

Antigenotoxic Potential of Dayak Onion Bulb and Leaf (*Eleutherine bulbosa* Urb.) Ethanol Extract with *Allium cepa* Assay

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ABSTRACT

Dayak onion (*Eleutherine bulbosa* Urb.) has the potential as an antigenotoxic agent capable of counteracting the effects of damage to DNA due to ROS (Reactive Oxygen Species). The bioactive compounds in Dayak onions have antiradical scavenging activity that plays a role in counteracting free radicals so that they can improve the genotoxicity that occurs. The purpose of this study was to determine the antigenotoxic potential of the ethanol extract of Dayak onion bulb and Dayak onion leaf using the *Allium cepa* assay. This study used a completely randomized design with various treatments of extract concentration (0.5, 1, 5, and 10 ppm) after immersion of 3% hydrogen peroxide (H₂O₂) with 3 replications. Chromosome observations of shallot (*Allium cepa* L.) roots were carried out between 09.00-10.00 am and then the mitotic index and percentage of inhibition were determined. Data were analyzed descriptively qualitatively, quantitatively, and through the ANOVA test followed by the 5% DMRT test. The ethanolic extract of 10 ppm Dayak onion bulb showed the highest antigenotoxic activity with a mitotic index reaching 6.78% and an inhibition percentage of 92.25%. The antigenotoxic activity of the ethanolic extract of Dayak onion bulb was higher than the ethanolic extract of Dayak onion leaf. These results are related to the antiradical scavenging activity of the ethanolic extract of Dayak onion bulb which is also higher than the ethanolic extract of Dayak onion leaf.

Keywords: *Allium cepa* assay, *Eleutherine bulbosa* Urb, genotoxic, hydrogen peroxide

Introduction

The back to nature lifestyle trend makes use of plants as the primary elements in herbal therapy, which has few adverse effects. The World Health Organization (WHO) has estimated that 80% of the world's population uses herbal treatment (herbal medicine, phytotherapy, phytomedicine, or botanical medicine) to maintain primary health since 1985 (Peters and Whitehouse, 1999). In the twenty-first century, WHO employs a new health paradigm philosophy, namely preventive and promotive, which mostly pertains to herbal medicine (Syaaf, 2018).

Indonesia possesses 7500 medicinal plant species, 940 of which have been recognized, accounting for almost 90% of the total number of medicinal plants in the Asian region (Puslitbangtri, 1992). According to the 2017 National Socio-Economic Survey or Susenas, over 22.3% of Indonesians still utilize herbal remedies derived from plants to cure a variety of conditions (Syaaf, 2018).

Herbal medications play a significant role in combating the effects of ROS (Reactive Oxygen Species), which can lead to a variety of disorders and diseases. ROS is a byproduct of biological activities that, if excessive and unnaturalized by the antioxidant system, can lead to degenerative disorders such as cancer, aging, diabetes, and so on (Sharma et al., 2018). Genotoxicity is the result of DNA damage that alters the nucleotide base order

or the links between DNA sugar-phosphates. Not only is DNA damaged, but so are cell components crucial to the function and activity of chromosomes in cells. Hydrogen peroxide (H₂O₂) is a non-reactive radical that acts as a ROS agent. According to Prajitha and Thoppil (2016), H₂O₂ induction induced genetic harm to the roots of *Allium cepa* L., as evidenced by a reduction in the mitotic index.

To counteract genotoxic effects, researchers have begun to develop bioactive molecules from natural compounds that can neutralize mutagenic and carcinogenic effects. According to Uttara et al. (2009), antioxidant compounds are responsible for counteracting free radicals and reducing ROS formation because the body's cellular defense mechanism is incapable of providing overall protection.

