

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

Siti Nuryanti, Nurul Latifasari, Rifda Naufalin*, Rumpoko Wicaksono and Erminawati

Department of Agricultural Technology, Jenderal Soedirman University, Karangwangkal
dr Soeparno street, Purwokerto 53123, Indonesia

*Corresponding author email: rifda.naufalin@unsoed.ac.id

Received January 08, 2020; Accepted March 05, 2021; Available online July 20, 2021

ABSTRACT. Kecombrang (*Etlingera elatior*) is one spices which widespread in Indonesia and has many uses. Kecombrang extract has 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 aimed to determine the effect of extraction on bioactive components of flowers, stems and leaves of kecombrang in different types of solvents and determine 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, has the potential as a source of natural antioxidants. Spices generally used as seasonings by the community to make food 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 candlenut (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 even used as an insecticide. Plant extract obtained by the extraction process.

Extraction is the process of separating materials using certain solvents. After the extraction process, the solvent separated from the sample by filtering (Mukhriani, 2014). When 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 detected

into some fractions that have the molecular dimensions and similar polarity (Mukhriani, 2014).

Kecombrang (*Etlingera elatior*) is one of spice plants which has many uses. These herbs are used as food and also 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; Hanifah, 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, triterpenes, saponins, tannins, steroids, alkaloids, and glycosides. Crude water extract of kecombrang flower 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 water extract flower showed that there are 6 main compound groups, namely alkanes, alkenes, alcohols, fatty acids and phenols. Three of the dominant are 1-dodecanol,

3-methyl-1-okso-2-buten 1-(2¹,4¹,5¹-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 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 it is also intended the antioxidant compounds 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 (*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%, NaHCO₃ 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, 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, 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). 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 is allowed to stand for 48 hours while stirring every 6h, for 2-5 min, then filtered with filter paper and filter cloth (Savitri, Suhendra, & Wartini, 2017). The filtrate obtained 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%), 0.06 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 Ciocalteu and allowed to stand for 5 minutes at room temperature. Then added 1.5 ml of sodium bicarbonate (NaHCO₃) 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 (NaHCO₃) 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 / (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 % = Abs control - Abs sample / 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 (%) = initial sample weight (g) / 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 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 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	<i>n</i> -hexane	0.202
	ethyl acetate	3.379
	distilled water	36.248
Stems	<i>n</i> -hexane	8.574
	ethyl acetate	15.395
	distilled water	32.757
Leaves	<i>n</i> -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 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.

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 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 (Hue, Boyce, & Somasundram, 2012). The results of research on the antioxidant activity of flowers, stems and leaves kecombrang are presented in **Table 2**.

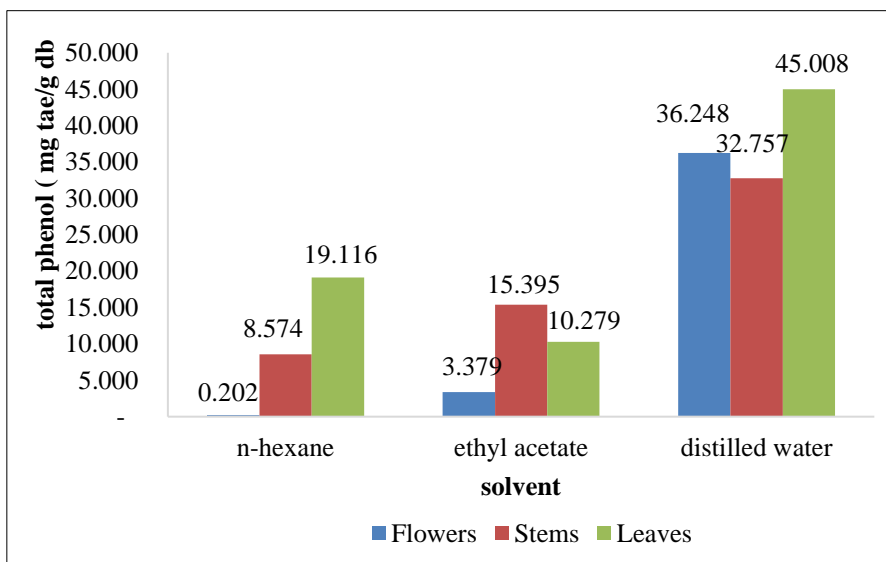


Figure 1. Total phenol content of flowers, stems and kecombrang leaves with 3 types of solvents

