[ICMSC-2018] Template Revision

Yahoo/Inbox

admin <conference.fmipa@gmail.com>
To: Dwi Lestari, Rudi Kartika, Eva Marliana

Fri, Dec 21, 2018 at 3:07 PM

Dear Author

We have checked the templates in your article before this article entered the reviewer and we found several things that need to be corrected in the author's article. (see attachment)

Regard
ICMSC Proceeding Team

International Conference on Mathematics, Science, and Computer Science
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[ICMSC-2018] Editorial Decision on Paper

Yahoo/Inbox

admin <conference.fmipa@gmail.com>
To: Dwi Lestari
Cc: Rudi Kartika, Eva Marliana

Fri, Mar 22, 2019 at 3:27 PM

Dwi Lestari:

After a careful review of your submission, "ANTICANCER ACTIVITY FROM Eleutherine bulbosa (Mill.) Urb ON LEUKEMIA CELLS L1210" will be considered for publish at International Conference on Mathematics, Science, and Computer Science proceedings if the following revisions are successfully implemented. please send the revise by March 25, 2019

Thank you and looking forward to your participation in this event.

admin
conference.fmipa@gmail.com

Reviewer A:

Does the writing title reflect the content, specific and effective?:
Acceptable but needs some
improvements

Abstracts and keywords, do you describe the contents of the writing as a

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Abstracts and keywords, do you describe the contents of the writing as a whole? :
acceptable but needs some improvements

Is the purpose of the article stated clearly?:
acceptable but needs some improvements

Are the study methods described clearly?:
acceptable but needs some improvements

Does the article introduce a new idea that might stimulate others to do related research? :
Good

Does the article offer a valuable contribution to a certain area of research?:
Good

Are the results clearly presented?:
acceptable but needs some improvements

Are the conclusions accurate and supported by the content?:
Good

Is the article correctly written (in terms of grammar, style and register)?:

acceptable but needs some improvements

Is the literature used in support of Research sufficiently comprehensive and current?:
acceptable but needs some improvements

Specific Comments and Suggestions : :
See the comments

Reviewer Recommendation : (Choose One) :
Publish, but suggest changes to the article as specified in this review.

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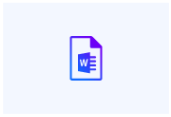

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[ICMSC-2018] Letter of Acceptance for Publication 2

Yahoo/Drafts

Rita Hairani <conference.fmipa@gmail.com>
To: Dwi Lestari
Cc: Rudi Kartika, Eva Marliana

Sat, Jun 1, 2019 at 11:50 AM

Dear Author

Congratulations, your paper entitled:
"Antioxidant and anticancer activities of Eleutherine bulbosa (Mill.) Urb on leukemia cells L1210" has been accepted for publication in Journal of Physics: conference series, IOP Science, a conference proceeding for the 2nd International Conference on Mathematics, Science and Computer Science (ICMSC 2018) which was held in Balikpapan, East Kalimantan, Indonesia in October 24th, 2018 at Novotel Hotel, Balikpapan (LOA Attached).

Please be advised that the article can only be published in the conference proceeding if the publication fee has been paid. please make a payment by today, 1st June 2019:

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Editorial Board of ICMSC 2018

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THE 2nd INTERNATIONAL CONFERENCE ON
MATHEMATICS, SCIENCE, AND COMPUTER SCIENCE
ICMSC 2018
FACULTY OF MATHEMATICS AND NATURAL SCIENCES
Jl. Barong Tongkok No. 04 Kampus Gunung Kelua, Samarinda – East Kalimantan, 75123, Indonesia

Samarinda, 01st June 2019

Letter of Acceptance

Dear Dwi Lestari, Rudi Kartika and Eva 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

Congratulations, your paper entitled:
"Antioxidant and anticancer activities of *Eleutherine bulbosa* (Mill.) Urb on leukemia cells L1210" has been accepted for publication in Journal of Physics: conference series, IOP Science, a conference proceeding for the 2nd International Conference on Mathematics, Science and Computer Science (ICMSC 2018) which was held in Balikpapan, East Kalimantan, Indonesia in October 24th, 2018 at Novotel Hotel, Balikpapan.