One of the plants that have high antioxidant potential is Dayak onion (*Eleutherine bulbosa* Urb.). The results of previous studies stated that the IC₅₀ value of the ethanolic extract of Dayak onion bulb was 25,3339 µg/ml (Kuntorini and Astuti, 2010), while the IC₅₀ value of the ethanolic extract of the Dayak onion leaf was 31,97437 g/ml, this value indicates a very high antiradical scavenging activity (less than 50µg/mL) (Pratiwi et al., 2013). Empirically, Dayak onions have been used by the community to treat various diseases such as breast cancer, colon cancer, abdominal pain after childbirth, hypertension, cholesterol, ulcers, and stroke (Galingging, 2009). Lestari et al. (2019)

stated that the ethanol extract of Dayak onion contains several secondary metabolites such as alkaloids, tannins, flavonoids, and quinones. Flavonoids are good antioxidants because they can ward off free radicals by liberating hydrogen atoms from their hydroxyl groups. This compound is also able to prevent the bond between carcinogenic molecules and DNA to prevent DNA damage, stimulate the DNA repair process, and prevent the starting point of the formation of cancer cells.

This study aimed to evaluate the activity of the ethanolic extract of Dayak onion (the bulb and leaf) in overcoming the effects of exposure to hydrogen peroxide (H₂O₂) by observing the mitotic index and chromosomal aberrations in shallot roots. Shallots are used as genotoxic indicators because they have high sensitivity to various substances that can affect changes in chromosomal structure and fast root growth and can be in direct contact with the test solution to predict DNA damage through chromosomal aberrations (Tedesco and Laughinghouse IV, 2012).

Research Method

Chemical, Reagents, and Instruments

The instruments used were Rotary Evaporator (RE), UV-Vis Spectrophotometry, oven, vortex mixer, beaker glass (size of 100 and 1000 ml), volumetric flask (size of 10 and 100 ml), test tube, cuvette, cover slip, glass slip, light microscopes and cameras, knives, and scissors.

The materials used in this study were Dayak onion bulbs and leaves aged 5 months with seeds from Pasir Besar, South Pontianak, Pontianak, shallot bulbs, 3% hydrogen peroxide (H₂O₂), 96% ethanol, ethanol PA, distilled water, HCl 1N, DPPH (2,2-diphenyl-1-picrylhydrazyl), 0.2% colchicine, vitamin C, 2% aceto-orcein, and glacial acetic acid 45%.

Experiments

This research is an experimental study with a Completely Randomized Design (CRD). This design was used to observe the antigenotoxic effect of the ethanol extract of Dayak onion bulb and leaf at various concentrations with 3 replications.

1. Plant authentication

Plant determination is carried out at Biology Laboratorium, Faculty Mathematics and Science, Sebelas Maret University, Surakarta and was authenticated by Mr. Suratman (a taxonomist) to ensure that the plant used are correct with Reference no: 063 /UN27.9.6.4/Lab/2022 for future reference.

2. Extraction

Dayak onion bulbs and leaves are cleaned and then cut into small pieces. Samples were oven-dried at room temperature. The dried

simplicia was crushed to obtain simplicia powder. Extraction was carried out by maceration technique using 96% ethanol solvent with a ratio of 1:6 for bulb samples and 1:10 for leaf samples. The 96% ethanol solvent is a polar compound that is often used as a solvent because it evaporates easily. The solvent change was done every 3 days. The results of the macerate were concentrated with a Rotary evaporator (RE) at a temperature and then evaporated again to remove the remaining solvent so that a concentrated extract was obtained. The yield was calculated and stored in a refrigerator at a temperature for use in the next test.

3. DPPH scavenging assay test

The radical scavenging activities of Dayak onion bulb and leaf extracts against the DPPH radical were determined by the method of Sharon et al. [2013]. Determination procedures were as follows: 2 mL of 40 ppm DPPH radical solution (prepared daily) was mixed with 2 ml of ethanolic solutions of 90 ppm Dayak onion bulb and leaf extracts (maximum dissolved concentration). After 30 min incubation at 37 °C, the absorbance decrease of the mixture was monitored at 517 nm (A_s). During reduction by the antioxidant, the solution colour changed from violet to yellow pale. DPPH radicals have an absorption maximum of 517 nm. Blank samples were prepared and measured daily at the same wavelength (A_b). Ascorbic acid was used as a positive control. The experiment was carried out in triplicate. Radical scavenging activity was calculated using the following formula.

$$\text{Inhibition rate} = \frac{(A_b - A_s)}{(A_b)} \times 100\%$$

The parameter inhibitory concentration (IC₅₀) is defined as the concentration of substrate that causes 50% loss of the DPPH activity and is used for the interpretation of results from the DPPH method.