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

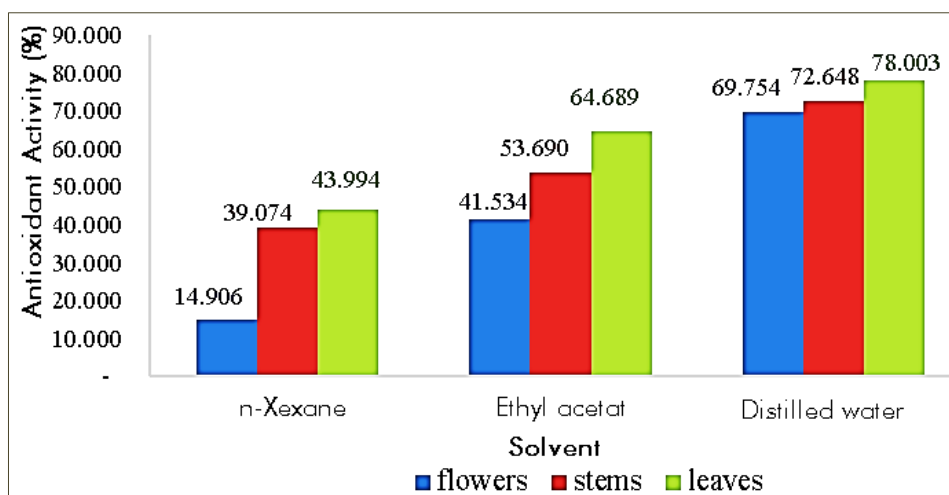


Figure 2. Antioxidant activity of flowers, stems and leaves with distilled water

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 of $0.339\% \pm 0.006$ 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 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 at 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	15.90
Stems	20	<i>n</i> -hexane	0.10	0.4	0.50
	20	ethyl acetate	0.11	0.4	0.55
	20	distilled water	3.32	0.4	16.60
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

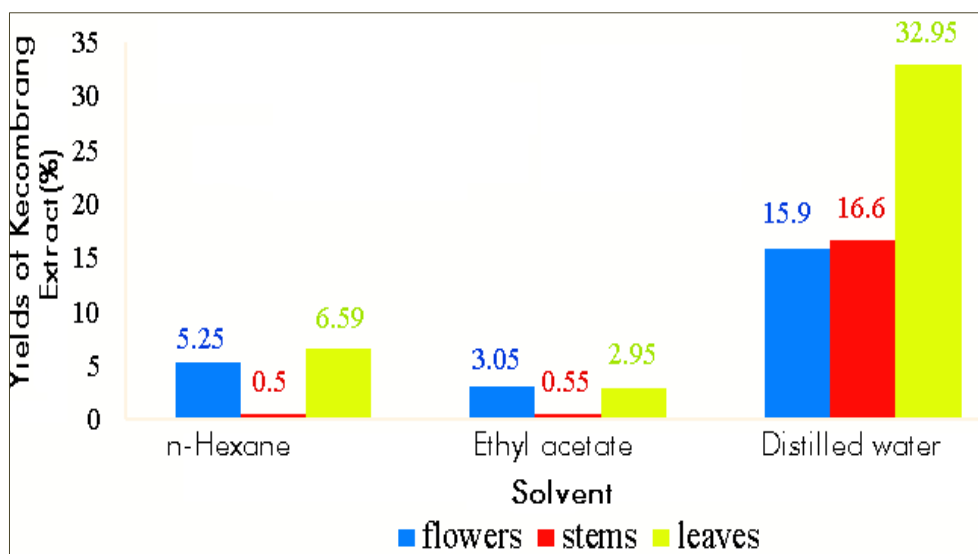


Figure 3. Yield of flowers, stems and leaves kecombrang with distilled water

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. 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 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

The author and researcher would like to thanks to DRPM Republic of Indonesia Research and Technology Ministry which has provided research funding through 2019 Thesis Research Grant Scheme and to the Institute for Research and Community Service at

Jenderal Soedirman University for their support during the course of this research.

