Antioxidant and anticancer activity of *Eleutherine bulbosa* (Mill.) Urb on leukemia cells L_{1210}

IOP Conf. Series: Journal of Physics: Conf. Series **1277** (2019) 012022 doi:10.1088/1742-6596/1277/1/012022

Antioxidant and anticancer activity of *Eleutherine bulbosa* (Mill.) Urb on leukemia cells L_{1210}

D Lestari*, **R Kartika and E Marliana**
Department of Chemistry, Faculty of Mathematics and Natural Sciences, Mulawarman University, Jl. Barong Tongkok No.4 Kampus Gunung Kelua Unmul, Samarinda 75123, East Kalimantan, Indonesia

*Corresponding author: rieka4827@gmail.com

Abstract. Antioxidant and anticancer activity test on Leukemia cells L_{1210} were carried out on four fractions (ethanol extract, *n*-hexane, chloroform, and water fraction) of *Eleutherine bulbosa* [Mill.] Urb. The purpose of this research is to obtain IC_{50} antioxidant and anticancer activity of the active fraction of *Eleutherine. bulbosa* [Mill.] Urb on Leukemia cells L_{1210}. The research method includes antioxidant activity test with DPPH (2,2-diphenyl-1-picrylhydrazyl) method, anticancer activity test of active fraction on Leukemia cell L_{1210}, and GC-MS analysis to determine the active compound. Chloroform fraction has the best antioxidant activity with IC_{50} value 19.694 ppm (very strong category). The IC_{50} value of the chloroform fraction on L_{1210} Leukemia cells was 9.56 ppm (very strong category). Based on GC-MS analysis of the chloroform fraction, some compounds such as hexadecanoic acid, 9,12-octadecadienoic acid, linolenic acid, octadecanoic acid, androstan-17-one, and 1-(2,3,5,6-tetramethylphenyl)-ethanone were detected.

1. Introduction

Leukemia is a proliferation that occurs irregularly or a buildup of white blood cells in the bone marrow so that the elements in the normal bone marrow are replaced [1]. Old cells will die and be replaced by new cells, but this regular process does not work properly, due to the growth of abnormal white blood cells [1]. Eleutherine is a plant that is known to contain naphthoquinone and has been used as a drug and poison since prehistoric times. Naphthoquinone compounds are known to be very toxic that can be used as antimicrobial and antioxidant [2]. "Bawang Tiwai" (*Eleutherine bulbosa*) is widely used by the Dayak and Kutai tribes as traditional medicines that can treat various diseases including colon cancer, breast cancer, diabetes mellitus, hypertension, cholesterol-lowering, stroke, boils, anti-bleeding and abdominal pain. [3]. Secondary metabolite of *Eleutherine bulbosa* has the potential as a medicinal plant with various properties. Currently, the use of *Eleutherine bulbosa* as an additive in cooking is increasing, but studies of *Eleutherine bulbosa* about its activity as an anti-cancer blood or leukemia have not been widely studied. Several studies have shown that *Eleutherine bulbosa* has moderate potential antibacterial activity against Escherichia coli, with a strong category and has potential as an antidiabetic agent that is useful in the prevention and protection of diabetes mellitus [2,4,5]. *Eleutherine bulbosa* is widely used in Kalimantan to cure breast cancer, heart problems, increase endurance, anti-inflammatory, anti-tumor and can stop bleeding [5]. This article discusses the antioxidant and anticancer activity test of *Eleutherine bulbosa* on Leukemia cells.

2. Materials and methods

2.1. Materials

Plant material (*Eleutherine bulbosa*) was obtained in Samarinda. The identification of the plant was conducted at the Anatomy and Plant Systematics Laboratory, the Faculty of Mathematics and Natural Sciences, Mulawarman University. Other materials used were distilled water, ethanol, *n*-hexane, chloroform, HCl, Meyer reagent, Bouchardat reagent, Dragendorff reagent, ether, Lieberman Burchard reagent, Mg powder, amyl alcohol, NaOH, $FeCl_3$, sea salt, NaCl, 0.5% CMC-Na, DPPH, Vitamin C, RPMI-1640, $NaHCO_3$, serum bovine calf, tryphan blue, methanol, and L_{1210} Leukemia cells.

