydroxy] phenyl, and 1-tetradecene (Sukandari,

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ABSTRACT. Kecombrang (*Etlingera elatior*) is one of the spices which is quite widespread in Indonesia and has many uses. Kecombrang extract has the potential as an antioxidant and natural antimicrobial to extend the shelf life of food products. Extraction was carried out by multilevel maceration method with different types of solvents. This study aims to determine the effect of extraction on the bioactive components of flowers, stems and leaves of kecombrang in different types of solvents and determine the antioxidant activity and total phenols of each type of kecombrang plant extract. The results showed that extraction with distilled water produced the highest total phenol, antioxidant activity and yield on kecombrang leaves. The total phenol extracts of n-hexane leaves, leaves of ethyl acetate, and leaves of kecombrang distilled water were 19.116, 10.276, and 45.008 (mg TAE g db⁻¹) respectively. The antioxidant activity value of flowers, stems and leaves of distilled water solvent kecombrang are 69.754, 72.648, and 78.003 (%) respectively. The yield for flowers, stems and leaves with distilled water solvent is 15.9; 16.6 and 32.95 (%) respectively.

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INTRODUCTION

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Radiastutu, Jayanegara, Muawanah, & Hudaya, 2011). Antioxidant (% inhibition) stem extracts with *n*-hexane, ethyl acetate, and ethanol as solvent are 15.7864; 14.7692 and 17.7707 respectively (Susana & Bahri, 2018).

The process of extracting antioxidant compounds from plants generally uses different types of solvents with different polarity levels, from non-polar, semi-polar and polar. Solvents used such as *n*-hexane, ethyl acetate, ethanol and distilled water. The selection of solvents based on the level of polarity is very useful to get extracts with a greater yield and is also intended so that the group of antioxidant compounds that have the highest activity can be extracted. This study aims to determine the effect of extraction on the bioactive components of kecombrang flowers, stems and leaves in different types of solvents and determine the total phenols, antioxidant activity and yield of each type of kecombrang plant extract.

EXPERIMENTAL SECTION

Materials and Experimental Design

This research was conducted in Technology Agriculture Laboratory, Agriculture Faculty, Jenderal Soedirman University from March to August 2019. The materials used in this study were flowers, stems and leaves of kecombrang (*Etlintera elatior*) from farmers in Baturraden, multilevel solvents: technical solvent of *n*-hexane (nonpolar), technical solvent of ethyl acetate (semi polar), and distilled water (polar). Material of analysis: DPPH, Folin Ciocalteu 10%, tannic acid, methanol p.a, 95% ethanol and 70%, NaHCO_3 0.556 M.

The tools used in this study were 40x40cm stainless steel pan, cabinet dryer, tissue paper (Nice), wipes, plastic and stainless steel spoons, plastic jerry cans, knives and cutting boards, grinders (Philips), 60 mesh sieves, clear plastic clips, vortex mixers, shakers, centrifuge, rotary evaporator (RE-200), UV-VIS spectrophotometer (Shimadzu-1800), cuvette, water bath, showcase (Polytron), 10 mL vial bottles, test tubes (Pyrex), test tube racks, aluminum foil, erlenmeyer (Pyrex and Iwaki) 100 mL, 250 mL and 500 mL, measuring cups (Pyrex and Iwaki) 50 mL and 100 mL, beaker glass (Pyrex) 50 mL and 100 mL, measuring flask (Pyrex) 50 mL and 100 mL spatula, plastic funnel, paper Whatman filters, filter cloths, measuring pipettes (Pyrex), drip pipettes, fillers, mini scale platform (OEM), digital scales, analytical scales, plastic containers.

This research was an experimental laboratory research. The study design uses a non factorial complete randomized design (CRD) with 3 treatments, namely B1: non-polar kecombrang flower extract, B2: semi-polar kecombrang flower extract, B3: polar kecombrang flower extract. Each treatment was repeated five times so that 15 experimental units were obtained.

Powder Processing

Kecombrang flowers, stems and leaves are washed in running water then cut into small pieces (2x3cm). After that, dried process with cabinet dryer at a temperature of 50°C until it breaks dry, then mashed using a grinder and sieved (60 mesh) (Naufalin & Herastuti, 2013).