REFERENCES

- Boga, Y. (2014). *Kitchen of Indonesia: 300 Recipes of Popular Indonesian Cuisine*. Gramedia Pustaka Utama, Jakarta.
- Carolia, N., and Noventi, W. (2016). Potential of Green Betel Leaf Extract (*Piper betle* L.) as an Alternative Therapy for Acne vulgaris. *Majority Journal*, 5(1), 140-145.
- Handayani, V, Ahmad, A.R, and Sudir, M. (2014). Antioxidant activity test of methanol extract of patikala flower and leaf (*Etingera elatior* (Jack) R.M.Sm) using the DPPH method. *Pharmaceutical Sciences & Research*, 1(2), 86-93.
- Hanifah, M.R., Naufalin, R., and Wicaksono, R. (2019). The Effect of Edible Coating Contained Kecombrang Leaves Concentrate on Gourami Fish Fillet Quality. (Vol. 250 (2019), p.012057: 18). IOP Conf. Series: Earth and Environmental Science.
- Hue, S.M, Boyce, A. N, and Somasundram, C. (2012). Antioxidant Activity, Phenolic and Flavonoid Contents in the Leaves of Different Varieties of Sweet Potato (*Ipomoea batatas*). *Australian Journal of Crop Science*, 6(3), 375-380.
- Jahangiri, Y., Ghahremani, H., Torghabeh, J.A., and Salehi, E. A. (2011). Effect of temperature and solvent on the total phenolic compounds extraction from leaves of *Ficus carica*. *Journal of Chemical and Pharmaceutical Research*, 3(5), 253-259.
- Katrin & Bendra, A. (2015). Antioxidant Activity of Extracts, Fractions and Chemical Compounds

