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Antioxidant and Anticancer Potential Test of Alikokop (*Dischidia nythesiana*) Leaf Methanol Extract

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Abstract: ***This study aimed to determine the antioxidant and anticancer activity of methanol extract of Alikokop leaves (Dischidia nythesiana). The extraction method used is maceration extraction using methanol solvent. The antioxidant activity test was carried out using the DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical deterrent method and the anticancer activity assay was carried out using the MTT (Microculture Tetrazolium) method using P-388 cancer cells. From this study, the antioxidant test results showed that the methanol extract of Alikokop leaves had antioxidant activity with moderate concentration, with IC50 of 67.136 µg / mL, and the results of anticancer tests showed that methanol extract of Alikokop leaves had anticancer activity on leukemia cells P388 with concentration inhibition values medium, with IC50 of 24.88 µg / mL. The methanol extract of Alikokop leaves has the potential as an antioxidant and anticancer leukemia.***

Keywords: Antioxidant, Anticancer, Alikokop, Methanol Exstract, *Dischidia nythesiana*

1. Introduction

The Asclepiadaceae tribe consists of about 250 genera with 2000 species, spread in the tropics, subtropics and slightly in temperata [1]. One of the genera of plants found in the Asclepiadaceae is Dischidia. This genus is classified as an epiphytic plant whose root is not on the ground but lives by wrapping or hanging its body on other plants. It is known that there are 80 species from the genus Dischidia spread across Asia. Several species of the Dischidia genus are endemic, such as *Dischidia torricellensis* in Papua New Guinea and *Dischidia cleistantha* in the Philippines. while there are also several species that are classified as conservation status as Critically Endangered species, such as *Dischidia acutifolia* and Dischidia nummularia [2]. Several species of the Dischidia genus are used traditionally in the treatment of diseases, such as *Dischidia pectinoides* which is used to treat respiratory diseases [3] and Dischidia imbricate which are believed to treat tumor and cancer by the Minahasa tribe (Nurrani et al., 2014). The use of D. imbricate plants as traditional medicine has been supported by research on the content of compounds found in this plant. *D. imbricate* is known to contain secondary metabolic compounds in the form of alkaloids, flavonoids, steroids and triterpenoids which have the potential to be used as antioxidants and anticancer [4]. In addition to *D. imbricate* in Minahasa, Bolaang Mongondow Community also utilizes one of the plants from the genus Dischidia in their area as traditional medicine, namely *Dischidia nythesiana* or known as the local name Alikokop. By drinking a decoction from Alikokop leaves, the community believes that this plant can treat cancer [5]. Similarly, *D. nythesiana* is known by the community of Sangihe Regency with the local name Lintakuhi, local people also use lintakuhi plants to treat cancer [6,7)

Cancer is a disease caused by the accumulation of free radicals. Free radicals are atoms or molecules that have unpaired electrons. The unpaired electrons cause free radicals to be very reactive and can attack healthy cells, causing them to lose their function and structure [8]. The negative effects of free radicals on the body can be prevented by compounds called antioxidants, because antioxidant compounds have the ability to give electrons, bind and end free radical reactions [8]. The compounds that have the ability as antioxidants (alkaloids, flavonoids, steroids, saponins, and terpenoids) in Dischidia plants, are known to have the potential to be developed as anticancer, because antioxidant performance is closely related to the performance of anticancer. As research conducted by [9], showed that methanol extract from *S. delicatula* leaves containing alkaloid compounds had IC50 values of 82.81 µg / mL (Inhibition Concentration 50%) which were able to inhibit the growth of cancer cells P388 [10]. In this study methanol solvents were used because it is known that methanol solvents are good solvents to attract alkaloids, steroids, saponins, and flavonoids [11]. Until now there has been no scientific research on the plant D. nythesiana as a treatment for cancer, so that in this study scientific evidence of the potential of *D. nythesiana* as a treatment for cancer by testing antioxidant and anticancer activities contained therein will be carried out.

Based on the background above, the formulation of the problem in this study is (1). Does the methanol extract of Alikokop leaves (*Dischidia nythesiana*) have the potential as an antioxidant with the DPPH (1,1-diphenyl-2-picrylhydrazyl) method? (2). Does the methanol extract of Alikokop leaves (*Dischidia nythesiana*) have potential for P388 leukemia cells? Based on the background and formulation of the problem above, the purpose of this study is as follows: (1). To test the potential of antioxidant activity contained in the methanol extract of Alikokop leaves (*Dischidia nythesiana*) through parameters of IC50 values using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method. (2). To test the potential of anticancer activity of methanol

extract of Alikokop leaves (*Dischidia nythesiana*) on P388 leukemia cells. This research is expected to be a source of scientific information about the potential of *Dischidia nythesiana* plants as an anticancer and antioxidant. In addition, it is expected to be a study of science for deeper and broader researches.

