[JM] Inbox

Dr. Amin Fatoni 06/01/2020
to Lecturer, me, Nurul, Ermina...

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Salam,
Editor

Editor in Chief of Journal Molekul
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siti nuryanti 07/01/2020
to Amin

From siti nuryanti • sitinuryanti47@gmail.com
To Dr. Amin Fatoni • aminfatoni@unsoed.ac.id
Date 7 Jan 2020, 07:43
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Terima kasih atas sarannya, akan kami perbaiki segera

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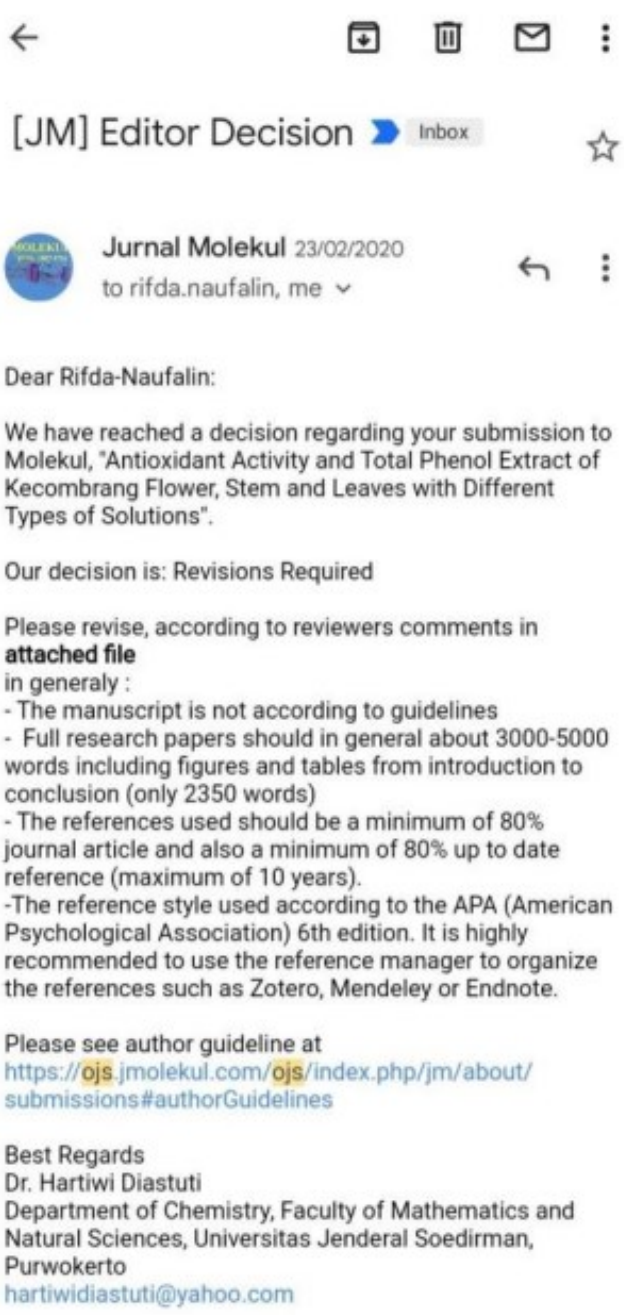

[JM] Editor Decision Inbox

Jurnal Molekul 23/02/2020
to rifda.naufalin, me

Dear Rifda-Naufalin:

We have reached a decision regarding your submission to Molekul, "Antioxidant Activity and Total Phenol Extract of Kecombrang Flower, Stem and Leaves with Different Types of Solutions".

Our decision is: Revisions Required

Please revise, according to reviewers comments in **attached file**
in generaly :
- The manuscript is not according to guidelines
- Full research papers should in general about 3000-5000 words including figures and tables from introduction to conclusion (only 2350 words)
- The references used should be a minimum of 80% journal article and also a minimum of 80% up to date reference (maximum of 10 years).
-The reference style used according to the APA (American Psychological Association) 6th edition. It is highly recommended to use the reference manager to organize the references such as Zotero, Mendeley or Endnote.

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Best Regards
Dr. Hartiwi Diastuti
Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Jenderal Soedirman, Purwokerto
hartiwidiastuti@yahoo.com

REVIEW 1

Dear Rifda-Naufalin:

We have reached a decision regarding your submission to Molekul, "Antioxidant Activity and Total Phenol Extract of Kecombrang Flower, Stem and Leaves with Different Types of Solutions".

