



The Effect of Temperature and Length of Heating on the Antioxidant Activity of Onion Dayak Bulb Extract (*Eleutherine palmifolia*)

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Abstract :

Onion Dayak (*Eleutherine palmifolia*) is a typical plant of center Kalimantan which has been used hereditary as medicine by Dayak people. The part of this plant that is used as medicine is the bulb. Empirically, the bulb of onion Dayak can heal diabetic disease, hypertension, cancer and lower cholesterol level. The aimed of this study is to know the effect of heating temperature and heating time to the antioxidant activity of onion Dayak bulb extract (*Eleutherine palmifolia*). This study was using a completely randomized design (CRD) with factorial pattern. Two factor used in this study were heating temperature (75, 85, 95 °C) and heating time (5, 10, 15 minutes). This study was performed in three replications. The result showed that heating temperature and heating time has a significance effect on the yield, antioxidant activity value and IC₅₀. The treatment of 75°C for 5 minutes of heating was the best combination of heating temperature and heating time with the value yield (76,50%), antioxidant activity value (63,72%), IC₅₀ (33,05µg/ml), color: L* 43,25, a*-2,01, b* 18,4. Phytochemical analysis showed that the extract of onion Dayak bulb contains phenolic and flavonoid compounds.

Keywords: Dayak Onion, Temperature, Antioxidant

1. Introduction

Dayak onions (*Eleutherine palmifolia*) is a typical of Central Kalimantan plant that has been used by Dayak people as a medicinal plant for generations. The part used in this plant is the bright red tuber, 1-6 cm long which split into layers. The green leaves are ribbon-shaped, almost similar to the leaves of oil palm plants and have white flowers (Firdaus, 2006). Dayak people consume Dayak onion bulbs by boiling the bulbs in water and drinking the boiled water. This simple way of consuming Dayak onion bulbs is still used today. Empirically, it is known that boiled water from Dayak onion bulbs can prevent and cure various degenerative diseases such as cancer, heart disease, osteoarthritis, diabetes mellitus, hypertension, lower cholesterol, cure ulcers and stomach ache after giving birth.

(Galingging, 2007). Antioxidants are chemical compounds that can donate one or more electrons to free radicals, so that the free radicals can be suppressed and have a partner (Setiawan Dalimartha, 1999). Antioxidants are very sensitive to processing using high temperatures.

Extraction is the separation of one or several materials originating from a solid or liquid using a solvent. Extraction is based on the mass transfer of solid components into the solvent where the transfer begins to occur at the interfacial layer and then diffuses into the solvent (Directorate General, 2000). Extraction involving heat is often used to obtain optimal extracts in a short time, but extraction using heat is thought to cause a decrease in the antioxidant activity value of the material. This is because the antioxidant component is a bioactive compound that is very sensitive to light, temperature and pH (Nur, 2011). Apart from temperature, extraction time also greatly influences the type and amount of compounds extracted. According to Budiyo (2008), the right extraction time will produce the optimal type and amount of active

compounds in the extract. An extraction time that is too long will cause the extract to hydrolyze, while an extraction time that is too short will cause not all active compounds to be extracted from the material.

Research conducted by Rachmawati et al., (2013) stated that the antioxidant activity of red guava jam decreases at high temperatures. Guava jam made with variations in time (30, 60, and 90 minutes) and heating temperature (70.80, and 90°C) experienced a decrease in susceptibility from 96.73 - 82.04%. The higher the temperature and the longer the heating time, the secondary metabolite compounds which act as antioxidants become damaged.

Based on this description, researchers conducted research with the aim of determining the effect of temperature and heating time on the antioxidant activity of Dayak onion (*Eleutherine palmifolia*) tuber extract.

2. Research Methods

Materials and Tools

Dayak onion bulbs (*Eleutherine palmifolia*) were obtained from Kerinci. Dimethyl sulfoxide (DMSO), methanol, DPPH, Mayer's reagent, concentrated ammonia chloroform, 1% iron (III) chloride solution, and Lieber Mann – Burchard reagent (acetic acid and concentrated sulfuric acid) were purchased from local store.