4. Antigenotoxic test

The antigenotoxic effect of Dayak onion bulb and leaf extracts was tested against 3% H₂O₂. Shallot roots were grown in distilled water at room temperature and dark conditions for 3 days. The grown roots were then soaked in 3% H₂O₂ for 1 hour. The roots were washed and then treated with ethanol extract of Dayak onion bulb and leaf with the lowest concentration series (0.5 ppm, 1 ppm, 5 ppm, 10 ppm) for 24 hours. Treatment with distilled water only served as a negative control, while the 3% H₂O₂ treatment functioned as a positive control. All treatments were carried out in 3 replications.

The root tips were then cut at 9-10 cm and then washed with distilled water (Prajitha and Thoppil, 2016). Pre-treatment was carried out by soaking the root pieces in 0,2% colchicine at 4°C for 4 hours. Washing was carried out with distilled water 3 times. Root pieces were fixed in 45% glacial acetic acid at 4°C for 15 minutes, then washed again. Root pieces were hydrolyzed in 1N HCl in a temperature oven for 5 minutes, and then washed again. Root sections were stained with 2% aceto-orcein for 60 minutes at room temperature. The tip of the root is placed on a glass object and dripped with glycerin and then squashed. Cytological analysis was carried out by observing 300 cells for each treatment using a binocular microscope to see various types of chromosomal aberrations that occurred. The mitotic index value and the percentage of inhibition were calculated using the following formula (Prajitha and Thoppil, 2016).

$$\text{Mitotic index (\%)} = \frac{\text{Number of dividing cells}}{\text{Total number of cells}} \times 100\%$$

$$\text{Genotoxic Inhibition Rate (\%)} = \frac{a - b}{a - c} \times 100\%$$

With a = number of cells with aberrations induced by positive control, b = number of cells with aberrations induced by extract and 3% H₂O₂, c = number of cells with aberrations induced by negative control

Data Analysis

Data analysis was done by interpreting the data in the form of pictures, tables, and graphs. The IC₅₀ value from the standard curve linear equation was determined as an antiradical scavenging activity with a range of IC₅₀ values <50 ppm (very strong), 50-100 ppm (strong), 100-150 ppm (moderate), 150-200 ppm (weak), and >200 ppm (very weak).

Mitotic index data and percentage of inhibition were presented as mean ± SE (Standard Error) of each dose treatment with three replications. The relationship between dose and mean yield was obtained from regression and correlation analysis. Data were analyzed using analysis of variance (one way ANOVA) and then continued with Duncan's test for statistical significance.

Results and Discussion

Antiradical Scavenging Activity

The ethanol extract of the Dayak onion bulb had a higher yield (7%) than the ethanol extract of the Dayak onion leaf (2.44%). Several studies have shown the comparison of differences in IC₅₀ values between bulb extract and leaf extract. The difference in IC₅₀ value can be influenced by several factors, such as geographical conditions, age of harvest, and the solvent used in the maceration process which affects the condition of the sample extract.

The antioxidant test showed that the percentage of inhibition of the ethanol extract of Dayak onion bulb increased at a concentration of 90 ppm by 67.1±0.01% (Table 1). The antiradical scavenging activity of the ethanolic extract of the Dayak onion bulb showed an IC₅₀ value of 20.12 ppm (very strong). The percentage of inhibition of the ethanol extract of Dayak onion leaf increased at a concentration of 90 ppm by 55.76±0.02% (Table 2). The IC₅₀ value of the ethanol extract of Dayak onion leaf is 47.41 ppm (very strong).

The percentage of inhibition of ascorbic acid increased at a concentration of 10 ppm by 75.93±0.09% (Table 3), while the IC₅₀ value of ascorbic acid was 1.71 ppm (very strong). The antiradical scavenging activity of vitamin C was higher than in the two samples.