Our decision is: Revisions Required

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Best Regards

Dr. Hartiwi Diastuti

Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Jenderal Soedirman, Purwokerto

hartiwidiastuti@yahoo.com

REVIEW 2

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

Siti Nuryanti[1], Nurul Latifasari[1], Rifda Naufalin[1]*), Rumpoko Wicaksono[1] and Erminawati[1]

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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.

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

INTRODUCTION

Kecombrang (*Etlingera elatior*) is one of the spice plants which has many uses. These herbs are used as food and are also used for medicine. The results of the research by (Naufalin, 2019; Naufalin & Herastuti, 2017; Latifasari, Naufalin, & Wicaksono 2019; Hanifah, Naufalin, & Wicaksono 2019), stated that the active compounds contained in the kecombrang parts include alkaloids, flavonoids, steroids, saponins and essential oils. 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, triterpenes, saponins, tannins, steroids, alkaloids, and glycosides. 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

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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.

MATERIALS AND METHODS

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 (*Etlingera 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, wipes, plastic and stainless steel spoons, plastic jerry cans, knives and cutting boards, grinders, 60 mesh sieves, clear plastic clips, vortex mixers, shakers, centrifuse, 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, erlemeyer (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, 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 process

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 1 ml of each dilution series was taken and added 1.5 ml of 10% Folin Ciocalteau 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.

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- 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-Ciocalteau 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 × X (g) × (V) / (sample weight (g))

X = sample concentration

FP = dilution factor

Antioxidant activities 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 % = Abs control-Abs sample)/Abs control × 100

Kecombrang extract yield calculation (Nurhaen, Winarsii & Ridhay, 2016)

The yield of kecombrang extract produced is determined using the following equation:

Yield (%) = initial sample weight (g)/extract weight (g) × 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

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**.

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 $^{-1}$)
1.	Flowers	*n*-hexane	0.202
		ethyl acetate	3.379
		distilled water	36.248
2.	Stems	*n*-hexane	8.574
		ethyl acetate	15.395
		distilled water	32.757
3.	Leaves	*n*-hexane	19.116
		ethyl acetate	10.279
		distilled water	45.008

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.

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 by (Naufalin & Herastuti, 2011), the largest average value of total phenols is shown by the interaction interactions 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.

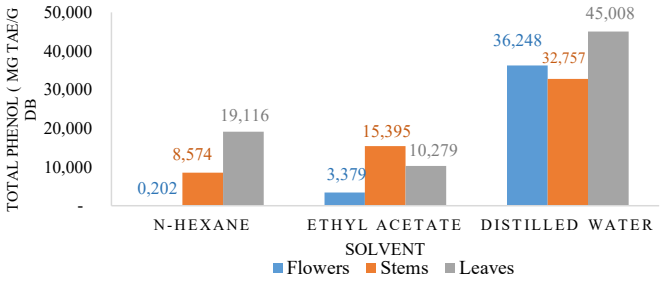

Figure 1. Total phenol content of flowers, stems and kecombrang leaves with 3 types of solvents

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.

The results of research on the antioxidant activity of flowers, stems and leaves kecombrang are presented in **Table 2**.

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

	Parts	Solvents	Antioxidant (% inhibition)
1	Flowers	*n*-hexane	14.906
		ethyl acetate	41.534
		distilled water	69.754
2	Stems	*n*-hexane	39.074
		ethyl acetate	53.690
		distilled water	72.648
3	Leaves	*n*-hexane	43.994
		ethyl acetate	64.689
		distilled water	78.003

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 of 0.339% ± 0.006 and $LC_{50} \leq$ 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 sporacid.

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**).

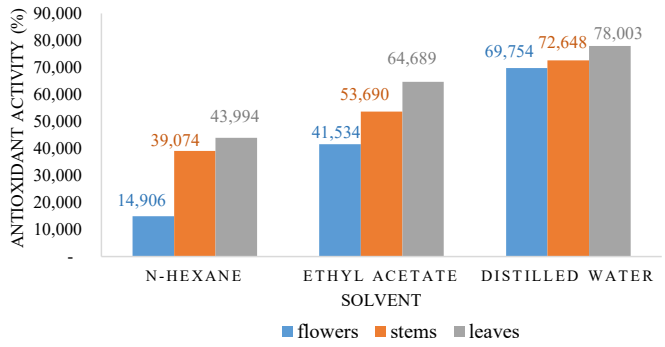

Figure 2. Antioxidant activity of flowers, stems and leaves with distilled water

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, Naufalin, & Wicaksono, 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 (%)
1.	Flowers	20	n-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
2.	Stems	20	n-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
3.	Leaves	20	n-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

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 were15.9; 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**).

