Anatomical structure and antioxidant activity of red bulb plant (Eleutherine americana) on different plant age

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Anatomical structure and antioxidant activity of red bulb plant (*Eleutherine americana*) on different plant age

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Abstract. *Kuntorini EM, Dewi M, Misrina. 2016. Anatomical structure and antioxidant activity of red bulb plant (*Eleutherine americana*) on different plant age. Biodiversitas 17: 229-233. Eleutherine americana* Merr is an medicinal herb named "red bulb" or "bawang dayak". Red bulb plant is common used as anti-breast cancer in Kalimantan, which may be linked to their bioactive naphtoquinone-derivatives properties. The aim of this study was to characterize the anatomical structure and antioxidant activity in red bulb of different ages. The anatomical structure of bulb was fixed and prepared following paraffin embedding techniques. Measurement of antioxidant activity carried out using the DDPH method. The anatomical structure of red bulb showed that the P2 (12 week after planting (WAP)) bulb is thicker in the upper and lower epidermis, parenchyma, and vascular bundles than P1 (6 WAP) bulb. The result antioxidant activity of P2 (12 WAP) with IC50 = 50.42 ppm have stronger antioxidant activity than the P1 (6 WAP) with IC_{50} = 93 ppm, but weaker than vitamin C (IC_{50} = 3.03 ppm) and BHT (IC50 = 5.52 ppm) as a control.

Keywords: Anatomical structure, antioxidant activity, *Eleutherine americana,* red bulb

INTRODUCTION

Red bulb plant or "bawang dayak" (*Eleutherine americana* Merr) grow in Kalimantan forest and has been used for traditional medicine (Galingging 2008). Bulbs of plant contain naphthoquinone group (elecanacine, eleutherine, elutherole, eleutherinone) (Hara et al. 1997; Alves et al. 2003; Nielsen and Wege 2006; Han et al. 2008), which is known has function as anti-cancer as well as antioxidant (Babula et al. 2009). Our previous research showed that bulb ethanol extract of red bulb from Banjarbaru has antioxidant activity of IC_{50} for 25.33 ppm. The same research reported the phytochemical screening red bulb contain triterpenoid and quinone (Kuntorini and Astuti 2010).

Triterpenoid and quinone are bioactive compounds present naturally in many plants. The formation process of bioactive compounds is complex, which involves interaction between biosynthetic processes, degradation, and can only be found in certain organisms or organism groups (Dewick 2002). The formation process highly depends on the physiological condition such as age and growth steps on different plants (Baikar and Malpathak 2010; Soetarno 1997). Research previously investigated the effect of ages and the leaf position on the quality and the quantity of Japanese mint oil production (Duriyaprapan and Britten 1982). The result showed the most of the oils and the main component (menthol) were synthesized for the first 2 weeks of the growth, yet it degraded after 2 weeks.

Bioactive compounds produced by plants as one of the ways to keep predators away (Dewick 2002). Plants do not always produce bioactive compound in each cell. Plants usually do biosynthetic on specific organs (Cseke et al. 2006), whereas the product can be accumulated inside the vacuole of the cell (Dixon 2001).

Our previous research showed the thickness of leaf mesophyll at the age of 6 and 12 weeks which were 52.3 µm and 66.9 µm respectively showed a marked difference, as well as on the bulb length showed that there was a difference at the age of 6 and 12 weeks which were 3.1 cm and 4.02 cm respectively. Through the observation of anatomical structure on the leaf, although there was a significant increase of the mesophyll thickness and the size of the constituent cells, it was not followed by the increase of naphthoquinone group content, whereas on bulb it was followed by the increase of the content of bioactive compound in naphthoquinone group. It can be assumed that naphthoquinone group was translocated to the bulb as the stockpiling organ (Kuntorini 2008). According to Babula *et. al.* (2009) the naphthoquinone compound is known to be highly toxic, commonly used as antimicrobial, antifungal, antiviral, anti parasite, and antioxidant.

However, despite the fact that red bulb is interesting from a medicinal and pharmacological point of view, the antioxidant activity on different plant ages uninvestigated. Therefore, the aim of this study was to investigate anatomical structure and the antioxidant activity of red bulb (*Eleutherine americana* Merr.) on different plant age.

MATERIALS AND METHODS

Nine hundreds of red bulb with the diameter of 0.3-0.5 cm were planted in a 6 m x 1.5 m x 15 cm wooden container with the spacing of 10 cm x 10 cm. The soil was analyzed to show the N, P, and K content at the soil

laboratory of Wetlands Agricultural Research Bureau (BALITTRA), Banjarbaru, South Kalimantan, Indonesia. The soil analysis result was used as a supporting data.