Please remember that the article can only be published in the conference proceeding if the publication fee has been **paid**.

Again, we would like to congratulate you on submitting an article of such high quality and we look forward your contribution in the next conference.

Yours faithfully,
The Editorial Board of ICMSC 2018

Dr. R R Dirgarini Julia N Subagyono, M.Sc

Anticancer activity from *Eleutherine Bulbosa* (mill.) urb on leukemia cells L_{1210}

D Lestari, **R Kartika and E Marliana**
Departement Master of Chemistry FMIPA Universitas Mulawarman
Email correspondence: rieka4827@gmail.com

Abstract. Anticancer activity has been tested against L_{1210} Leukemia cells from 4 fractions of *Eleutherine bulbosa* (Mill.) Urb, named ethanol extract, *n*-hexane fraction, chloroform fraction, and water fraction. The purpose of this study was to obtain pharmacological information from the active fraction of *E. bulbosa* bulbs, including antioxidant and anticancer properties. The study included antioxidant testing using DPPH method, anticancer activity of L_{1210} cell from active fraction, and GC-MS analysis. Chloroform fraction is the best fraction that has antioxidant activity IC_{50} = 19.694 ppm (very strong category) and IC_{50} value for Leukemia cells L_{1210} was 9.56 ppm (very strong category). Based on GC-MS analysis of chloroform fraction, it showed that some compounds such as 9,12-octadecadienoic acid, di-n-octyl phthalate, and 1-(2,3,5,6-tetramethyl phenyl) ethanone.

1. Introduction

Leukemia is a proliferation that occurs irregularly or the accumulation of white blood cells in the bone marrow so that the elements in the normal bone marrow are replaced (Smeltzer, 2001). Old cells will die and be replaced by new cells, but this process that occurs regularly does not work properly, which is the occurrence of pressure from other cells due to abnormal growth of white blood cells (Smeltzer, 2001) One of the natural ingredients used is "Bawang Tiwai" (*Eleutherine bulbosa*) because it is empirically widely used by Dayak and Kutai people as traditional medicine that can treat various diseases including colon cancer, breast cancer, diabetes mellitus, hypertension, cholesterol lowering, stroke, boils, anti-bleeding and abdominal pain. (Galingging, 2009). The chemical content of E. bulbosa bulbs has the potential as a medicinal plant with a variety of properties that are very large. Currently the use of *E. bulbosa* as an additional ingredient in cooking is also increasingly well known, but research on *E. bulbosa* bulbs has not been widely carried out, especially about its efficacy as an anti-cancer blood or leukemia. Several studies have shown that "Bawang Tiwai" has moderate potential antibacterial activity against *Escherichia coli*, as an antioxidant with a strong category and has the potential as an antidiabetic agent that is useful in the prevention and protection of diabetes mellitus (Amanda, 2014; Kuntorini, 2010; Febrinda, 2013). Bulbus (layer tuber) Dayak onion or *E. bulbosa* by the local people of Kalimantan is widely used as a cure for breast cancer, heart problems, increase endurance, anti-inflammatory, antitumor and can stop bleeding (Saptowaluyo, 2007).