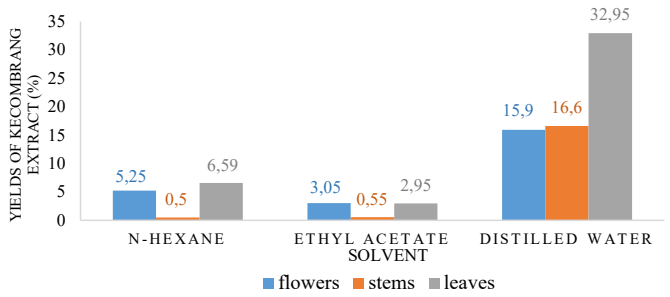

Figure 3. Yield of flowers, stems and leaves kecombrang with distilled water

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.

ACKNOWLEDGEMENTS

Authors wish to thanks to the DRPM Kemenristek Republic of Indonesia which has provided research funding through Thesis Magister Research Grant Scheme 2019 also to Institute for Research and Community Service of Jenderal Soedirman University for the support.

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REVIEW 3

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

Siti Nuryanti[1], Nurul Latifasari[1], Rifda Naufalin[1]*), Rumpoko Wicaksono[1] and Erminawati[1]

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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.

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

INTRODUCTION

Kecombrang (*Etlingera elatior*) is one of the spice plants which has many uses. These herbs are used as food and are also used for medicine. The results of the research by (Naufalin, 2019; Naufalin & Herastuti, 2017; Latifasari, Naufalin, & Wicaksono 2019; Hanifah, Naufalin, & Wicaksono 2019), stated that the active compounds contained in the kecombrang parts include alkaloids, flavonoids, steroids, saponins and essential oils. 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, triterpenes, saponins, tannins, steroids, alkaloids, and glycosides. 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). According to Sukandar, Radiastutu, Jayanegara, Muawanah & Hudaya (2011), kecombrang flower water extract has antioxidant compounds such as

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Commented [A8]: Tambahkan literatur mengenai **senyawa spesifik/yang khas struktur kimianya dari kecombrang** yang bersifat antioksidan. Karena senyawa fenolik, flavonoid, tannin dan seterusnya, bukan nama suatu senyawa, tetapi merupakan golongan atau kelompok senyawa yang masing2 memiliki struktur beragam. Dan kelompok senyawa tersebut terdapat di semua tumbuhan.

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.

MATERIALS AND METHODS

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 (*Etlingera 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, wipes, plastic and stainless steel spoons, plastic jerry cans, knives and cutting boards, grinders, 60 mesh sieves, clear plastic clips, vortex mixers, shakers, centrifuse, 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, erlemeyer (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, 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 process

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 1 ml of each dilution series was taken and added 1.5 ml of 10% Folin Ciocalteau 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-Ciocalteau 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 × X (g) × (V) / (sample weight (g))

X = sample concentration

FP = dilution factor

Antioxidant activities 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 % = Abs control-Abs sample)/Abs control × 100

Kecombrang extract yield calculation (Nurhaen, Winarsii & Ridhay, 2016)

The yield of kecombrang extract produced is determined using the following equation:

Yield (%) = initial sample weight (g)/extract weight (g) × 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

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**.

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 $^{-1}$)
1.	Flowers	*n*-hexane	0.202
		ethyl acetate	3.379
		distilled water	36.248
2.	Stems	*n*-hexane	8.574
		ethyl acetate	15.395
		distilled water	32.757
3.	Leaves	*n*-hexane	19.116
		ethyl acetate	10.279
		distilled water	45.008

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.

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 by (Naufalin & Herastuti, 2011), the largest average value of total phenols is shown by the interaction interactions 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.