The bulb was taken after 6 weeks after planting (WAP) and 12 WAP. Five bulbs were collected in each harvesting time for study the anatomical structure, while 100 grams of gross weight of bulb were used to determine the anti-oxidant activity. Single-stain paraffin embedding method (Ruzin 1999) was applied in preparation of microscopic for examination of bulb slides included the thickness of paren-chyma tissue, the thickness of upper and lower epidermis tissue, and the diameter of vascular bundle on bulb.

Sample preparation for antioxidant analyses

Sample extraction of bulb used maceration method. Bulbs powder as much as 31 grams was put into 200 mL erlenmeyer and ethanol as much as 70 mL was added as a solvent. The extract was macerated for 24 hours at a room temperature. The extract was filtered using filtering paper after 24 hours maceration. The processes were repeated for three times. The first, the second, and the third filtrates were collected and evaporated using *rotary vacuum evaporator* until the thick extract was obtainable.

Antioxidant activity test

Antioxidant activity test was assessed using DPPH method. The concentrated extract of bulbs were weighed as much as 0.0085 grams as P1 sample (6 WAP) and 0.005 grams as P2 sample (12 WAP), then each of them was put into a beaker glass and dissolute using methanol. After that, the sample was put into the flask and methanol was added up to the marked sign, until the concentration of extract solution on P1 sample (6 WAP) reached 170 ppm and P2 sample (12 WAP) reached 100 ppm. Starting from concentration of 170 ppm, P1 sample (6 WAP) was then diluted to get the standard series with the concentration of 160 ppm, 120 ppm, 80 ppm, and 40 ppm. For concentration of 100 ppm, P1 sample (12 WAP) was diluted to 70 ppm, 50 ppm, 30 ppm, and 10 ppm. This dilution was done using methanol in a 10 ml measuring flask. It was then transferred into a beaker glass and was put into 1 ml of DPPH 1 mM, incubated for 30 minutes and later the absorption was measured on 515 nm wave lengths. As a positive control and for the comparison, ascorbic acid was used (concentration of 2, 3, 4 and 5 ppm) and BHT (concentration of 2, 4, 6 and 8 ppm) which was done by the same way as the extract (Andayani et al. 2008).

The antioxidant activity was estimated according to Andayani et al. (2008). The sample was determined by the amount of DPPH radical absorption resistance using % antioxidant power calculation in this formula:

$$\% \text{ of resistance} = \frac{(A\ blanko - A\ sampel)}{A\ blanko}\ 100\%$$

Where:

A Blank: DPPH radical absorption of 1 mM in methanol on 515 nm wave length

A sample: DPPH radical absorption of 1 mM treated as sample in methanol on 515 nm wave length

In DPPH method concept, antioxidant activity determination by looking at value of IC_{50} which is test compound concentration having antioxidant power to resist free radical is as much as 50 %. The value of IC_{50} was obtained using linier regression equation which stated the relation between sample concentration (test compound) and percentage of antioxidant power (Cholisoh and Utami 2008). The measurement of sample concentration in this research followed that concept, where the X value (concentration) has to be in the range % antioxidant power as much as 50%.

Phytochemical test

Phytochemical test was done as a qualitative support data. The test of some chemical compounds on the ethanol extract of bulb were done according to Harboren (1987) which consisted of steroid group, triterpenoid group, alkaloid group, flavonoid group, saponin group, tannin group and quinone group using appropriate reactant.

Data analysis

Qualitative data analysis was done in the phytochemical test, while quantitative data analysis was done for the measurement of parenchyma tissue thickness, upper and lower epidermis, as well as vascular bundle diameter using T Test. Antioxidant activity test was done by looking at the value of IC_{50}.

RESULTS AND DISCUSSION

Anatomical structure of red bulb

Anatomical structure of each layer of bulb cross section consisted of epidermis followed by parenchyma tissue and vascular bundle which were distributed randomly. Epidermis tissue consisted of upper surface (adaxial) and lower surface (abaxial), formed by cells with a thin wall, arranged closely each other in a small four-sided shape. According to Esau (1977) epidermis tissue functions as a cover for the tissue inside.

The observation showed that parenchyma tissue was formed by parenchyma cells in irregular hexagonal form as far as nearly round. According to Hidayat (1995) most parenchyma cells have a thin wall, yet some have a thicker wall. The most significant characteristic of parenchyma cells is that they can split and specialize to be a tissue with specific function. Parenchyma cells usually form the base tissue on plants, which is the reason why they are called as the base tissue (Fahn 1991). Moreover, parenchyma cells are known to play a role as food storage. The food can be found in vacuole.