Table 1. Phytochemical of *Eleutherine* Plants

Spesies	Golongan Senyawa	Nama Senyawa	Bagian Tumbuhan	Pustaka
E. bulbosa	Poliketide	(R)-11-hydroxyeleutherin	Bulb	Gallo dkk, 2010
E. bulbosa	Poliketide	Eleutherinon	Bulb	Hara dkk, 1997
E. bulbosa	Naftokuinon	Eleutherinone	Bulb	Alves dkk, 2003
E. bulbosa	Naftokuinon	Hongconin	Bulb	Gallo dkk, 2010
E. bulbosa	Naftokuinon	Eleutherine	Bulb	Han dkk, 2008
E. americana	Naftokuinon	Eleuthenin	Bulb	Tangyueyengwatana, 2015
E. americana	Naftokuinon	Eleutherinoside A	Bulb	Tangyueyengwatana, 2015
E. americana	Naftokuinon	Isoeleutherine	Bulb	Han dkk, 2008
E. americana	Naftokuinon	Elecanacin	Bulb	Hara dkk, 1997
E. americana	Naftokuinon	Eleutherol	Bulb	Tangyueyengwatana, 2015
E. palmifolia	Naftokuinon	Eleutherobine	Bulb	Shibuya dkk, 1997
E. palmifolia	Naftokuinon	Eleuthosides A	Bulb	Shibuya dkk, 1997
E. palmifolia	Naftokuinon	Eleuthosides B	Bulb	Shibuya dkk, 1997
E. palmifolia	Naftokuinon	Eleuthosides C	Bulb	Shibuya dkk, 1997
E. palmifolia	Flavonoid	Kuersetin	Bulb	Rosa, 2013
E. palmifolia	Flavonoid	Rutin	Bulb	Sofic, dkk, 2011

2. Materials and Methods

2.1.*Material*

E. bulbosa bulbs, distilled water, ethanol, n-hexane, chloroform, 2 N HCl, Meyer reagent, Bouchardat reagent, Dragendorff reagent, ether, Lieberman Burchard reagent, Mg powder, HCl (p), amyl alcohol, 1% HCl, NaOH 1 N, 1% FeCl3, yeast, sea salt, NaCl, 0.5% CMC-Na, DPPH, Vitamin C, RPMI-1640, NaHCO3, serum bovine calf, tryphan blue, methanol, L1210 Leukemia cells.

EQUIPMENT???

2.2. *Phytochemical Screening*

2.2.1**.** *Identification of alkaloids*. The extracts and fractions were weighed as much as 0.05 g, then added 1 mL of. 2 N HCl and 9 mL of distilled water, heated over tangas of water for 2 minutes, cooled and filtered, and the filtrate was tested for alkaloids. , namely: first taken 3 drops of filtrate, then added 2 drops of mayer reagent to produce white / yellow precipitate, secondly taken 3 drops of filtrate, then added 2 drops of bouchardat reagent to produce brown to black precipitate and thirdly the filtrate was sprayed on the KLT plate sprayed dragendorff reagent shows brick red spots. Alkaliod is considered positive if there are deposits or positives of at least two or three of the above experiments (MOH, 1979).

2.2.2. *Identification of steroid and triterpenoid groups*. The extracts and weighed fractions of 0.05 g each were then added 10 mL of ether and 0.5 mL of burchard lieberman reagent to form blue or green which showed the presence of steroids and green or purple color indicating triterpenoid (Harborne, 1987).

2.2.3. *Identification of flavonoids***.** The extracts and fractions were weighed as much as 0.05 g each and then each dissolved in 10 mL of hot distilled water until a solution was then formed from each extract solution and fractions were added 100 mg of Mg powder into a tube and added 1 mL concentrated HCl and add 3 mL of amyl alcohol, beaten vigorously and allowed to separate. If red, yellow, orange forms in the amyl alcohol layer indicate flavonoids (Harborne, 1987).

2.2.4. *Identification of saponins*. The weighed extracts and fractions of 0.05 g were then dissolved in 10 mL of hot distilled water until a solution was formed and then shaken vertically for 10 seconds to

form a stable foam, left for 10 minutes then in each extract and fraction. Fraction was added 1 drop of 1% HCl if the foam was not lost indicating the presence of saponins (Harborne, 1987).
2.2.5 *Quinone group identification*. The extracts and fractions weighed as much as 0.05 g each then dissolved in 10 mL of hot distilled water until a solution was then formed in each extract solution and the fractions were added a few drops of 1 N NaOH, if the filtrate formed color red indicates the presence of quinone (Harborne, 1987).