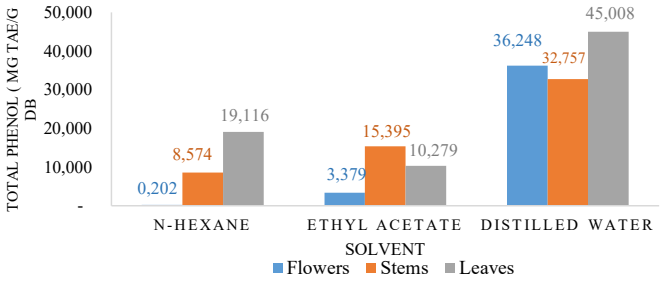

Figure 1. Total phenol content of flowers, stems and kecombrang leaves with 3 types of solvents

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.

The results of research on the antioxidant activity of flowers, stems and leaves kecombrang are presented in **Table 2**.

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

	Parts	Solvents	Antioxidant (% inhibition)
1	Flowers	*n*-hexane	14.906
		ethyl acetate	41.534
		distilled water	69.754
2	Stems	*n*-hexane	39.074
		ethyl acetate	53.690
		distilled water	72.648
3	Leaves	*n*-hexane	43.994
		ethyl acetate	64.689
		distilled water	78.003

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 of 0.339% ± 0.006 and $LC_{50} \leq$ 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 sporacid.

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**).

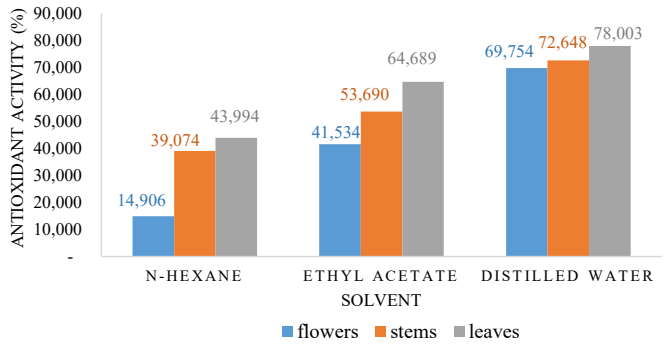

Figure 2. Antioxidant activity of flowers, stems and leaves with distilled water

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, Naufalin, & Wicaksono, 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 (%)
1.	Flowers	20	n-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
2.	Stems	20	n-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
3.	Leaves	20	n-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

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 were15.9; 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**).

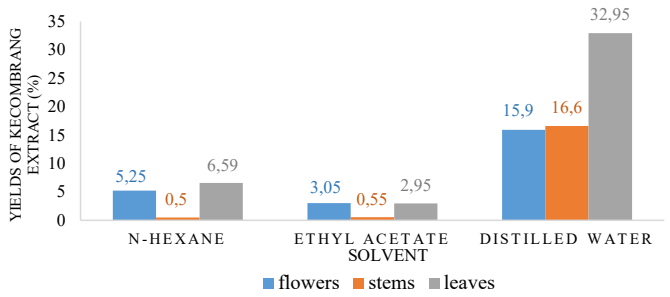

Figure 3. Yield of flowers, stems and leaves kecombrang with distilled water

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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REVIEW 4 (FINAL)

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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 (*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 aimeds 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 extractions with distilled water produced the highest total phenol, antioxidant activity and yield on kecombrang leaves. The total phenol of n-hexane extract, ethyl acetate extract, and distilled water extract of kecombrang leaves are: 19.116, 10.276, and 45.008 (mg TAE g db $^{-1}$); respectively. The antioxidant activity value of distilled water extract of kecombrang flowers, stems and leaves are: 69.754, 72.648, and 78.003 (%); respectively. The yield for the distilled water extract of flowers, stems and leaves are: 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, can be a safe source of antioxidants to reduce free radical. Spices, ~~are~~ generally used as seasonings by the community to make ~~them~~ food delicious. This type of spice comes from certain parts of the plant or spices such as bark, roots, stems, leaves, flowers, fruit, and seeds. Plants included in the spice category includecardamom and its family, cloves, coriander, pepper, nutmeg, cinnamon, cumin, and candlenut (Boga, 2014). Spices contain bioactive compounds and can produce extracts and essential oils that have variousbenefits, 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~~ by 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 (Mukhriani, 2014). 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. Using of a simple separation technique to isolate single compound from the extract will be very hard to do in early extract. Because of this, the early extract needs to be detached into some fractions that have the molecular dimensions and similar polarity (Mukhriani, 2014).