Kecombrang Extraction

This research uses maceration method. Maceration is a multilevel extraction method using various types of solvents based on their level of polarity (Purwanto, Bahri, & Ridhay, 2017). Kecombrang powder was weighed, 100 grams of flowers, 100 grams of stems and 100 grams of leaves were then added as much as 1.000 mL of solvent (1:10), but for the extract of the stem was added as much as 2.000 mL of solvent (1:20). The mixture is allowed to stand for 48 hours while stirring every 6 hours, for 2-5 minutes, then filtered with filter paper and filter cloth (Savitri, Suhendra, & Wartini, 2017). The filtrate obtained by the solvent is separated by a vacuum rotary evaporator so that a thick kecombrang extract is obtained. The residue obtained is air dried, and then put into an erlenmeyer to be extracted again with ethyl acetate solvent and then distilled water with the same treatment on the extract using *n*-hexane.

Modified Total Phenol of Singleton & Rossi's method (Othman, Ismail, Ghani, & Adenan 2007)

Curve preparation of tannic acid 10 mg was dissolved in 95% ethanol as much as 100 ml, then dilution was carried out with 0.02 dilution series; 0.04; 0.06; 0.08 and 0.1 mg/ml. 0.02 mg/ml = (2 ml of stock solution + 8 ml of ethanol 95%), 0.04 mg/ml = (4 ml of stock solution + 8 ml of ethanol 95%), 0.06 mg/ml = (6 ml of stock solution + 8 ml of ethanol 95%), 0.08 mg/ml = (8 ml of stock solution + 8 ml of ethanol 95%), 0.1 mg/ml = (10 ml of stock solution + 8 ml of ethanol 95%). A total of 5 ml of each dilution series was taken and added 1.5 ml of 10% Folin Ciocalteu and allowed to stand for 5 minutes at room temperature. Then added 1.5 ml of sodium bicarbonate (NaHCO_3) 0.556 M, shaken, and left in a dark room for 90 minutes, then absorbance was measured using a spectrophotometer at λ 725 nm.

Sample preparation

A total of 100 mg of sample added 4 ml of 70% ethanol were then shaken with a shaker for 2 hours at a speed of 200 rpm, then centrifuged for 15 minutes at a speed of 1000 rpm. The obtained supernatant is an extract for determining the sample. The sample is then diluted with 0 ppm and 10 ppm dilutions.

Sample analysis

A total of 400 μL supernatant was added with 1.5 ml of 10% Folin-Ciocalteu and allowed to stand for 5 minutes at room temperature. Then added 1.5 ml of sodium bicarbonate (NaHCO_3) 0.556 M is shaken and left in a dark room for 90 minutes. Furthermore

absorbance was measured using a spectrophotometer at λ 725 nm. Total phenol (mg / g) = $FP \times X (g) \times (V) / (\text{sample weight (g)})$

X = sample concentration

FP = dilution factor

Antioxidant Activities by DPPH Method (Shekhar & Anju, 2014)

DPPH (1,1-diphenyl-2-picrylhydrazyl) is dissolved in methanol at a concentration of 0.15 mM. An amount of 2 ml of sample solution was added to 2 ml of DPPH solution. The mixture was incubated at room temperature in dark conditions for 30 minutes. Reduction in absorbance was measured using a spectrophotometer at a wavelength of 517 nm. The control solution is made from 2 ml of methanol p.a and 2 ml of DPPH solution. Percentage of DPPH free radical capture expressed by percentage inhibition (Naufalin & Herastuti, 2016). The percentage of DPPH radical capture during incubation is calculated by the following equation: Radical capture % = $(\text{Abs control} - \text{Abs sample}) / \text{Abs control} \times 100$

Kecombrang Extract Yield Calculation (Nurhaen, Winarsii & Ridhay, 2016)

The yield of kecombrang extract produced is determined using the following equation: Yield (%) = $(\text{initial sample weight (g)} / \text{extract weight (g)}) \times 100$

Statistical Analysis

Data analyzed using F-test and with Duncan's Multiple Range Test (DMRT) if the result shows diversity.