2.2.6. *Identification of tannin groups*. The extracts and fractions weighed as much as 0.05 g each then dissolved in 10 mL of hot distilled water until a solution was then formed in each extract solution and fractions were added a few drops of 1% $FeCl_3$ solution, if the filtrate was formed dark blue or blackish green proof of tannin (Harborne, 1987).

2.3. *Antioxidant Activity Test with DPPH Damping Method (1,1-diphenyl-2-picrylhydrazyl)*
Antioxidant activity test was carried out by first making 40 ppm DPPH solution which was 0.004 g DPPH added ethanol to 100 mL, then the 2 mL DPPH 40 ppm solution was observed in the range of 450-600 nm to determine its maximum wavelength. Preparation of 100 ppm vitamin C main solution was 0.01 g of vitamin C added ethanol to 100 mL then 100 ppm of vitamin C mother liquor was pipetted as much as 1, 1.5, 2, and 2.5 mL, respectively, then added ethanol to 10 mL then obtained a concentration of 10, 15, 20, and 25 ppm then to determine the absorbance of the concentration series of vitamin C with DPPH by way of, from each series of concentration of vitamin C was pipetted as much as 2 mL and added 4 ml of DPPH 40 ppm solution then observed absorption occurs at each concentration. Preparation of mother liquor from ethanol extract, n-hexane fraction, chloroform fraction, and water fraction of 200 ppm, respectively by weighing extracts and fractions of 0.02 g each, then adding ethanol to 100 ml, then making a series of concentrations. from ethanol extract, n-hexane fraction, chloroform fraction, and water fraction by means of the 200 ppm mother liquor pipetted as much as 0, 0.625, 1.25, 2.5, 5, and 10 ml ethanol added up to 10 ml except the concentration of 200 ppm without the addition of ethanol to obtain a concentration of 0, 12.5, 25, 50, 100, and 200 ppm, then determine the absorbance of the concentration series of ethanol extract, n-hexane fraction, chloroform fraction, and water fraction plus DPPH that is, from each series of concentrations dipipet as much as 2 mL, then added 4 mL of 40 ppm DPPH solution, allowed to stand for 30 minutes observed absorption that occurred at the massing of each concentration on UV Vis spectrophotometry, then calculated free radical inhibition activity using the following formula:

$$\%Inhibition = \frac{Absorbance\,control - Absorbance\,material}{Absorbance\,control} x100\% \qquad (1)$$

2.4. *Cytotoxicity Test for L1210 Leukemia Cells*
2.4.1. *Preparation of media*. RPMI-1640 weighing 10.4 g containing L-glutamine was dissolved in 1 L of sterile water (solution A). A total of 1.3 g of NaHCO3 was dissolved in 50 mL of sterile water (solution B). A solution of B as much as 25 mL was added to 475 mL of solution A, then obtained 500 mL of media (solution C). In the test a fresh media solution was made, which was 15 mL of serum bovine calf added to 85 mL of C solution. All work was done in a sterile room.

2. 4. 2. *Cell planting and testing of cytotoxicity activity*. L1210 leukemia cells were added to the media which contained serum bovine calf so that a number of cells were around 2x105 cells / mL. Leukemia cancer cells that will be used initially come from The Institute of Chemical Research Japan. Testing of cytotoxic activity was carried out on chloroform extract from E. bulbosa. Testing of the cytotoxic activity of the extract was carried out with a variety of doses of 5, 10, 20, 40, 80 µg / mL. The media containing L1210 leukemia cell suspension (2 x 105 cells / mL) and the test substance were inserted

into the multi well plate tissue culture so that the total volume was 1 mL in each well. As a control 10 µL of methanol was used which added 990 µL of cell suspension. The experiment was carried out duplo, then the cell suspension which was filled with the test substance was incubated for 48 hours at 37°C in a 5% CO_2 incubator.