Kecombrang (*Etlingera elatior*) is one of ~~the~~ spice plants which has many uses. These herbs are used as food and ~~are~~ also ~~used~~ for medicine. Flower of kecombrang indicated rich natural antioxidants, powder formulations of flower contain bioactive compounds, namely phenolic (Naufalin & Herastuti, 2016; Naufalin, Erminawati, Wicaksono, Febryani, & Latifasari, 2021). The results of the research, stated that the active compounds in the all of kecombrang parts include alkaloids, flavonoids, steroids, saponins and essential oils (Naufalin, 2019; Naufalin & Herastuti, 2017; Latifasari, Naufalin, & Wicaksono 2019; Hanifah, Naufalin, & Wicaksono 2019). All parts of kecombrang such as part of flowers, stems, leaves, fruits and rhizomes have the potential as a source of naturalantioxidants. Antioxidant compounds in kecombrang include phenolic, flavonoids, triterpenes, saponins, tannins, steroids, alkaloids, and glycosides. Crude water extract of kecombrang flower ~~water~~ has the potentialas an antioxidant. Compounds that are thought to beantioxidants are compounds that contain phenol groups. GCMS (Gas Chromatography Mass Spectrometry) analysis results of kecombrang ~~flower~~ water extract flower showed that there are 6 main compoundgroups, namely alkanes, alkenes, alcohols, fatty acidsand phenols. Three of the dominant ~~ones~~ are 1- dodecanol, 3-methyl-1-okso-2-buten 1-(2^1,4^1,5^1-trihydroxyl phenyl), and 1-tetradekena (Sukandar, 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 of antioxidant compoundsfrom plants generally uses different types of solventswith different polarity levels, from non-polar, semi- polar and polar. Solvents used such as *n*-hexane, ethyl acetate, ethanol and aquadest. The selection of solvents build upon the polarity level is very useful to get extracts with a greater yield and it is also intended ~~so that~~ the ~~group of~~ antioxidant compounds ~~that have the~~ highest activity can be extracted. This study purposes to determine the effect ofextraction 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. This study were used flowers, stems andleaves of kecombrang (*Etlingera elatior*) obtained 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 stainlesssteel pan, cabinet dryer, tissue paper (Nice), wipes, plastic and stainless steel spoons, plastic jerry cans, knives and cutting boards, grinders (Philips), 60 meshsieves, clear plastic clips, vortex mixers, shakers, centrifuse, rotary evaporator (RE-200), UV-VISspectrophotometer (Shimadzu-1800), cuvette, water bath, showcase (Polytron), 10 mL vial bottles, glass test tubes (Pyrex), test tube racks, aluminum foil, erlemeyer(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 with water flow further cut into size of 2x3cm. After that, dried process with cabinet dryer at 50˚C until it breaks dry or crispy, then mashedusing 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). Weighed a 100 g of kecombrang powder of flowers, stems, and leaves then added 1.000 mL of solvent (1:10), except for kecombrang stem use 2.000 mL of solvent (1:20). The mixture allowed to stand for 48 hours while stirring every 6h, for 2-5min, then filtered with filter paper and filter cloth (Savitri, Suhendra, & Wartini, 2017). The filtrate obtained ~~by the solvent is~~ then 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%), 0.06mg/ml = (6 ml of stock solution + 8 ml of ethanol 95%), 0.08 mg/ml = (8 ml of stock solution + 8 ml ofethanol 95%), 0.1 mg/ml = (10 ml of stock solution +8 ml of ethanol 95%), A total of 1 ml of each dilutionseries was taken and added 1.5 ml of 10% Folin Ciocalteau and leaved it for 5 minutes at room temperature. Then added 1.5 ml of sodium bicarbonate ($NaHCO_3$) 0.556 M, shaken. The next step, left in the dark room for 90 minutes, further absorbance was measured using a spectrophotometer at λ 725 nm.