On the observation of parenchyma cells of bulbs, amylum grain was found. Amylum is a food reserve most commonly found on plants, specifically inside the fruits, seeds, and roots (Hidayat 1995). On the red bulb layers, secretion cells were unavailable. It can be assumed that naphthoquinone group compound formation as a secondary metabolite takes place at cytosol and are stored inside the vacuole. Babula et al. (2005) pointed naphthoquinone group compound are usually stored inside the vacuole in the

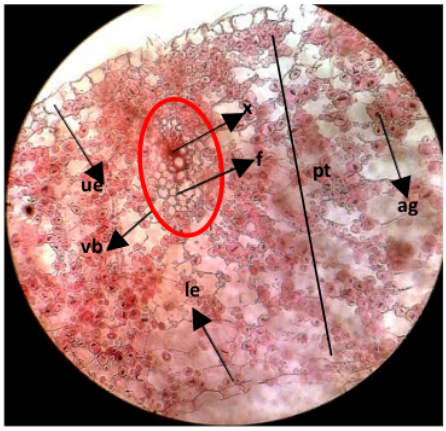

Figure 1. Cross section of Dayak onion bulbous layers at the age of 12 WAP (P2). Note: ue (upper epidermis), le (lower epidermis), vb (vascular bundle), x (xilem), f (floem),pt (parenchyma tissue), ba (amylum grain) 10x20 magnification

Table 1. The average of upper epidermis tissue thickness, lower epidermis tissue thickness, and vascular bundle diameter

Observation	Tissue thickness			Vascular bundle diameter (μm)
	Upper epidermis (μm)	Lower epidermis (μm)	Parenchyma (μm)	
P1 (6 WAP)	17.30[a]	14.20[a]	290.50[a]	44.50[a]
P2 (12 WAP)	28.00[b]	17.60[b]	497.00[b]	73.80[b]

form of glycoside. Hidayat (1995) suggested most parenchyma cells contain tannin and mineral salt in a crystallized form.

Based on the result of T test on the observation of parenchyma tissue thickness, upper epidermis tissue thickness, lower epidermis tissue thickness, and vascular bundle diameter, it showed that there was a significant difference between P1 (6 WAP) and P2 (12 WAP) because the significant value on the T test was less than the value of α (0.025). The average anatomic measurement data of red bulb can be seen below

Description

The same number followed by the same letter in the same column showed that there was no significant difference on the T Test at test level α (0.025). The observation result of anatomical structure of bulb layers showed the thickness of parenchyma tissue was increased at P2 (12 WAP), and it also happened in the thickness of upper epidermis and lower epidermis tissue. On the vascular bundle structure of P1 (6 WAP), the number of the constituent cells was still low and smaller in size compared to P2 (12 WAP), thus influencing the size of vascular bundle diameter.

The growth of the plants was indicated by the increasing size because multicellular organisms grow from a zygote. The increasing number is not only about the volume, but also in weight, as well as in the number of cells, protoplasm, and the complexity. The growth and development process are the result of 3 basic phenomena at cellular level. The first step is the cellular fission: one of the fully-grown cells splits into two individual cells, which are not always similar from one to another. The second step is the development of the cells: The cells grow bigger in volume. The third step is the differentiation of cells: cells with certain volume are specialized in particular ways. Many kinds of cells split, grow, and specialize resulting in a wide variety of tissue and organs as well as into many kinds of plants (Salisbury and Ross 1995).

The growth of the cell itself is a process of absorbing liquids into the expanding vacuole, which later space out the cell wall. In that process, the growth power is the turgor pressure. The pressure inside the cell is caused by the mechanical resistance of cell wall against a tense. If the resistance is reduced, the wall loosens. The wall stretch causes decreasing pressure, reduced liquid potential, and increasing water gradient potential. Therefore, liquid comes into the cell causing the increasing size of the cell itself (Salisbury and Ross 1995).

In this research, the bulb development was visible at the age of 12 WAP (P2) on the thickness of parenchyma tissue, upper and lower epidermis, and the diameter of vascular bundle. Referring on the soil elements contained in the used soil, it was ascertainable that N was 0.126%, P was 0.041%, and K was 0.036%. Compared to the soil sample on the natural habitat in Kalimantan, It can be inferred that the NPK on this sample is higher than at the soil in the natural habitat in Kalimantan.

Based on the result of the research above, it showed the red bulb can grow better in the soil with higher soil elements as in the natural habitat Kalimantan as well as lower soil elements than the macro soil elements concentration which is considered as sufficient (0.1%) as mentioned before. According to Heyne (1987) red bulb are usually found in many places, growing freely between 600 and 1500 m above sea level, sometimes in a huge number at the grassy paths, between tea plants, quinine, and rubber trees. This wide spreading indicates that the contents of NPK are diverse or the range of the NPK is wide.

The amount of NPK needs is related to the needs of plants to grow well. If the NPK is insufficient, the growth will be interfered. The increasing amount of the thickness of upper and lower epidermis tissue, parenchyma tissue, and vascular bundle diameter on P2 (12 WAP) showed that the needs of NPK is sufficient (Lakitan 2004).