2.4.3. *Calculation of cells*. Cell calculations were performed using Haemocytometer Neubauer Improved. To differentiate between living cells and dead cells, before counting, 90 µL of suspension was put into sero cluster plate (96 wells) and added 10 µL of 1% tryphan blue solution and homogenized. A total of 10 µL of solution is channeled into an improved Neubauer haemocytometer. After that the number of living cells is calculated under a microscope. Living cells are seen as clear spheres with blue spots of the nucleus of the cell in the center of the sphere, while dead cells are seen as concentrated blue spots that are irregular in shape.
2.4.4. Percentage of inhibition of test substances on the growth of L1210 leukemia cells is calculated as follows:

$$\%Inhibition = (1-\frac{A}{B})x100\% \quad (2)$$

Information:
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. *Identification of Plant*
Bawang Tiwai plant used in this study was obtained from farmers in the area of Jalan Kadrie Oening Samarinda. Onion Tiwai identification was conducted at the MIPA Faculty of Anatomy and Systematics Laboratory, Mulawarman University. This identification aims to ensure the correctness of the material used in the research is true E. bulbosa with the species name *Eleutherine bulbosa* (Mill.) Urb.

3.2. *Phytochemical Screening*

Table 2. Phytochemical Screening of *Eleutherine bulbosa* (Mill.) Urb.

No	Compound	Reagent	Result			
			Ethanol	*n*-Hexane	Chloroform	Water
1	Alkaloid	Mayer	-	-	+	-
		Bouchardat	+	+	+	+
		Dragendrof (KLT)	+	+	+	+
2	Flavonoid	Mg powder + 1 mL HCl (p) + 3 mL amyl Alcohol	+	+	+	+
3	Tannin	+ 1-2 drops FeCl3 1%	+	+	-	-
4	Saponin	+ H_2O + 1 drops HCl 1%	-	-	-	-
5	Terpenoid	+ 10 mL ether + 0,5 mL *Lieberman Burchard*	-	-	-	-

6	Quinone	+ 10 ml H_2O + NaOH	1 N	+	-	+	-

3.3. *Antioxidant Activity Test*

Antioxidant activity was performed using DPPH radical (1,1-diphenyl-2-picrylhydrazyl) method. This method is a commonly used method and very simple to measure the antioxidant activity of extracts and each fraction by using stable free radical compounds, DPPH. Antioxidant activity test was carried out on ethanol extract, n-hexane fraction, chloroform fraction and water fraction of *E. bulbosa* tuber. Vitamin C was used as positive control. The principle of the method of radical capture is the measurement of free radical capture in solvents such as ethanol / methanol at room temperature by a compound that has antioxidant activity (Pokorny, 2001).