Research Design and Statistical Analysis

This research used a completely randomized design (CRD) with two factors. The first factor was temperature which consists of 3 levels (75, 85 and 95 °C). The second factor was the length of heating time which also consists of 3 levels (5, 10, 15 minutes) with 3 repetitions to obtain 27 experimental units.

Preparation of Extract

Fresh Dayak onion bulbs were sorted, peeled and washed thoroughly with running water. The Dayak onion bulbs that have been washed were sliced into small pieces to increase the surface area. About 100 gram Dayak onion bulbs were weighed and heated in 200 ml of water at certain temperature of (75, 85 and 95°C) for 5, 10 and 15 minutes, and leave to cool down. The Dayak onion bulb extract was filtered using filter paper to separate the filtrate from impurities.

Parameter

The parameters observed were yield, antioxidant activity (Selvi et al., 2003), IC50, color analysis with a color reader (Andarwulan et al., 2011), descriptive tests and qualitative phytochemical tests (Mubarokah, 2011).

Data Analysis

To determine the effect of treatment on the observed parameters, the data obtained were analyzed statistically using analysis of variance at the 1% and 5% levels. If it shows a significant effect, then Duncan's New Multiple Range Test (DNMRT) was applied.

3. Results and Discussion

The Extract and The Yield

The description of the Dayak onion bulb extract is presented in Table 1. The extracts have variation in red color depending on the temperature and length of time applied during the preparation of the extract. The temperature and length of extraction are also influence the aroma and viscosity of the extract obtained. Yield is the percentage of the final weight of the sample per initial weight of the sample. The yield calculation is used to determine the loss that occurs during the processing process. The yield is expressed in percent (%) (Apriyanto et al., 1989). The results of the analysis of variance showed that temperature had a very significant effect on the yield of Dayak onion bulb extract. The length of heating time has a significant effect on the yield of Dayak Dayak onion bulb extract. There is an interaction between temperature and heating time on the yield of Dayak onion bulb extract. The average yield value of Dayak onion bulb extract at various temperature treatments and heating times can be seen in Table 2.

Table 1. The description of Dayak onion bulb extract at different temperature and length of heating.

Treatment		Description		
		Color	Aroma	Viscosity
75°C	5 Minutes	Dark red	Aroma kacang rebus	Cair
85 °C	5 Minutes	Dark red	Aroma kacang rebus	Agak kental
95 °C	5 Minutes	Dark red	Agak langu bercampur aroma kacang rebus	Kental
75 °C	10 Minutes	Dark red	Langu bercampur aroma kacang rebus	Agak kental
85 °C	10 Minutes	Very dark red (+)	Langu bercampur aroma kacang rebus	Kental
95 °C	10 Minutes	Very dark red (+)	Langu bercampur aroma kacang rebus	Sangat kental
75 °C	15 Minutes	Very dark red (++)	Langu	Sangat kental
85 °C	15 Minutes	Very dark red (++)	Langu	Gel
95 °C	15 Minutes	Very dark red (++)	Langu	Gel

Table 2. Average value of yield (%) of Dayak onion bulbs based on temperature treatment and heating time.

Temperature (°C)	Yield (%)		
	Length of Heating (minutes)		
	5	10	15
75	76,50a A	67,50a B	23,33a C
85	68,33b A	48,67b B	16,50b
95	46,33c A	30,33c B	10,67c C

Notes:

Numbers followed by the same letter are not significantly different at the 5% level according to the DNMRT test. Uppercase letters are read horizontally and lowercase letters are read vertically.

Table 2 shows that the highest yield value was obtained from the 75°C temperature treatment with a heating time of 5 minutes, namely 76.50% while the lowest was obtained in the 95°C treatment with a heating time of 15 minutes, namely 10.67. The interaction that occurs between temperature and length of heating time on the yield of Dayak onion bulb extract is caused because the higher the temperature and the longer the extraction time will cause the solvent used in the extraction in this case is water to evaporate at high temperatures and long heating times so that it affects the rate of evaporation of the solvent.

The decrease in yield value along with the higher temperature and length of heating time is due to the rate of evaporation is determined by the increase in temperature, the greater the difference between the temperature of the heater and the heated material, the greater the speed of heat transfer into the heated material so that evaporation will occur more and faster. Similarly, the longer the extraction heating time, the longer the heating is carried out, the more heat (heat) received by the heated material so that the material will evaporate more easily (Taib et al., 1988).