RESULTS AND DISCUSSION

Total Phenol

Total phenol levels used the Follin-Ciocalteu method. Follin-Ciocalteu is an inorganic reagent that can form complex solutions with phenolic compounds, namely molybdenum tungstic which is blue in color. The more intense the color intensity, the greater the phenol content in the fraction (Wungkana, Suryanto, & Momuat, 2013).

Phenol group compounds known to be a group of compounds which provides antioxidant activity (Kurnia, Rosliana, Juanda, & Nurochman, 2020). The higher the phenol content in the sample, the more

chromagen (blue) molecules formed as a result, the absorbance value increases. The results of the calculation of total phenols from flowers, stems and leaves of kecombrang are presented in Table 1.

High temperature during extraction increases the solubility of phenol (Wazir, Ahmad, Muse, Mahmood, & Shukor, 2011). High temperatures are able to release cell wall phenolic compounds or bound phenolic compounds caused by the destruction of cell elements, causing more and more phenol compounds to be extracted. The total phenol of the fresh simplicia essential oil of kecombrang flower was higher than the dry simplicia essential oil. This can be caused by the evaporation of volatile compounds contained in kecombrang flowers during the drying process so that the phenol content of the dry simplicia essential oil of kecombrang flowers is less. According to Jahangiri, Ghahremani, Torghabeh, & Salehi (2011), the drying process can destroy some phenol compounds because in dry conditions all the components in the cell are fused so that phenol extraction from a material or sample becomes more difficult.

Based on the data obtained, polarity greatly affects the total phenol in kecombrang extract. Polar solvents (distilled water) are able to extract higher phenols so that the total phenol value of distilled water extracts is higher. Phenol is a compound that has the highest solubility in polar solvents. According to Naufalin (2019), phenol compounds are substances that have an aromatic ring with one or more hydroxyl groups so that they dissolve in polar solvents. The highest total phenol value of flowers, stems and kecombrang leaves with distilled water solvent was 36.246; 32.757 and 45.008 (mg TAE g db⁻¹). This is supported by the research of Naufalin & Herastuti (2011), which states that the average total phenol value of the ethyl acetate fraction during storage ranges from 522.08 - 1776.08 mg 100 g⁻¹ and for the ethanol fraction ranges between 854.10 - 4851.30 mg 100 g⁻¹. The n-hexane fraction is composed of non-polar compounds while ethyl acetate is composed of semi-polar compounds which may still contain non-polar compounds. These non-polar compounds can inhibit the extracted phenolic compounds.

Table 1. Results of total phenol analysis of flowers, stems and leaves of kecombrang with various solvents

Parts	Solvents	Total phenol (mg TAE g db ⁻¹)
Flowers	n-hexane	0.202
	ethyl acetate	3.379
	distilled water	36.248
Stems	n-hexane	8.574
	ethyl acetate	15.395
	distilled water	32.757
Leaves	n-hexane	19.116
	ethyl acetate	10.279
	distilled water	45.008

Besides being influenced by the type of solvents, the parts of the kecombrang also influence the total phenol value (Figure 1). The total phenol value of *n*-hexane leaf, ethyl acetate leaf and distilled water leaf extract were 19.116; 10.276 and 45.008 (mg TAE g db⁻¹) respectively. Total phenol in kecombrang leaves has the highest value than the flower and stem of kecombrang. This is linear with the results of research (Naufalin & Herastuti, 2011), the largest average value of total phenols is shown by the interaction of ethanol leaf fractions, ranging from 2047.58-15894.07 mg 100 g⁻¹, while other interactions do not significantly affect the total phenol. This is presumably because the components in kecombrang leaves are generally polar and contain a lot of chlorophyll.

Antioxidant Activity

DPPH is characterized as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole (Sagar & Singh, 2011). The

working principle of this method is the process of reducing DPPH free radical compounds by antioxidants. Antioxidant activity is expressed by an Inhibition Concentration of 50% or IC₅₀, namely the concentration of the sample that can reduce DPPH radicals by 50%. The IC₅₀ value is obtained from the x value after replacing y with 50 (Katrin & Bendra, 2015).

Antioxidants an important role in order for the body to avoid the bad effects caused by free radicals. There are two main types of antioxidants, namely primary and secondary antioxidants. Both have different mechanisms of action. Primary antioxidants scavenge free radicals and provide a hydrogen atom or electron to stabilize the free radical. On the other hand, secondary antioxidants work by suppressing the formation of free radicals which then prevent oxidative damage (Hus, Boyce, & Somasundram, 2012). The results of research on the antioxidant activity of flowers, stems and leaves kecombrang are presented in Table 2.