2.2. Sample preparation

The simplisia of mashed *Eleutherine bulbosa* bulbs were put in a bottle. Ethanol was added to the bottle and the sample was soaked for 24 hours, filtered, and the filtrate was concentrated. The maceration process in the same sample was repeated twice. The remaceration results were mixed together. All concentrated filtrates are called ethanol extracts. The ethanol extract was partitioned with n-hexane:methanol:water (5:9:1) using a separating funnel until 2 layers were formed, then each part was concentrated with a rotary evaporator to obtain n-hexane fraction and methanol-water fraction. The extract from methanol-water fraction was partitioned with chloroform:water (1:1) using a separating funnel until 2 layers were formed, then each part was concentrated with a rotary evaporator to obtain chloroform fraction and water fraction. Identification of the compounds in the active fraction was conducted using a Gas Chromatography-Mass spectrophotometer (GC-MS) Shimadzu QP 2010.

2.3. Phytochemical Screening

The ethanol extract and all fractions were subjected to phytochemical screening tests, including identification of alkaloids, steroid and triterpenoid groups, flavonoids, saponins and tannin groups with a method describe in Harborne [7] and Meyer et al. [8].

2.4. Antioxidant activity test with DPPH (2,2-diphenyl-1-picrylhydrazyl)

The antioxidant activity test with DPPH was conducted with a method described in Sundu et al. [9] Antioxidant activity test was carried out on ethanol extract, *n*-hexane fraction, chloroform fraction and water fraction of *Eleutherine bulbosa*. Vitamin C was used as a positive control. Determination of absorbance of the samples was carried out using Spectrophotometer UV Vis Shimadzu 1800. The values of free radical inhibition activity were calculated.

2.5. Cytotoxicity test for L_{1210} leukemia cells

The cytotoxicity test for L_{1210} leukemia cells was carried out with a method described in Hayshi et al. [10].

2.5.1. Preparation of media. RPMI-1640 (10.4 g) containing L-glutamine was dissolved in 1 L of sterile water (solution A). $NaHCO_3$ (1.3 g) was dissolved in 50 mL of sterile water (solution B). Twenty-five mL of solution B was added to 475 mL of solution A to obtain 500 mL of media (solution C). In the testing process, 15 mL of serum calf bovine was added to 85 mL of solution C. All work was carried out in a sterile room.

2. 5. 2. Cell planting and testing of cytotoxicity activity. Leukemia cells L_{1210} were added to the media containing bovine calf serum with $2x10^5$ cells/mL. Leukemia cancer cells used were from The Institute of Chemical Research Japan. Testing of cytotoxic activity was carried out on chloroform extract from tiwai onions. The dosage variations of the extract were 5, 10, 20, 40, 80 µg/mL. The media containing L1210 leukemia cell suspension ($2x10^5$ cells/mL) and the test substance were inserted into a multi well plate tissue's culture so that the total volume was 1 mL in each well. As a control, 10 µL methanol

was added with 990 µL cell suspension. The experiment was conducted twice, then the cell suspension filled with the test substance was incubated for 48 hours at 37 °C in an incubator (5% CO_2).

2.5.3. Calculation of cells. Cell calculations were carried out using the Haemocytometer Neubauer Improved. To differentiate between living cells and dead cells, before counting, 90 µL of the suspension was inserted into the sero cluster plate (96 wells) and was added with 10 µL of 1% tryphan blue solution and homogenized. A total of 10 µL of the solution was flowed into the Haemocytometer Neubauer Improved. The number of surviving cells was calculated under a microscope. Living cells were seen as clear spheres with blue spots of the cell nucleus in the middle of the circle, while dead cells appeared as dark blue patches that were irregular in shape.