According to Sa`adah (2015) in the comparison of ethanol and water solvents in the manufacture of tiwai onion bulb extract (*eleutherine americana merr*) extraction with water solvents produces the largest yield compared to other solvents because water is an excellent solvent for ionic compounds.

Phytochemical Composition

Phytochemical tests were carried out to determine the presence of specific chemical compounds such as alkaloids, phenols, steroids, saponins, triterpenoids, tannins and flavonoids. These compounds are secondary metabolites in plants that can be further utilized for medicinal purposes. Some of these

compounds have been isolated and some of them provide physiological and pharmacological effects better known as active chemical compounds (Copriyadi J, 2005). The results of phytochemical screening of Dayak onion bulb extract can be seen in Table 3.

Table 3. Phytochemical screening results of Dayak onion bulb extracts

Chemical Compound	Assay Results
Flavonoids	++
Phenolic	+++
Saponins	-
Triterpenoid/Steroids	-
Alkaloids	-

Notes: - = negative, + = positive, ++ = strong positive, +++ = very strong positive.

Based on Table 3, it can be seen that Dayak onion bulb extract positively contains secondary metabolite compounds, namely flavonoids and phenolics, and negatively contains saponins, steroids and alkaloids. Flavonoids are large group of plant polyphenolic compounds, the content of flavonoid compounds in plants is very low at around 0.25%. These components are generally found bound or conjugated with sugar compounds (Winarsi, 2007). It is known that flavonoids have a number of abilities, namely to trap and inhibit the formation of hydroxyl free radicals, superoxide anions, peroxy radicals, alkoxy radicals, singlet oxygen, hydrogen peroxide (Widowati et al., 2005).

Phenolic compounds have been known to have various biological effects such as antioxidant activity through mechanisms as reducers, free radical capturers, metal chelators, absorbers of singlet oxygen formation and electron donors (Karadeniz et al., 2005). Plants are known to have different phenolic component structures, there are phenolic components that have many -OH groups and there are also phenolic components that have few -OH groups. The difference in the number and position of hydroxyl groups in an antioxidant compound such as phenols and flavonoids, can affect its antioxidant activity. Bettuzi (2009) stated that compounds from the polyphenol group have very strong antioxidant activity. The antioxidant activity of polyphenolic components is characterized by relatively high activity as hydrogen or electron donors and the ability of polyphenolic radical derivatives to stabilize and move unpaired electrons (Sandasari, 2009). The phytochemical compounds identified in this Dayak onion bulb extract act as antioxidants, namely phenolics and flavonoids.

Antioxidant Activity

The results of analysis of variance showed that temperature had a significant effect ($p < 1\%$) on the antioxidant activity of Dayak onion bulb extract. Heating time significantly affects the antioxidant activity of Dayak onion bulb extract. There is no interaction between temperature and heating time on the antioxidant activity of Dayak onion bulb extract. The average value of antioxidant activity of Dayak onion bulb extract at various temperature treatments and length of heating time can be seen in Table 4.

Table 4. Mean value of antioxidant activity (%) of Dayak onion bulb extract based on temperature treatment and heating time.

Heating Temperature (°C)	Length of Heating (minutes)		
	5	10	15
75	63,72c B	62,72c A	62,73c A
85	62,53b B	54,85b A	59,19b A
95	46,52a B	43,29a A	41,65a A

Notes:

Numbers followed by the same letter are not significantly different at the 5% level according to the DNMR test. Uppercase letters are read horizontally and lowercase letters are read vertically.

Based on Table 4, it can be seen that the antioxidant activity values obtained in this study ranged from 41.65-63.72%. The highest antioxidant activity was found at a temperature of 75 °C with a heating time of 5 minutes, namely 63.72%, while the lowest was found at a temperature of 95 °C with a heating time of 15 minutes, namely 41.65%. The antioxidant activity of Dayak onion bulb extract is due to the presence of secondary metabolite compounds, namely flavonoids and phenolics in it. According to Nur (2011), fresh Dayak onion bulbs produce the highest antioxidant activity value compared to chips and simplisia, this is because fresh Dayak onion bulbs have not undergone thermal processes such as drying. Antioxidant components are bioactive compounds that are very sensitive to light, temperature and pH.