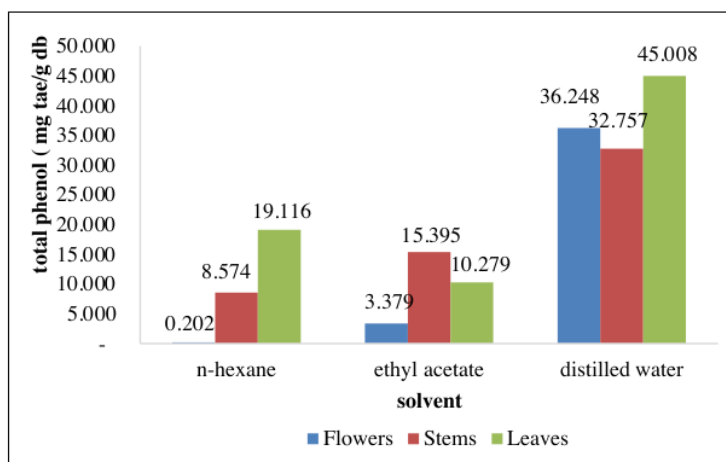


Figure 1. Total phenol content of flowers, stems and kecombrang leaves with 3 types of solvents

Table 2. Antioxidant activity of flowers, stems and leaves of kecombrang

Parts	Solvents	Antioxidant (% inhibition)
Flowers	<i>n</i> -hexane	14.906
	ethyl acetate	41.534
	distilled water	69.754
Stems	<i>n</i> -hexane	39.074
	ethyl acetate	53.690
	distilled water	72.648
Leaves	<i>n</i> -hexane	43.994
	ethyl acetate	64.689
	distilled water	78.003

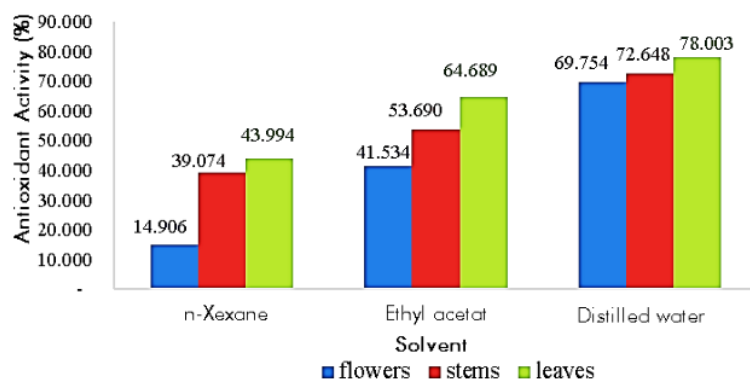


Figure 2. Antioxidant activity of flowers, stems and leaves with distilled water

According to Hanifah et al., (2019), kecombrang has antioxidant content which is thought to be able to inhibit the rate of oxidation in gourami fillets, namely flavonoids. Based on the data that has been obtained, parts of kecombrang and solvents affect the antioxidant activity of the extract. The highest antioxidant activity is extract with polar solvents (distilled water). The value of antioxidant activity of flowers, stems and kecombrang leaves in a row that is 69.754; 72.648 and 78.003 (%). This is directly proportional to the phenol content in kecombrang, the higher the phenol value, the greater the antioxidant activity. This statement is in accordance with the results of research Kusriani et al. (2017), reported that kecombrang leaf ethanol extract showed the best antioxidant activity and the highest total phenol content compared to kecombrang flower and rhizome extracts with total phenol and antioxidant levels $0.339\% \pm 0.006$ and $IC_{50} \leq 1000$ ppm. A combination of several phenol groups is called polyphenols. The content of phenol compounds allows the ingredients they contain to act as antioxidants. This is because phenol can act as a reducing agent by donating H^+ ions from hydroxyl group. The ability to donate H^+ ions is what makes phenol compounds can act as antioxidant (Naufalin, Wicaksono, Erminawati,

Arsil, & Gulo, 2019). Other research on total phenols according to Carolia & Noventi (2016), betel leaf has a distinctive aroma because it contains 1-4.2% essential oil. The phenol is not for sporadic. The other result of research by Handayani, Ahmad, & Sudir (2014) mentioning that testing antioxidants with the DPPH method on methanol extract of flowers and leaves kecombrang has an IC_{50} value of $30.65 \mu g mL^{-1}$ for leaves and flowers $101.84 \mu g mL^{-1}$.