2.5.4. Calculation of percentage of inhibition. Percentage of inhibition of test substances on the growth of L_{1210} leukemia cells was calculated as follows:

$$\%Inhibition = (1 - \frac{A}{B})x100\% \quad (1)$$

Where:
A: the number of living cells in a medium containing test substances.
B: number of living cells in a medium that does not contain a test substance (control).

3. Result and Discussion

3.1. *Phytochemical screening*

Phytochemical screening aims to determine the secondary metabolites contained in the extract and fractions of *Eleutherine bulbosa*. The results of the phytochemical screening are presented in Table 1.

Table 1. Results of phytochemical screening of four fractions of *Eleutherine bulbosa* (Mill.) Urb.

Compound	Reagent	Result			
		Ethanol	*n*-Hexane	Chloroform	Water
Alkaloid	Mayer	-	-	+	-
	Bouchardat	+	+	+	+
	Dragendorff	+	+	+	+
Flavonoid	Mg powder + 1 mL HCl (p) + 3 mL amyl Alcohol	+	+	+	+
Tannin	+ 1-2 drops $FeCl_3$ 1%	+	+	-	-
Saponin	+ H_2O + 1 drops HCl 1%	-	-	-	-
Terpenoid	+ 10 mL ether + 0,5 mL *Lieberman Burchard*	-	-	-	-
Quinone	+ 10 ml H_2O + NaOH 1 N	+	-	+	-

From table 1, it can be seen that tiwai onions contain secondary metabolites such as alkaloids, flavonoids, tannins, and quinones. These compounds are known to have a very broad biological

activity including antioxidants and anticancer. Flavonoids are known to be good antioxidants because they have at least two hydroxyl groups in ortho positions and para which can capture free radicals by freeing hydrogen atoms from their hydroxyl groups. Flavonoids as antioxidants have a higher potential as anticancer drugs than vitamins and minerals. Flavonoid compounds can prevent the reaction of carcinogen molecules joining cell DNA so as to prevent cell DNA damage; the bioactive components of flavonoids can prevent the initial process of cancer cell formation. Flavonoids can stimulate the regenaration process of mutated DNA cell so that the cells become normal again. In addition, quinone compounds have also been reported to have antioxidant activity. It is likely that quinone originates from the oxidation of the corresponding phenols namely catechol to form ortho-quinones and quinol producing para-quinones.

3.2. Antioxidant activity test

The antioxidant activity test was performed using DPPH (2,2-diphenyl-1-picrylhydrazyl) method. This method is a simple and common method to measure the antioxidant activity of extract and each fraction. The principle of this method is the measurement of scavenging free radical in solvents such as ethanol/methanol at room temperature by a compound that has antioxidant activity [11].

Table 2. Results of antioxidant activity

Group	Concentration (ppm)	Absorbance		% Inhibition	IC_{50} (ppm)
		Control	Sample		
Vitamin C	2	0.6866	0.5573	18.832	8.265
	4		0.4883	28.881	
	8		0.3416	50.248	
	16		0.0885	87.110	
Ethanol extract	25	0.7864	0.6986	11.165	157.598
	50		0.6226	20.829	
	100		0.4893	37.780	
	200		0.3141	60.058	
n-Hexane fraction	12.5	0.6803	0.6582	3.249	408.151
	25		0.6468	4.924	
	50		0.6279	7.702	
	100		0.5742	15.596	
	200		0.5108	24.915	
Chloroform fraction	5	0.6658	0.573	13.938	19.649
	10		0.473	28.958	
	20		0.2795	58.020	
	40		0.0583	91.244	
Water fraction	12.5	0.6739	0.6507	3.443	218.547
	25		0.6303	6.470	
	50		0.5749	14.691	
	100		0.5076	24.677	
	200		0.3692	45.214	

The IC_{50} value of chloroform fraction was 19.649 which categorized as very strong antioxidant activity. Chloroform fraction was detected to have very strong antioxidant activity because it contains a lot of compounds such as flavonoids, phenolic and alkaloid. Those compounds are known to exhibit antioxidant activity. Flavonoids can provide one electron to the free radical compounds and form complex compounds with metal.