Table 3. 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	

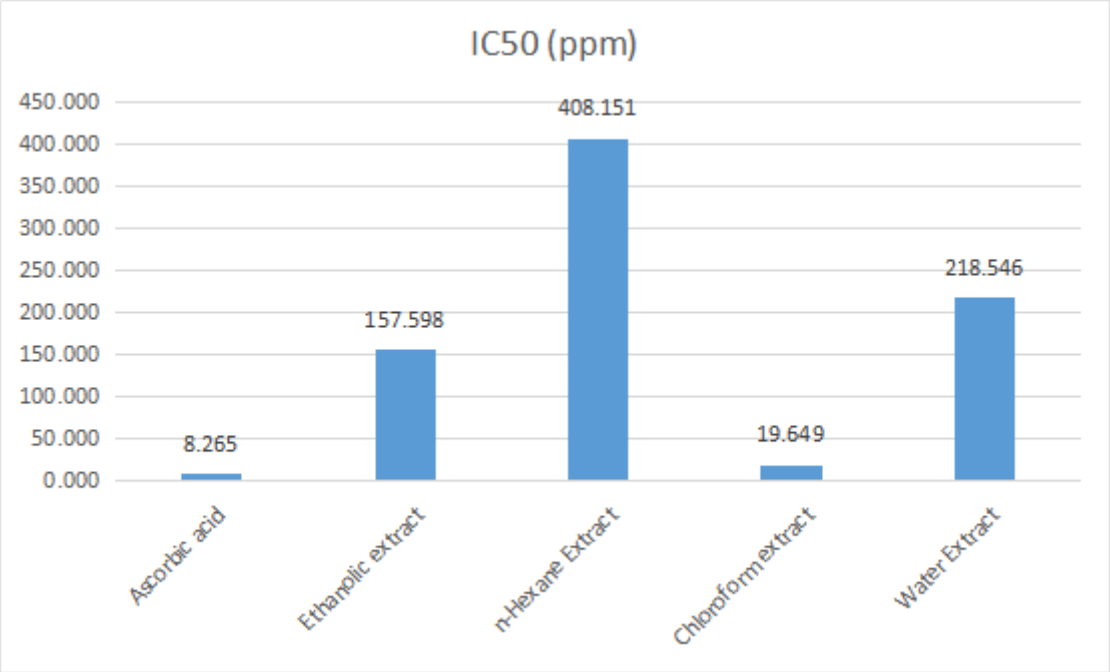


Figure 1. Antioxidant Activity (IC50)

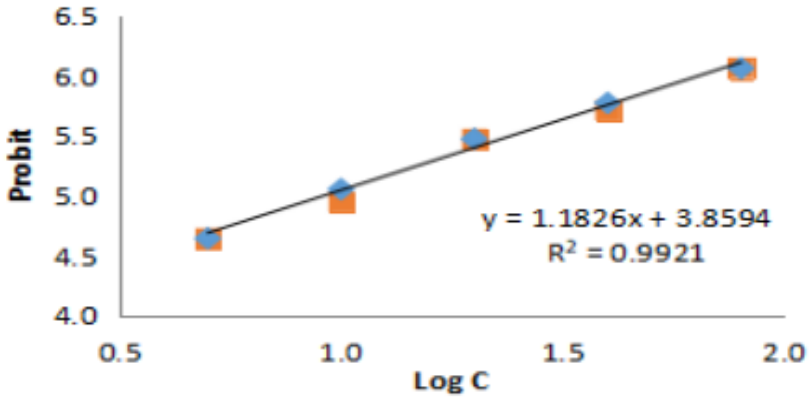
3.4. *Anticancer Activity on L1210 Cells*

Based on the results of the antioxidant test, the chloroform fraction of *E. bulbosa* bulb 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 L1210 leukemia cancer cells which is one of the leukemia cell lines derived from inoculation of rat spleen. This method was chosen because it is a bioassay method of tissue culture which is the right initial screening to quickly determine the potential for inhibition by using relatively few samples because with a percentage calculation of the sample it can provide inhibition potential value and a fast time to know the potential of cytostatics from a sample is a saving in terms of time which is very meaningful to decide quickly whether the sample has the potential to be developed further or not. Anticancer activity testing of *E. bulbosa* chloroform fraction 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 *E. bulbosa* tuber using doxorubicin as a positive control on L1210 leukemia cancer cell growth can be seen in table 4.

Table 4. Anticancer activity on L1210 cells

Anticancer activity on cell L1210							
Test 1				Test 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 testing of the chloroform fraction of *E. bulbosa* tuber on L1210 leukemia cells in this study showed an average IC_{50} value of 9.56 ppm including a very strong



Picture 2. Graph of linearity equation of IC_{50} of chloroform fraction

The IC_{50} anticancer activity test results were carried out in duplicate and obtained values of 9.22 ppm and 9.90 ppm with an average value of 9.56 ppm, indicating that the chloroform fraction had high anticancer potential against L_{1210} leukemia cells, to distinguish Trypan blue solution is used for dead cells, where this solution can enter the cytoplasm of dead cells. Figure 4.12 shows the difference in L_{1210} leukemia cells, namely living cells that have a clear circle on the outside and inside a black spot while the dead cell is blackish blue and there is no clear circle on the outside.