Table 1. The percentage of inhibition and IC₅₀ of Dayak onion bulb using the DPPH method

Sample	Concentration (ppm)	Absorbance	% Inhibition	IC ₅₀ (ppm)
Blank	40	0.7799	-	-
Ethanolic extract of Dayak onion bulb	50	0.3379±0.00036	56.67±0.05	20.12
	60	0.3098±0.00048	60.27±0.06	
	70	0.3012±0.00058	61.38±0.07	
	80	0.2908±0.00029	62.72±0.04	
	90	0.2566±0.00009	67.1±0.01	

Table 2. The percentage of inhibition and IC₅₀ of Dayak onion leaf using the DPPH method

Sample	Concentration (ppm)	Absorbance	% Inhibition	IC ₅₀ (ppm)
Blank	40	0.5249	-	-
Ethanolic extract of Dayak onion leaf	50	0.2620±0.00015	50.09±0.03	47.41
	60	0.2511±0.00018	52.16±0.03	
	70	0.2466±0.00016	53.02±0.02	
	80	0.2358±0.00040	55.08±0.08	
	90	0.2322±0.00010	55.76±0.02	

Table 3. The percentage of inhibition and IC₅₀ of the ascorbic acid using the DPPH method

Sample	Concentration (ppm)	Absorbance	% Inhibition	IC ₅₀ (ppm)
Blank	40	0.8366	-	-
Ascorbic acid	2	0.4017±0.00044	51.98±0.05	1.71
	4	0.3620±0.00028	56.73±0.03	
	6	0.3304±0.00046	60.51±0.06	
	8	0.3083±0.00057	63.15±0.07	
	10	0.2013±0.00075	75.93±0.09	

The antiradical scavenging activity in the extracts of Dayak onion bulb and leaf was determined by the secondary metabolite compounds that depend on it. Several compounds that are thought to play a role in the antiradical scavenging activity of a plant extract are flavonoids, phenolics, and tannins. The ability of these compounds has been proven in the research of Pratiwi et al. (2013), which stated that the phytochemical screening of Dayak onion leaf extract contains flavonoid, phenolic, and tannin compounds which are a group of phenolic compounds. Phenolic compounds are compounds with an -OH group attached to an aromatic ring carbon. Phenol compounds can donate hydrogen atoms so that the DPPH radical can be reduced to a more form. The free radical scavenging activity of phenolic compounds is influenced by the amount and position of phenolic hydrogen in the molecule. The more the number of hydroxyl groups, the greater the antiradical scavenging activity produced. Kuntorini and Astuti (2010), stated that the phytochemical screening of Dayak onion bulb extract contains triterpenoid and quinone compounds, as well as naphthoquinone phenolic compounds such as elecanacin, eleutherin, isoeleutherin, eleutherol, and eleutherinone which have very strong antiradical scavenging activity.

Antigenotoxic Potential

The analysis of the genotoxic effect of 3% H₂O₂ showed that the positive control treatment of 3% H₂O₂ was proven to affect the division of shallot roots so that the mitotic index value obtained the lowest yield of 3±0,58%. This indicates that 3% H₂O₂ is a genotoxic agent capable of inhibiting cell division in shallot roots as previously described. The negative control (aquadest) showed the highest mitotic index of 19.22±3.80%. Improvement of the genotoxic effect of 3% H₂O₂ using ethanol extract of Dayak onion showed that the highest mitotic index occurred after treatment with a concentration of 10 ppm at 6.78±0.40%. Meanwhile, in the leaf extract group, the highest mitotic index was shown by a concentration of 10 ppm at 14.67±0.33%. However, these two values were not significantly different from the results at a concentration of 5 ppm (Table 4).