3.3. Anticancer activity on L_{1210} cells

Based on the results of the antioxidant test, chloroform fraction of *Eleutherine bulbosa* has IC_{50} value which is categorized as very strong antioxidant. Chloroform fraction was chosen because of the potential for anticancer activity testing on L_{1210} cells. This test used L_{1210} leukemia cancer cells which is one of the leukemia cell lines derived from inoculation of rat spleen. Anticancer activity test of chloroform fraction of *Eleutherine bulbosa* was carried out at the Health Materials Laboratory, the Center for Radioisotope and Radiation (PAIR) of the National Atomic Energy Agency (BATAN) Jakarta. Anticancer activity test results from the chloroform fraction of *Eleutherine bulbosa* using doxorubicin as a positive control on L_{1210} leukemia cancer cell growth can be seen in table 3.

Table 3. Anticancer activity on L_{1210} cells.

Anticancer activity on cell L_{1210}							
Replicate 1				**Replicate 2**			
C (ppm)	**Log C**	**Inhibition (%)**	**Probit**	**C (ppm)**	**Log C**	**Inhibition (%)**	**Probit**
5	0.6990	36.00	4.64	5	0.699	36	4.64
10	10.000	52.00	5.05	10	1	48	4.95
20	13.010	68.00	5.47	20	1.301	68	5.47
40	16.021	78.00	5.77	40	16.021	76	5.71
80	19.031	86.00	6.06	80	19.031	86	6.06
r	0.998			R	0.993		
Log C	0.9645			Log C	0.9958		
IC_{50}	9.22			IC_{50}	9.90		
Average IC_{50} = 9.56 ppm							

Anticancer activity test of chloroform fraction of *Eleutherine bulbosa* on L_{1210} leukemia cells in this study showed an average IC_{50} value of 9.56 ppm (very strong activity). The value of IC50 on anticancer activity test result was carried out in duplicate and obtained values of 9.22 ppm and 9.90 ppm with an average value of 9.56 ppm. This value indicated that the chloroform fraction had high anticancer potential against L1210 leukemia cells. GC-MS analysis of chloroform fraction of *Eleutherine bulbosa* was performed to determine its chemical profile (Figure 1). In this study, there were several compounds identified such as hexadecanoic acid, 9,12-octadecadienoic acid, linolenic acid, octadecanoic acid, androstan-17-one, and 1-(2,3,5,6-tetramethylphenyl)-ethanone.