- in Leaves *Premna oblongata* Miq *Pharmaceutical Science Original Article*, 2(1), 21-31.
- Kurnia, D., Rosliana, E., Juanda, D., and Nurochman, Z. (2020). Antioxidant Activity and Determination of Total Phenol Levels from Marine Microalgae *Chlorella vulgaris*. *Journal of Research Chemistry*, 5(1), 4-21.
- Kusriani, H., Subarnas, A., Diantini, A., Iskandar, Y., Marpaung, S., Juliana M., and Silalahi, F. (2017). Antioxidant and Cytotoxic Activities and Determination of Levels of Total Phenol Compounds in Leaf, Flower, and Rhizome Extracts of Kecombrang (*Etligeria elatior*). *Pharmacy Journal*, 14(1), 51-63.
- Latifasari, N., Naufalin, R., and Wicaksono, R. (2019). Edible Coating Application of Kecombrang Leaves to Reduce Gourami Sausage Damage. (Vol. 250 (2019), p. 012055: 1-7). IOP Conf. Series: Earth and Environmental Science.
- Mukhriani. (2014). Extraction, Compound Separation, and Identification of Active Compounds. *Journal of Health*, 7(2), 361-367.
- Naufalin, R., and Herastuti, S. R. (2011). Antioxidant Potential of Kecombrang (*Nicolaia speciosa* Horan) Extraction During Storage. *On-line*. <https://www.researchgate.net/publication/257265941>.
- Naufalin, R., and Herastuti S. R. (2016). Antioxidant Activity and Physicochemical Properties of *Nicolaia speciosa* Flower Extract. *Agriculture and Agricultural Science Procedia* 9, 297 –303.
- Naufalin, R. (2017). *Kecombrang: Antimicrobial and Use as a Food Preservative*. Purwokerto: Jenderal Soedirman University.
- Naufalin, R., Wicaksono, R., Erminawati, Arsil, P., and Gulo, K. I. T. (2019). Application of Concentrates Flower Kecombrang on Edible Coating as Antioxidant to Suppress Damage on Gourami Sausage. (Vol. 255 (2019), p.012040). IOP Conf. Series: Earth and Environmental Science.
- Naufalin, R. (2019). Natural preservation opportunities and challenges in improving food safety. In *AIP Conference Proceedings*. (Vol. 2094, No. 1, p. 020032). AIP Publishing.
- Naufalin, R., and Herastuti, S. R. (2017). Antibacterial activity of *Nicolaia speciosa* fruit extract. *International Food Research Journal*, 24(1), 379-385.
- Naufalin, R., and Herastuti, S. R. (2013). Microcapsule application of kecombrang flower extract: effect of concentration, type of fraction, pH of medium and NaCl on microbiological properties of minced beef. *Journal Animal Production*, 15(1), 8-14.
- Nurhaen, D., Winarsi, and Ridhay, A. (2016). Isolation and Identification of Chemical Components of Essential Oils from the Leaves, Stems and Flowers of the Salembangu Plant (*Melissa* sp.). *Online Journal of Natural Science*, 5(2), 149-157.
- Othman, A.A., Ismail, Ghani, N.A., and Adenan, I. (2007). Antioxidant capacity and phenolic content of cocoa bean. *Food Chemistry*, 100(4), 1523-1530.
- Purwanto, D., Bahri, S., and Ridhay, A. (2017). Antioxidant Activity Test of Purnajiwa Fruit Extract (*Kopsia arborea* Blume) with Various Solvents. *KOVALEN*, 3(1), 24-32.
- Sagar, B. K., and Singh, R.P. (2011). Genesis and Development of DPPH Method of Antioxidant Assay. *J. Food Science Technology*, 84(4), 2011.
- Sani, R.N., Fithri, C.N., Ria, D.A., and Jaya, M.M. (2014). Yield Analysis and Phytochemical Screening of Ethanol Extract of *Tetraselmis chuii* Marine Microalgae. *Food and Agroindustry Journal*, 2(2), 121-126.
- Savitri, I, Suhendra, L., and Wartini, N. M. (2017). Effect of Solvent on Maceration Method on *Sargassum polycystum* Extract Characteristics. *Journal of Agroindustry Engineering and Management*, 5(3), 93-101.
- Shekhar, T.C, and Anju, G. (2014). Antioxidant Activity by DPPH Radical Scavenging Method of *Ageratum conyzoides* Linn. Leaves. *American Journal of Ethnomedicine*, 1(4), 244-249.
- Sukandar, D., Radiastutu, N., Jayanegara, I., Muawanah, A., and Hudaya, A. (2011). Antioxidant Activity Test of Crude Extract of Kecombrang (*Etligeria elatior*) Flower Water as A Functional Food Ingredient. *UIN Syarif Hidayatullah of Jakarta Article*, ISSN 0853-2788.
- Susana, I., Ridhay, A., and Bahri, S. (2018). Study of Antioxidant Activity of Kecombrang Stem (*Etligeria elatior*) Extract based on Solvent Polarity Level. *Kovalen*, 4(1), 16-23.
- Wazir, D., Ahmad, S., Muse, R., Mahmood, M., and Shukor, M.Y. (2011). Antioxidant Activities of Different Parts of *Gnetum gnemon* L. *Journal Plant Biochemistry and Biotechnology* 20(2), 234-240.
- Wungkana, I., Suryanto, E., & Momuat, L. (2013). Antioxidant Activity and Sunscreen Phenolic Fraction of Corn Cobs Waste (*Zea mays* L.). *Pharmakon Jurnal Ilmiah Farmasi*, 2(4), 149–155.