Table 4. The antigenotoxic effect of the ethanolic extract of Dayak onion after exposure to 3% H₂O₂ was observed in the mitotic index of shallot root cells

Treatment group	Total of dividing cells	Mitotic index (%)
Negative control	57.67±11.38	19.22±3.80
Positive control	9±1.73 ^{ab}	3±0.58 ^{ab}
0.5 _A	10.67±1.76 ^{ab}	3.56±0.59 ^{ab}
0.5 _B	10.67±0.88 ^{ab}	3.56±0.29 ^{ab}
1 _A	17±1.16 ^{cd}	5.67±0.38 ^{cd}
1 _B	11±1.16 ^b	3.67±0.38 ^b
5 _A	20±2.08 ^d	6.67±0.70 ^d
5 _B	14±0.58 ^{bc}	4.67±0.20 ^{bc}
10 _A	20.33±1.20 ^d	6.78±0.40 ^d
10 _B	14.67±0.33 ^{bc}	4.89±0.11 ^{bc}

Description: A = bulb extract, B = leaf extract. The data shown are mean±SE. Values in the same column followed by the same letter are not significantly different in the 5% DMRT test

The genotoxic effect of hydrogen peroxide (H₂O₂) was observed through the *Allium cepa* test which evaluates the presence of chromosomal abnormalities in the shallot roots. The *Allium cepa* test has several advantages, one of which is the presence of a mixed oxidase function system in shallot root cells that can activate promutagens or genotoxic chemicals. This in vivo test is important because root growth is in direct contact with compounds that allow DNA damage and this condition is correlated with conditions in humans (Nefic et al., 2013). The 3% hydrogen peroxide reagent was used to induce severe cytogenetic damage in shallot root meristematic cells, while the lowest concentration series (0.5 ppm, 1 ppm, 5 ppm, 10 ppm) was used for antigenotoxic assay in the recovery of genotoxicity due to 3% H₂O₂ exposure. The administration of ethanol extract of Dayak onion aims to improve the possibility of DNA damage that occurs due to the genotoxic effect of 3% H₂O₂. In this case, the antigenotoxic effect of the ethanol extract of Dayak onion bulb and leaf can be analyzed by comparing the mitotic index and the percentage of inhibition in each series of plant extract concentrations.

Exposure to 3% H₂O₂ in shallot roots resulted in severe nuclear lesions because cells were subjected to oxidative stress due to genotoxic exposure. Active oxygen (O) which is formed due to oxidative stress is known to affect the cytoskeleton structure in cells (Prajitha and Thoppil, 2016). H₂O₂ is able to pass through the membrane and then causes the oxidation of biomolecules which results in cell damage (Sinaga, 2016). This damage can include damage to DNA, proteins, or oxidation of important enzymes.

Cytological aberrations in the form of chromosomal aberrations occurred in shallot roots due to exposure to 3% H₂O₂ (positive control) with a total of 11.33±0.8 cells experiencing aberration cells, while the percent aberration was 3.78±0.29%. Some chromosomal aberrations due to exposure to 3% H₂O₂ are shown in Figure 1. Immersion with distilled water (negative control) is a normal condition when the roots are not exposed to genotoxic agents which show a total cell aberration of 2.33±1.45 cells with an aberration percentage of 0.78±0.49%. The results of improvements with plant extracts showed that the most effective concentration in restoring cytological aberrations was 10 ppm Dayak onion bulb extract which was indicated by a reduction in the number of aberrated cells up to 3±0.58 cells with an aberration percent of 1±0.19%. The percentage value of inhibition of the extract of Dayak onion bulb at a concentration of 10 ppm reached 92.25%. In the leaf extract group, a concentration of 10 ppm showed the best improvement in cytological aberrations with a reduction in the number of cell aberrations reaching 6±1.00 with aberration percent of 2±0.33% and the inhibition value reaching 59.22%. The antigenotoxic potential of both samples showed strong activity with an inhibitory value of more than 40% (Table 5).

Cell aberrations that occur can be classified as physiological or clastogenic cell aberrations. Physiological aberrations are caused by the inhibition of the spindle thread while clastogenic aberrations are caused by the direct cytotoxic action of chromosomes (Sharma et al., 2018). Physiological aberrations such as c-mitosis, laggard, delayed anaphase, stickiness, and vagrant

chromosomes, while clastogenic aberrations such as chromosome fragment, chromosomal break, chromatin bridge, coagulated chromosome, nuclear lesion, and ring chromosomes.