Sample preparation

Sample 100 mg added 70% ethanol as much as 4 mL were then shaken with a shaker for 2 hours at 200 rpm speed, then centrifuged for 15 minutes at a speed of 1000 rpm. The obtained supernatant isan extract for determining the sample. The sample isthen 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-Ciocalteau and leaved it for 5 minutes at room temperature. Then added 1.5 ml ofsodium bicarbonate ($NaHCO_3$) 0.556 M is shaken and left in a dark room for 90 minutes. Furthermore absorbance was measured at λ 725 nm using spectrophotometer. Total phenol (mg / g) = FP × X (g) × (V)/ (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. Anamount of 2 ml of

sample solution was added to 2 mlof DPPH solution. The blend solution was incubated in the dark conditions and room temperature for 30 minutes. Spectrophotometer used to measured reduction in absorbance at a wavelength of 517 nm. The control solution is made from 2 ml of methanol p.a and 2 ml of DPPH solution. DPPH free radical percentage declrared by percentage inhibition (Naufalin & Herastuti, 2016). The percentage of DPPH radical capture during incubation is calculated by thefollowing equation: Radical capture % = Abs control-Abs sample)/Abs control × 100

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

The yield of kecombrang extract produced isdetermined using the following equation: Yield (%) =initial sample weight (g)/extract weight (g) × 100

Statistical Analysis

This research used F-test data analyzed, if the result shows diversity continued by Duncan's Multiple Range Test (DMRT).

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 tungstant 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 ofcompounds which provides antioxidant activity (Kurnia, Rosliana, Juanda, & Nurochman, 2020). Thehigher 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 thesolubility of phenol (Wazir, Ahmad, Muse, Mahmood,& Shukor, 2011). High temperatures are able torelease cell wall phenolic compounds in consequence of the cellelements destruction, inducing more phenol to be extracted. The total phenol of the fresh simplicial 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 thatthe phenol content of the dry simplicia essential oil ofkecombrang 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. The highest total phenol of kecombrang extract obtained by using polar solvents (distilled water). Phenol is a compound that has the highest solubility in polar solvents. Accordingto Naufalin (2019), phenol compounds are soluble in polar solvents because they have an aromatic ring or more hydroxyl groups. 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), showed that the total phenol level value of the ethyl acetate fraction during storage time ranges 522.08 - 1776.08 mg 100 g $^{-1}$ and for the ethanol fraction ranges between 854.10-4851.30 mg 100 g $^{-1}$. The fraction of *n*-hexane is structured by non-polar compounds while ethyl acetate is structured by semi-polar compounds whichstill possible contain non-polar compounds. Accordingly, non-polar compounds or non-polar solvents unable to extract phenol.

Table 1. Results of total phenol analysis of flowers, stems and leaves of kecombrang withvarious 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, theparts of the kecombrang also influence the value of total phenol (**Figure 1**). The result of 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. It is similar with the results of research by(Naufalin & Herastuti, 2011), the largest average total phenols value is proven by the interaction from ethanol leaf fractions, 2047.58-15894.07 mg 100 g $^{-1}$, whilst other interactions do not significantly affect the total phenol. Based on the result, the constituent in kecombrang leaves are generally polar and contain alot of chlorophyll.

Antioxidant Activity

Characterized compound as a stable free radical by good of the spare electron delocalization over the molecule as a whole can be interpreted as DPPH (Sagar & Singh, 2011). The working principle of this method is a process of reducing free radical of DPPH compounds because of antioxidants. Antioxidant activity is expressed by 50% Inhibition Concentration or IC_{50}, namely the sample concentration that can reduce DPPH radical compounds by 50%. The IC_{50} grade is obtained from the x grade 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. Thereare two main types of antioxidants, namely primary and secondary antioxidants. Both have different mechanisms of action. Primary antioxidants scavengefree radicals and provide a hydrogen atom or electronto stabilize the free radical. On the other hand, secondary antioxidants work by suppressing the formation of free radicals which then prevent oxidative damage (Hue, Boyce, & Somasundram, 2012). The research results on the antioxidant activity of flowers, stems and leaves kecombrang are presented in **Table 2.**

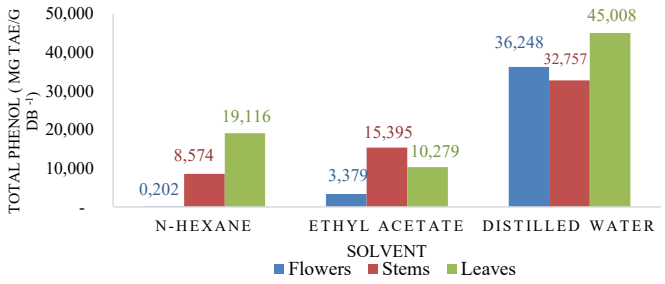

Figure 1. Total phenol content of flowers, stems and kecombrang leaves with 3 types of solvents