This study is in line with Estiasih's (2009) research on the antioxidant stability of keluwak powder (*Pangium edule* Reinw) during drying and cooking, which stated that the longer the cooking time, the lower the antioxidant activity of keluwak powder. The highest value was obtained from the treatment of 5 minutes boiling time (control) which amounted to 68.52%, while the lowest value was produced by the treatment of boiling time for 90 minutes which was 33.75%.

This research is in line with research conducted by Rachmawati et al., (2013) stated that the antioxidant activity of red guava fruit jam decreased in the treatment of high temperature cooking. Guava jam made with variations in time (30, 60 and 90 minutes) and heating temperature (70, 80 and 90°C) decreased in the range of 96.73 - 82.04%. The higher the temperature and the longer the heating time, the more the secondary metabolite compounds that act as antioxidants are damaged.

IC₅₀

IC₅₀ is the concentration of substrate solution or sample that is able to counteract free radicals by 50% (Molyneux, 2004). A compound is declared as a very strong anti-free radical if the IC₅₀ value is <10 µg/mL, strong if the IC₅₀ value is between 10-50 µg/mL, moderate if the IC₅₀ value ranges between 50-100 µg/mL, weak if the IC₅₀ value ranges between 100-250 µg/mL and inactive if the IC₅₀ is above 250 µg/mL (Phongpaichit et al., 2007).

The results of the analysis of variance showed that the heating temperature treatment had a significant effect on the IC₅₀ of Dayak onion bulb extract. The length of heating time has a very significant effect on the IC₅₀ of Dayak onion bulb extract. There is no interaction between temperature and length of heating time on the antioxidant activity of Dayak onion bulb extract. The average IC₅₀ value of Dayak onion bulb extract at various temperature treatments and length of heating time can be seen in Table 5.

Table 5. Mean IC₅₀ values (µg/mL) of Dayak onion bulb extract based on temperature treatment and length of heating.

Heating temperature (°C)	Length of heating time (minutes)		
	5	10	15
75	33,05a A	39,57a B	43,44a C
85	32,91b A	43,13b B	50,39b C
95	33,51b A	45,66b B	51,33b C

Notes: Numbers followed by the same letter are not significantly different at the 5% level according to the DNMRT test. Uppercase letters read horizontal and lowercase letters read vertical.

Based on Table 5, it can be seen that the average IC₅₀ value of Dayak onion bulb extract ranges from 32.91 – 51.33 µg/mL. The highest IC₅₀ value was obtained from extraction treatment at 85°C for 5 minutes, namely 32.91 µg/mL, while the lowest IC₅₀ value was obtained from treatment at 95°C for 15 minutes, namely 51.33µg/mL. The results of the IC₅₀ analysis of Dayak onion bulb extract are classified as strong antioxidants, ranging from 10 - 50 µg/mL.

The results of this study are in line with Kuntorini's (2010) research on the anatomical structure and antioxidant activity of Dayak onion bulbs (*eleutherine palmifolia*) from the South Kalimantan area. The IC₅₀

value of Dayak onion bulbs ranges from 25.3339–86.9039 $\mu\text{g/mL}$. This shows that the extract has strong antioxidant activity, when compared with the IC50 value in other plants, such as in Pratomo's (2016) research, the IC50 value of the functional drink of sambungjiwa leaves ranged from 76.45-109.99 $\mu\text{g/mL}$ and also according to Herliya's research. (2017) The average IC50 value of IV quality (fanning) black tea brewing water from the Kayu Aro tea factory ranges from 59.99-71.34 $\mu\text{g/mL}$.

Color

Color is an important parameter to determine the quality of the extract produced. The L* value indicates the brightness level, where if the value is close to 100, the color becomes whiter. The relationship between temperature treatment and heating time on the L* value in Dayak onion tuber extract can be seen in Figure 1.