Sticky chromosomes (Figure 1.i) are chromosomes that are sticky to each other so that when pulled by the microtubule spindle they will form an anaphase bridge. Sticky chromosomes occur due to increased contraction and condensation of chromosomes or can occur due to DNA depolymerization and partial dissolution of nucleoproteins (Prajitha and Thoppil, 2016). The chromatin bridge (Fig. 1.h) in anaphase causes the sticky chromosomes to break or break in random areas. Chromatin bridges can occur during translocation of unequal chromatid exchange and trigger various mutations, especially aneuploidy (Khanna and Sharma, 2013). Inhibition of the cytokinesis process of cell division results in the occurrence of binucleic cells, namely cells with double nuclei (Figure 1.l) (Prajitha and Thoppil, 2016). Vagrant chromatin (Figure 1.h) is a state of chromatin that moves from its clusters toward the poles, causing an uneven separation of the number of chromosomes in daughter cells (Khanna and Sharma, 2013). This deviation can occur due to failure of the spindle thread adjustment to function normally. Fragmentation (Figure 1.k) is also known as chromosomal damage which probably occurs due to the activation of endogenous nucleases that produce small inter-nucleosome fragments (Khanna and Sharma, 2013). The decrease in the mitotic index can occur due to inhibition of DNA synthesis or blocking in the G2 phase of the cell cycle to prevent cells from entering the mitotic phase.

Table 5. Antigenotoxic effect of ethanol extract of Dayak onion after exposure to 3% H₂O₂ in terms of the percentage of inhibition

Treatment Group	Total of Aberrated Cells	Aberrated Cells Percentage (%)	Inhibition Rate (%)
Negative control	2.33±1.45	0.78±0.49	-
Positive control	11.33±0.8 ^e	3.78±0.29 ^e	-
0.5 _A	6.67±0.67 ^{cd}	2.22±0.22 ^{cd}	51.78
0.5 _B	8.67±0.33 ^{de}	2.89±0.11 ^{de}	29.56
1 _A	5±1.00 ^{abc}	1.67±0.33 ^{abc}	70.33
1 _B	8.67±0.67 ^{de}	2.89±0.22 ^{de}	29.56
5 _A	3.67±1.20 ^{ab}	1.22±0.40 ^{ab}	85.11
5 _B	7.67±1.45 ^{cd}	2.56±0.48 ^{cd}	40.67
10 _A	3±0.58 ^a	1±0.19 ^a	92.25
10 _B	6±1.00 ^{bcd}	2±0.33 ^{bcd}	59.22

Description: A = bulb extract, B = leaf extract. The data shown are mean±SE. Values in the same column followed by the same letter are not significantly different in the 5% DMRT test.

Also, disrupted nucleoprotein synthesis and decreased ATP for spindle lengthening, microtubule changes, and chromosome movement lead to the same end (Prajitha and Thoppil, 2016). Overall, improvements with the ethanolic extract of Dayak onion bulb showed better results than the ethanolic extract of Dayak onions. This result is related to the antioxidant content of the Dayak onion bulb ethanolic extract which is higher than that of the

leaf ethanol extract. The follow-up test showed that the concentration of 10 ppm had no significant difference from the concentration of 5 ppm. According to Prajitha and Thoppil (2016), higher concentrations of an extract show lower antimutagenic and mutagenic activity. In this case, increasing the concentration can repair DNA damage to the optimum limit, so that concentrations higher than the optimum limit are

toxic. In addition, crude extracts are known to consist of a complex mixture of phytochemicals that can act synergistically, additively, or antagonistically, while the evaluation of the synergism of the active ingredients requires further experiments in a case-by-case approach to drug studies (Syahrir et al., 2016).

Previous studies have stated that the antigenotoxic potential of plant extracts is associated with the phenolic content of plants which can form strong lignan complexes with metal ions. Plant extracts were also reported to be able to adsorb mutagens like the adsorption of carcinogens associated with the presence of pyrrole pigments, such as hemin and chlorophyllin. Plant extracts induce DNA glycosylase enzymes playing a role in DNA repair by the alkylation of DNA bases (Prajitha and Thoppil, 2016).

According to Bouguellid et al (2020), the antigenotoxic mechanism of a plant extract that has been observed is influenced by phenolic compounds that interact with H_2O_2 reactive intermediates so that ROS products become more stable.