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

	Parts	Solvents	Antioxidant (% inhibition)
1	Flowers	*n*-hexane	14.906
		ethyl acetate	41.534
		distilled water	69.754
2	Stems	*n*-hexane	39.074
		ethyl acetate	53.690
		distilled water	72.648
3	Leaves	*n*-hexane	43.994
		ethyl acetate	64.689
		distilled water	78.003

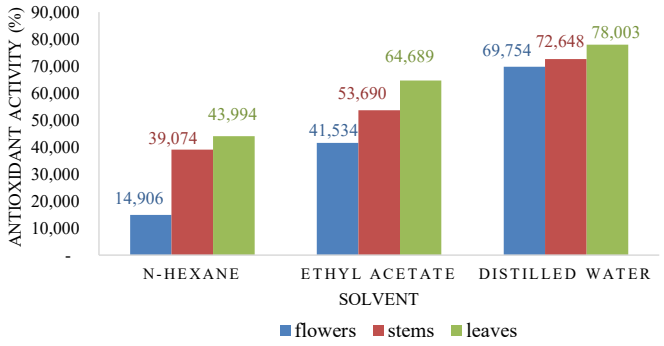

Figure 2. Antioxidant activity of flowers, stems and leaves with distilled water

According to Hanifah et al., (2019), kecombrang contains flavonoids which act as antioxidants and thought to be able to inhibit the rate of oxidation in gouramy fillets. Based on the data that has been obtained, the kecombrang and the solvent have an effect on the antioxidant activity of the resulting 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 are 69.754; 72.648 and 78.003 (%). These results is compatible with the phenol content in kecombrang, The value of phenol is strongly influenced by antioxidant activity. The statement is in accordance with the resultsof research Kusriani et al. (2017), showed 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 of 0.339% ± 0.006 and $IC_{50} \leq 1000$ ppm. Polyphenols are combinations of several phenol groups. Phenolic compounds can act as reducing agents by donating H^+ ions from the hydroxyl group. The ability to donate H^+ ions makes phenol compounds act as natural antioxidants especially for food (Naufalin, Wicaksono, Erminawati, Arsil, & Gulo, 2019). Other research on total phenolsaccording to Carolia & Noventi (2016), betel leaf hasa distinctive aroma because it contains 1-4.2% essential oil. The phenol is not for sporacid. 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 µg mL^{-1} for leaves and flowers 101.84 µg mL^{-1}.

Meanwhile, the highest % inhibition from kecombrang stem extract with various solvents each for *n*-hexane, ethyl acetate and distilled water 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 levelof polarity of a compound so that it would get extractswith a greater yield and also intended that the class of antioxidant compounds that have the highest activity can be extracted (Hanifah at al., 2019). How to calculate the extract yield that is based on the ratio of the final weight (weight of the resulting extract) to the early weight (weight of cell biomass used) multiplied by 100% (Sani, Fithri, Ria., & Jaya, 2014). Kecombrang extract yield result 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	*n*-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	*n*-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	*n*-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

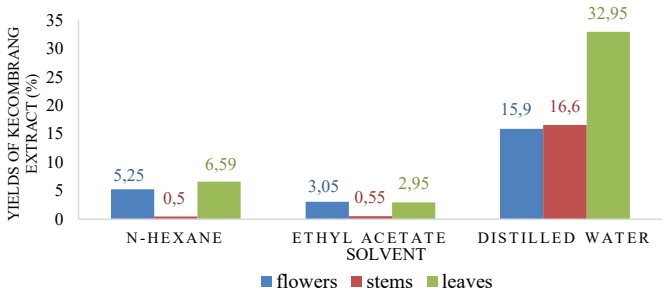

Figure 3. Yield of flowers, stems and leaves kecombrang with distilled water

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 ofkecombrang and polarity of the solvent greatly affectthe extract yield. The yield for flowers, stems and leaves with distilled water solvent were15.9; 16.6 and 32.95 (%). Polar solvents produce a lot of yield. The possibility of this is happened because the bioactive compounds in kecombrang are non-polar groups **(Figure 3).**

CONCLUSIONS

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

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