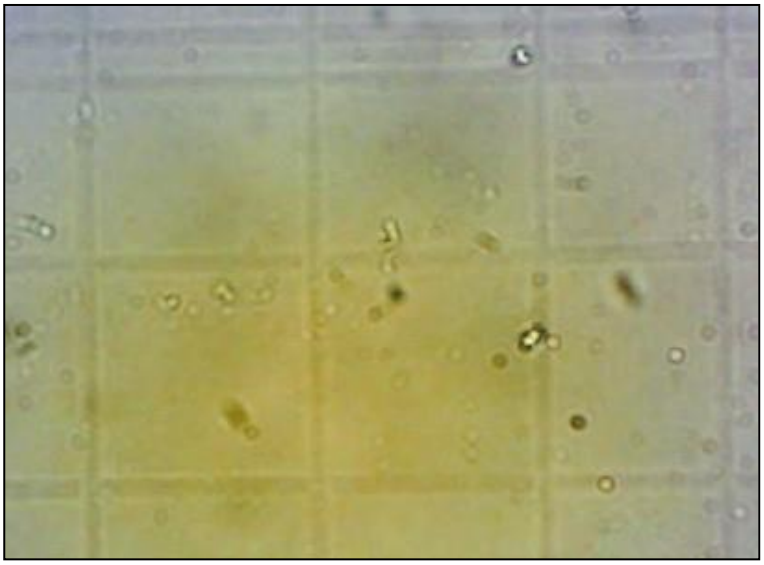
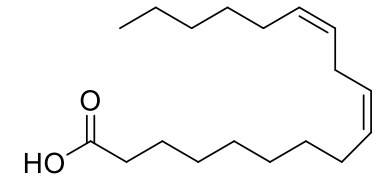
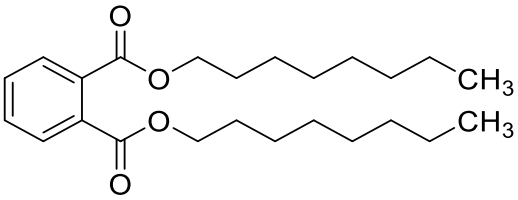


Figure 3. Photographs of living L1210 leukemia cells and dead cells

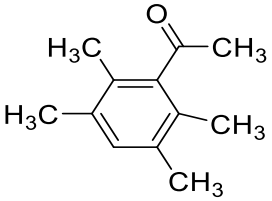
GC-MS analysis of chloroform fraction of *E. bulbosa* bulb was performed to determine its chemical profile, GC-MS testing was carried out. In this study, there were several compounds and when compared to the same base peak literature, 9,12-octadecadienoic acid (99%), di-n-octyl phthalate (78%), 1- (2,3,5,6-tetramethylphenyl) ethanone (55%).



9,12-Octadecadienoic Acid ($C_{18}H_{31}O_2$) m/z=280



Di-n-octyl phthalate ($C_{24}H_{38}O_4$) m/z=391



1-(2,3,5,6-Tetramethylphenyl) Ethanone ($C_{12}H_{16}O$) m/z=176

Figure 4. Chemical structure of *E. bulbosa* chloroform fraction in testing with GC-MS

4. Conclusion

- The chloroform fraction is the selected active fraction that has a value of antioxidan is 19.649 ppm (very strong category) and anticancer activity against L1210 Leukemia cells is 9.56 ppm (very strong category).
- The chemical compound profile of GCMS chloroform fraction of E. bulbosa bulbs are 9,12-Octadecadienoic Acid, Di-n-octyl phthalate, and 1- (2,3,5,6-Tetramethyl phenyl) Ethanone.

Acknowledgment

The author expresses their gratitude to the Dean of FMIPA, Chair of the Department of Chemistry and Chairperson of the Chemical Masters Study Program at Mulawarnan University, National Atomic Energy Agency in Jakarta, Director of the Samarinda Pharmacy Academy for opportunities and facilities in conducting this research.

Reference

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