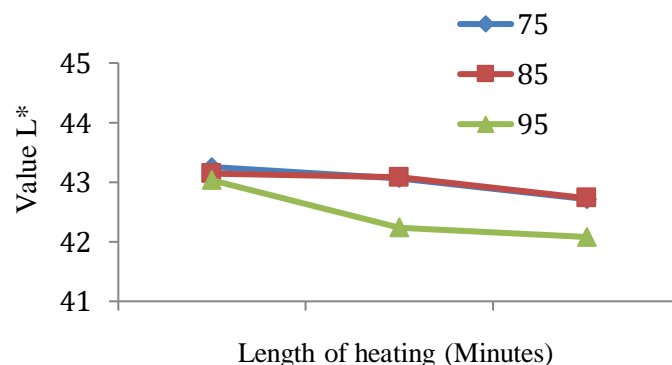


Figure 1. L* value of Dayak onion bulb extract at different temperature and length of heating

The analysis of variance shows that temperature and length of heating do not have a significant effect on the L* value of the extract. There was no interaction between temperature and heating time on the L* value of Dayak onion bulb extract. The L* value produced from Dayak onion tuber extract ranges from 42.08 – 43.25. The highest L* value was obtained at a temperature of 75°C with a heating time of 5 minutes, namely 43.25. The lowest L* value was obtained at a temperature of 85°C with a heating time of 15 minutes, namely 41.73. The L* value indicates the higher the temperature and the longer the time By heating Dayak onion bulb extract, it will turn dark red.

The a* value shows the color tendency from red to green. The greater the a* value indicates the tendency for the resulting color to be redder. The analysis of variance showed that the temperature and length of heating did not affect the a* value of Dayak onion bulb extract. The a* value produced from Dayak onion tuber extract ranges from (-1.32) – (-2.13). The highest a* value was obtained at a temperature of 95°C with a heating time of 15 minutes, namely -2.30, the lowest a* value was obtained at a temperature of 75°C and a heating time of 15 minutes, namely -1.32.

The b* value indicates the yellow-blue value in Dayak onion tuber extract. The b+ value ranges from 0-70 for yellow, while the b- value ranges from 0 - (-70) for blue. The relationship between temperature treatment and heating time on the b* value in Dayak onion tuber extract can be seen in Figure 2. The relationship between temperature treatment and heating time on the b* value in Dayak onion tuber extract can be seen in Figure 2.

The analysis of variance showed that temperature and heating time did not affect b* value and there was no interaction between temperature and length of heating on the b* value of Dayak onion bulb extract. Dayak onion bulbs contain anthocyanin compounds. Anthocyanin compounds are susceptible to high temperatures. According to Sari, et al (2013), anthocyanin decreases with increasing temperature and length of heating. The heat causes decomposition and changing in structure from aglycone to chalcone (colorless) (Sutrisno, 1987).

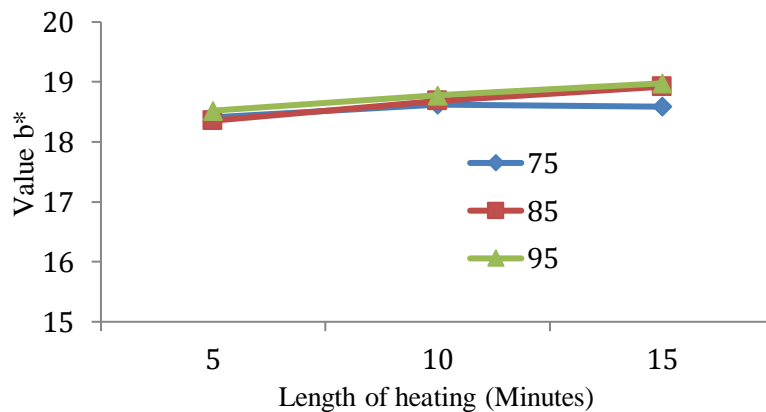


Figure 2. b^* value of Dayak onion bulb extract at different temperature and length of heating

4. Conclusions

Temperature and length of heating have a significant effect on the yield, antioxidant activity, IC50 of Dayak onion bulb extract but not on the color parameters of L^* , a^* , b^* . There is no interaction between temperature and heating time on antioxidant activity, IC50, yield, and color in Dayak onion tuber extract. The extract contain flavonoids and phenolics compounds. The temperature and length of heating suggested for Dayak onion bulb extract preparation is 75°C for 5 minutes which produce extract with an antioxidant activity of 63.72%, IC50 of 33.05 $\mu\text{g/ml}$ and 82.80% yield.

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