Meanwhile, the highest % inhibition of kecombrang stem extract with various solvents each for *n*-hexane, ethyl acetate and ethanol which is 15.7864; 14.7692 and 17.7707. The more polar, the antioxidant activity tends to be high (Figure 2).

Yield of Kecombrang Extract

Tiered solvents were selected to determine the level of polarity of a compound so that it would get extracts with a greater yield and also intended that the class of antioxidant compounds that have the highest activity can be extracted (Hanifah et al., 2019). Extract yield was calculated based on the ratio of final weight (weight of extract produced) to initial weight (weight of cell biomass used) multiplied by 100% (Sani, Fithri, Ria., & Jaya, 2014). Kecombrang extract yield results are presented in Table 3.

Table 3. Yields of kecombrang flowers, stems and leaves

Sample	Initial weight (g)	Solvent	Extract weight (g)	Amount of solvent (L)	Extract yield (%)
Flowers	20	<i>n</i> -hexane	1.05	0.2	5.25
	20	ethyl acetate	0.61	0.2	3.05
	20	distilled water	3.18	0.2	15.9
Stems	20	<i>n</i> -hexane	0.1	0.4	0.5
	20	ethyl acetate	0.11	0.4	0.55
	20	distilled water	3.32	0.4	16.6
Leaves	20	<i>n</i> -hexane	1.39	0.2	6.95
	20	ethyl acetate	0.59	0.2	2.95
	20	distilled water	6.59	0.2	32.95

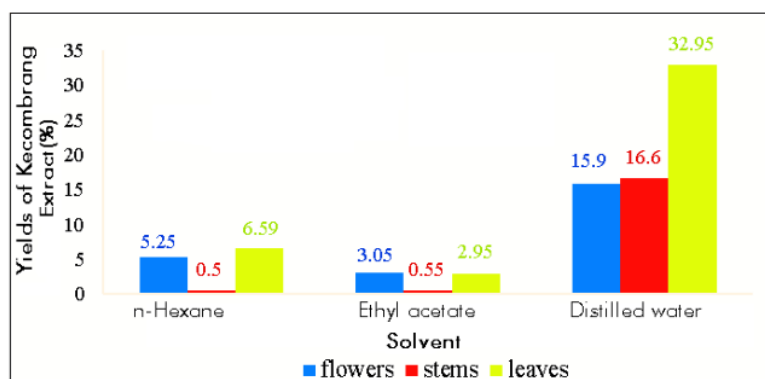


Figure 3. Yield of flowers, stems and leaves kecombrang with distilled water

The yield value is related to bioactive compounds contained in kecombrang (flowers, stems and leaves). Bioactive compounds in plants can function as antibacterial, anticancer, anti-inflammatory and antioxidant. Based on the results obtained, the parts of kecombrang and polarity of the solvent greatly affect the extract yield. The yield for flowers, stems and leaves with distilled water solvent were 15.9; 16.6 and 32.95 (%). Polar solvents produce a lot of yield. This is presumably because the bioactive compounds in kecombrang are non-polar (Figure 3).

CONCLUSIONS

Kecombrang flower, stem and leaf extracts have variations total phenol, antioxidant activity and yield. These variations are influenced by the physicochemical properties of kecombrang plants and the solvents used (nonpolar, semi-polar and polar). The more polar, bioactive compounds kecombrang plants, the more well extracted. The total phenol of *n*-hexane leaves, leaves of ethyl acetate and leaves of distilled water extract were 19.116; 10.276 and 45.008 (mg TAE g db^{-1}) respectively. The antioxidant activity of flowers, stems and leaves of distilled water solvent kecombrang were 69.754; 72.648 and 78.003 (%) respectively. Yields for flowers, stems and leaves with distilled water 15.9; 16.6 and 32.95 (%) respectively.

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