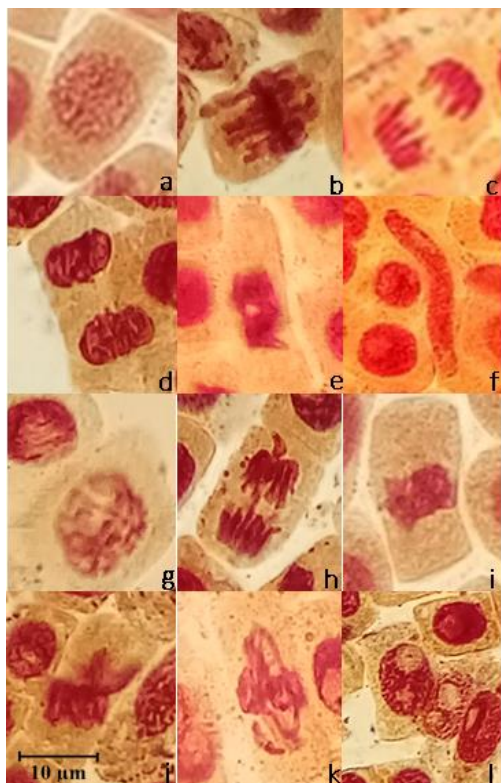


Figure 1. Normal mitotic phase in shallot roots and chromosomal aberrations resulting from 3% H_2O_2 induction. Description: a. Prophase, b. Metaphase, c. Anaphase, d. Telophase, e. Chromosomal coagulation in anaphase, f. Long cells with elongated nuclei, g. Nuclear lesions in prophase, h. Bridge chromatin and vagrant chromatin in anaphase, i. Chromosomes stick to metaphase, j. Chromosomes are damaged in metaphase, k. Chromatin bound and

fragmentation at metaphase, l. Binucleate. Dyes: Aceto-orcein 2%. Microscope magnification: 400x.

The DNA adducts formed can be repaired via the Base Excision Repair (BER) pathway. Phenolic compounds in plant extracts can induce DNA glycosylase, which initiates BER by catalyzing the hydrolysis of N-glycosidic bonds between the target base and deoxyribose (Krokan and Bjoras, 2013).

Determination of the antigenotoxic potential of a sample is assessed from the percentage of inhibition as follows: a value of less than 25% indicates a weak or nonantigenotoxic effect, a value of 25-40% indicates a moderate effect, and a value of more than 40% indicates a strong antigenotoxic activity (Verschaeve and Van Staden, 2008). In this case, the ethanolic extract of the Dayak onion bulb showed better antigenotoxic activity than the leaf extract with the percent inhibition value of all concentrations more than 40% (strong), while the ethanolic extract of the Dayak onion leaf at 0.5 ppm and 1 ppm was classified as moderate (25-40%), while the concentrations of 5 ppm and 10 ppm are quite strong (more than 40%).

Conclusion

The ethanolic extract of Dayak onion bulb has the potential to an antigenotoxic with the highest increase in the mitotic index reaching 6.78% at a concentration of 10 ppm and a decrease in the occurrence of cell aberrations reaching 1% with a percentage inhibition value of 92.25%. These results indicate that the antigenotoxic activity of the Dayak onion bulb extract is higher than that of the Dayak leaf extract.

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Reference

- Bhagyanathan, N.K. and Ernest, J. 2016. Genotoxic potential of *Cynanchum sarcomedium* Meve & Liede coupled with its modulatory action on oxidative-stress-mediated genotoxicity by hydrogen peroxide. *Turkish Journal of Biology*. 40:120-129.
- Bouguellid, G., Russo, C., Lavorgna, M., Piscitelli, C., Ayouni, K., Wilson, E., Kim, H.K., Verpoorte, R., Choi, Y.H., Kilani-Atmani, D., Atmani, D., Isidori, M. 2020. Antimutagenic, antigenotoxic and antiproliferative activities of *Fraxinus angustifolia* Vahl. leaves and stem bark extracts and their phytochemical composition. *PLoS ONE*. 15(4):1-21.
- Claudea, N. dan Yuswi, R. 2017. Ekstraksi antioksidan bawang dayak (*Eleutherine palmifolia*) dengan metode ultrasonic bath (kajian pelarut lama). *Jurnal Pangan dan Agroindustri*. 5(1):71-78.