Antioxidant activity of red bulb extract

The result of the research showed the red bulb extract on P2 (12 WAP) had a value of IC_{50} as much as 50.42 ppm, while on P1 (6 WAP) the value of IC_{50} is as much as 93 ppm which means the antioxidant activity on P2 (12 WAP) is stronger than the antioxidant activity on P1 (6 WAP). This showed that the growth phase (seed age) according to

Soetarno (1997) has an effect on the secondary metabolite which has compound with antioxidant activity. Compared to the positive control of vitamin C and BHT, the sample had weaker antioxidant activity.

P1 (6 WAP) had weaker antioxidant activity than P2 (12 WAP). This might happen because at that seed age the plant was still in the early phase of growth. On P1 (6 WAP) vascular bundle was found having smaller cells in a very small number, resulting in a small number of the transported and piled accumulation of secondary metabolite. Besides, the observation of the anatomical structure showed that there was an increasing of parenchyma tissue thickness on P2 (12 WAP), whereas the increasing thickness is seen in the increasing size of the cells. According to Salisbury and Ross (1995) the increasing size of cells is caused by the expanding vacuole. According to Hidayat (1995) vacuole is the biggest organelle on mature plants, containing liquids and solvents. On red bulb secretion cells are unavailable, thus it can be assumed that the piled secondary metabolite takes process in the vacuole. Babula et al. (2005) suggested in the plants family of Plumbaginaceae, Juglandaceae, Ebenaceae, Boranginaceae, and Iridaceae, the contained secondary metabolite especially naphthoquinone is usually stored inside the vacuole. Red bulb is classified into the Iridaceae family.

The difference of antioxidant activity between the two observations was caused by the different concentration of the secondary metabolite. The more secondary metabolites lead to the stronger antioxidant activity. It can be estimated that P2 (12 WAP) had higher concentration of secondary metabolite than P1 (6 WAP), thus the antioxidant activity is stronger. This is supported by our previous research showed on red bulb planted inside a glass house and in an open space at the age of 4 WAP and 12 WAP, showed the increasing of secondary metabolite (bioactive) naphthoquinone on bulb at the age of 12 weeks (Kuntorini and Nugroho 2009). It was ascertainable that P1 (6 WAP) and P2 (12 WAP) had the same result on the secondary metabolite such as positive steroid, tannin, quinone, and flavonoid. In this research, phytochemical testing were done qualitatively, with the result that the detected secondary metabolite were based on the color change while reacting.

Some species showing the increasing compound of secondary metabolite along with the aging are *Cinnamomum camphora*, which its camphor is accumulated inside the old stems and *Blumea balsamifera* which is harvested when the leaves are old enough (Soetarno 1997).

The secondary metabolites were detected in this research was quinone. Our previous research showed that red bulb ethanol extract from Banjarbaru contains quinone based on the phytochemical screening (Kuntorini and Astuti (2010). Quinone very likely comes from the appropriate oxidized components of phenol, such as catechol (1.2 dihydroxybenzen) producing *ortho*-quinone and quinole (1.4 dihydroxybenzen) producing *para* quinone, thus quinone can be formed from phenol system which is produced through acetate or shikimate pathway. Chimaphiline, plumbagin, eleutherine are the plants known to have naphthoquinone and have been used as medicine and poison since prehistoric era. Naphthoquinone compounds are known to be toxic, commonly used as antimicrobial, antifungal, antiviral, antiparasite, and antioxidant (Babula et al. 2005; Babula et al. 2009).

Our previous research showed the value of IC_{50} (antioxidant activity) red bulb from Tanah Laut Regency containing naphthoquinone ranges from 29.18 to 51,11 ppm. In this research, the value of IC_{50} on P2 (12 WAP) was as much as 50.42 ppm, so the value of antioxidant activity is categorized within the range similar to in its natural habitat (Tanah Laut) (Kuntorini et al. 2011).

In this research, in addition to naphthoquinone compound, flavonoid compound was also detected. Flavonoid group has the ability to transform in order to produce higher activity compounds with antioxidant activity. Flavonoid is known as a good antioxidant because it has at least two hydroxyl clusters on *orto* and *para* (Andayani et al. 2008).

In conclusion, anatomical structure of bulbs layers on the thickness of upper and lower epidermis tissue, parenchyma tissue, and vascular bundle diameter is bigger on bulb at the age of 12 WAP. Bulb at the age of 12 WAP (IC_{50} = 50.42 ppm) has stronger antioxidant activity than at the age of 6 WAP (IC_{50} = 93 ppm), yet is weaker than vitamin C (IC_{50} = 3.03 ppm) and BHT (IC_{50} = 5.52 ppm) as the control.

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