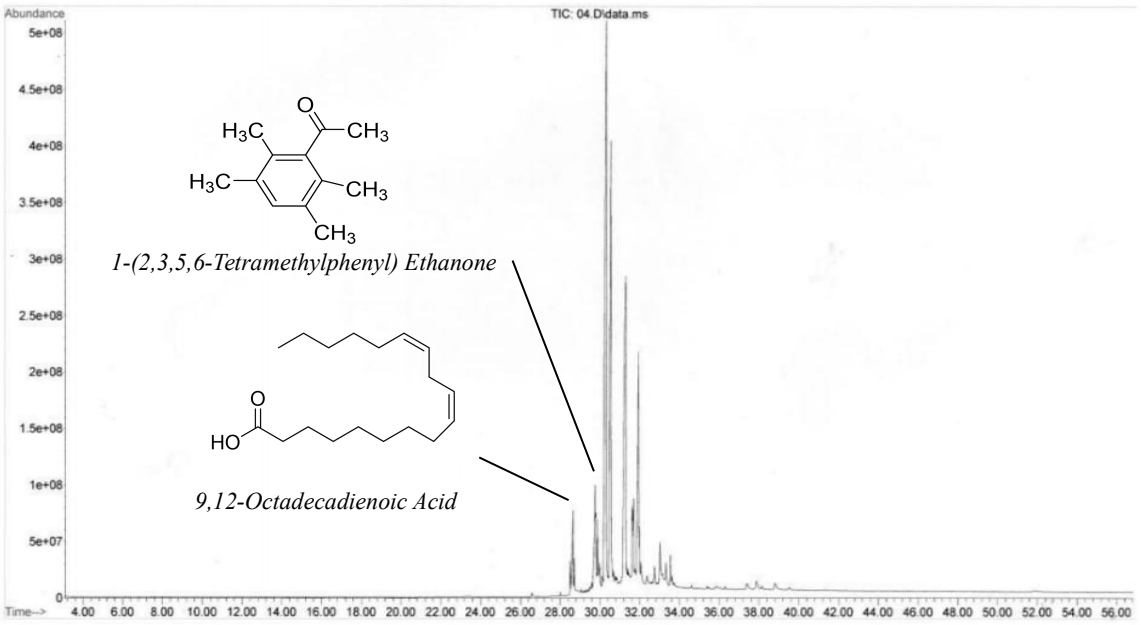

Figure 1. The chromatogram of the chloroform fraction of *Eleutherine bulbosa* and some compounds identified in the chromatogram

4. Conclusion

The chloroform fraction is the selected active fraction that has a value of antioxidan 19.649 ppm (very strong category) and anticancer activity against L_{1210} Leukemia cells 9.56 ppm (very strong category). Some chemical compounds identified in the chloroform fraction of *Eleutherine bulbosa* are hexadecanoic acid, 9,12-octadecadienoic acid, linolenic acid, octadecanoic acid, androstan-17-one, and 1-(2,3,5,6-tetramethylphenyl)-ethanone.

Acknowledgment

We would like to acknowledge National Nuclear Energy Agency of Indonesia and Director of the Samarinda Pharmacy Academy for opportunities and facilities in conducting this research.

Reference

[1] Smeltzer 2001 Buku Ajar Keperawatan Medikal Bedah Brunner & Suddarth.
[2] Kuntorini E M, Astuti M D, Nugroho L H 2010 Anatomical Structure and Antioxidant Activity of Dayak Onion Bulb (*Eleutherine americana* Merr) From South Kalimantan Region, *Penelitian Hayati* **16** 1-7
[3] Galingging R Y 2009 Dayak Onion (*Eleutherine palmifolia*) as a Multifunctional Medicinal Plant
[4] Amanda F R 2014 Effectiveness of Dayak Onion Extract (*Eleutherine palmifolia* L. Merr) in Inhibiting Escherichia coli Bacterial Growth
[5] Febrinda A E, Astawan M, Wresdiyati T, Yuliana N D 2013 Antioxidant Capacity and Alpha Glucosidase Inhibitors Extract of Dayak Onion Bulbs Journal of Technology and Food Industry 161
[6] Saptowaluyo C A 2007 Dayak Onions, Unresolved Cancer Medicinal Plants
[7] Harborne J B 1996 Phytochemical method. Second Edition. Translated by K. Radmawinata and I. Soediso. Bandung: ITB Press
[8] Meyer B N, Ferrigni N R, Putnam J E, Jacobsen L B, Nicholas D E, and Mc Laughlin J L 1982 Brine Shrimp: A Convenient General Bioassay for Active Plant Constituent, *Drug*

Information Journal **45** 31-34
[9] Sundu R, Sapri, Nurhasnawati H 2018 Uji Fitokimia dan Aktivitas Antioksidan Ekstrak Etanol Umbi Paku Atai Merah (Angiopteris ferox Copel*) Jurnal Ilmiah Ibnu Sina* **3**(1) 97-105
[10] Hayshi, Y, Nishikawa Y, Mori H, Matsushita, YI and Matsui T 1998 Antitumor activity of (10*E*, 12*Z*)-9-hydroxy-10,12- octadecadienoic acid from rice bran *Journal of Fermentation and Bioengineering* **86**(2) 149-153
[11] Tangyueyengwatana P and Gritsanapan W 2015 Standardization of Prasaplai, a Thai traditional preparation for antidysmenorrhea *Dove Press journal: Botanics: Targets and Therapy*