- Khanna, N., and Sharma, S. 2013. *Allium cepa* root chromosomal aberration assay: A review. *Indian Journal of Pharmaceutical Biological Research*. 1(3):105-119.
- Krokan, H.E., and Bjoras, M. 2013. Base excision repair. *Cold Spring Harbor Perspectives in Biology*. 5(4):1-22.
- Kuntorini, E.M., dan Astuti, M.D. 2010. Penentuan aktivitas antioksidan ekstrak etanol bulbus bawang dayak (*Eleutherine americana* Merr.). *Sains dan Terapan Kimia*. 4(1):15-22.
- Lestari, D., Kartika, R., Merliana, E. 2019. Antioxidant and anticancer activity of *Eleutherine bulbosa* (Mill.) Urb on leukemia cells L₁₂₁₀. *Journal of Physics: Conference Series*. 1277:1-7.
- Nefic, H., Musanovic, J., Metovic, A., Kurteshi, K. 2013. Chromosomal and nuclear alterations in root tip cells of *Allium cepa* L. induced by alprazolam. *Medical archives (Sarajevo, Bosnia and Herzegovina)*. 67(6):388-392.
- Peters, D., and Whitehouse, J. 1999. The role of herbs in modern medicine: Some current and future issues. Di dalam: *Herbs. Proceedings of the International Conference and Exhibition; Malaysia, 9-11 Nov 1999*. Malaysia: Malaysian Agricultural Research and Development Institute.
- Prajitha, V., and Thoppil, J.E. 2016. Genotoxic and antigenotoxic potential of the aqueous leaf extracts of *Amaranthus spinosus* Linn. using *Allium cepa* Assay. *South African Journal of Botany*. 102(4):18-25.
- Pratiwi, D., Wahdaningsih, S., Isnindar. 2013. Uji aktivitas antioksidan daun bawang mekah (*Eleutherine americana* Merr.) dengan metode DPPH (2,2-Difenil-1-Pikrilhidrazil). *Traditional Medicine Journal*. 18(1):9-16.
- Puslitbangtri. 1992. *Sepuluh Tahun Pusat Penelitian dan Pengembangan Tanaman Industri 1982-1991*. Sumbangan Penelitian dalam Pembangunan Perkebunan Rakyat. Deptan. R.I. Jakarta.
- Sharma, S., Sharma, S., Vig, A.P. 2018. Antigenotoxic potential of plant leaf extracts of *Parkinsonia aculeata* L. using *Allium cepa* assay. *Plant Physiology and Biochemistry*. 130:314-323.
- Sinaga, F.A. 2016. Stress oksidatif dan status antioksidan pada aktivitas fisik maksimal. *Jurnal Generasi Kampus*. 9(2):176-189.
- Syaaf, S. 2018. Daya Tarik Pengobatan Tradisional pada Era Modern. <https://lokadata.id/> [Diakses 03 April 2020].
- Syahrir, N.H.A., Farit, M.A., Susetyo, B. 2016. Efek sinergis bahan aktif tanaman obat berbasiskan jejaring dengan protein target. *Jurnal Jamu Indonesia*. 1(1): 35-46.
- Tedesco, S.B., and Laughinghouse IV, H.D. 2012. *Environmental Contamination. Chapter 8. Bioindicator of Genotoxicity: The Allium cepa Test*. IntechOpen. London
- Uttara, B., Singh, A.V., Zamboni, P., Mahajan, R.T. 2009. Oxidative stress and neurodegenerative diseases: A review of upstream and downstream antioxidant therapeutic options. *Current Neuropharmacology*. 7(1):65-74.
- Verschaeve, L., and Van Staden, J. 2008. Mutagenic and antimutagenic properties of extracts from South African traditional medicinal plants. *Journal of Ethnopharmacology*. 119(3):575-587.