2. Research Methods

This research was conducted at the Pharmacology Laboratory of FMIPA UNSRAT and Manado Health Polytechnic Health Analyst Laboratory for making extracts, for the antioxidant test the research was carried out at the Pharmacy Department Analyst Laboratory of FMIPA UNSRAT, and anticancer tests were carried out at the Natural Chemistry Laboratory (KOBA) Bandung Institute of Technology (ITB) The research begins in December 2018 until June 2019. 1. Making Extracts The tools used for making extracts are analytical scales, glass jars, knives, scissors, plastic containers, blenders, washcloths, label paper, stirring spoons, cloths, petri dishes, measuring cups, aluminum foil, rotary evaporators, desiccators, waterbaths , eppendorf tube. The ingredients used for making extracts are samples of *D. nythesiana* leaves and technical methanol.

2.1 Antioxidant Test

The tools used in the antioxidant test are measuring cups, erlenmeyer flasks, analytical scales, stirring spoons, stirring rods, micropipets, blue and yellow tips, cuvet, label paper, aluminum foil, small test tubes, tube racks, computers, and spectrophotometers UV-Vis. The material used in this study was *D. nythesiana* leaf extract, vitamin C injection (Ethica), methanol pa solution, 1,1-diphenyl-2-picrylhydrazyl (DPPH) crystal pa (Sigma-Aldrich code material No.D9132-1G). 3. Anticancer Test The tools used in anticancer tests are vial bottles, ovens, vortex, micropipets, small test tubes, small tube racks, 96-well plates, conical tubes, yellow tips, blue tips, culture dishes, hemocytometers, and ELISA readers. The ingredients used were D. nythesiana leaf extract, n-hexane, aquades, chloroform, anhydrous acetic acid, Mg metal, concentrated HCl, Dragendroff reagent, Mayer reagent, FeCl, concentrated H2SO4, Phosphate Buffered Saline (PBS), culture media Roswell Park Memorial Institute (RPMI), trypsin-Ethylene Diamine (EDTA), Dimethyl sulfoxide (DMSO), formaldehyde, Fetal Bovine Serum (FBS), Microculture Tetrazolium (MTT) 5 mg / mL (50 mg MTT and 10mL PBS), Sodium Dedocyl Sulfate (SDS) 10% in 0.1 N HCl, aluminum foil and zeolite NaX.

3. Research Procedure

3.1 Sampling and Processing

The samples used were leaves of D. nythesiana taken from Kombot Village, Pinolosian, Bolaang Mongondow Selatan, North Sulawesi. The leaves from the sample are washed, drained and sliced into small pieces, then weighed. The weighing results are expressed as wet weight. The leaves are then dried until dry and then blended until the sample becomes powder. After that, the dry weight is measured so that the water content parameters can be determined. The formula used to determine water content is as follows [10]:

Water content = (X-Y) / X x 100%
Information:
X = leaf wet weight (gram)
Y = leaf dry weight (gram)

3.2 Extraction

Extraction is done by maceration method. Samples that have become powder are sifted until fine powder is obtained, then weighed and soaked in a glass jar with the addition of 1000 mL of methanol. The sample is soaked seven times 24 hours and every six hours is stirred. The soaking results were then filtered using cloth and extracted from the filtering results and then evaporated using a rotary evaporator at a speed of 46 rpm for ± 20 minutes in a vapor temperature of 19ºC-21.2ºC and bath temperature 49.9ºC-50.0ºC. The evaporation extract then stirred using Waterbath for ± 60 minutes at a temperature of 30ºC to 80ºC until the extract became thick. The results of thick extracts were then weighed and put into Eppendorf tubes and stored until the samples were used for antioxidant tests and anticancer tests.

3.3 Test the Treatment of Extracts

3.3.1. Anticancer Test

Anticancer potential test of methanol extract of Alikokop leaves was carried out in vitro at the Bandung Institute of Technology's Organic Chemistry (KOBA) Laboratory with the following stages: (1) Preparation of sample solutions, preparation of sample test solutions, preparation of experimental materials, preparation and manufacture media buffer, (2) sterilization of tools & medium, (3) cell culture, (4) quantification of cell counts, (5) treatment of cancer cells with extracts, (6) measurement of anticancer activity with MTT Assay, (7) results of observation of this technique vitro anticancer activity test [12].

According to Ngama (2015) in detail the stages of anticancer test are as follows: Anticancer tests were carried out using P388 Leukemia cells on RPMI media in multiwall plates. Cell culture is carried out in sterile conditions. Leukemia cells P388 are maintained with 5 mL of maintenance medium in a 25 cm culture bottle. The maintenance medium was replaced every 2 days and the subculture was carried out when the cell culture had filled 80% of the substrate, then the maintenance medium was removed and the cells were washed with 1.5 mL FBS with three replications. After that the cells were rinsed with 1.5 mL EDTA 0.02%, then the cells were given 1.5 mL of 0.25% trypsin and then the cells were incubated at 37ºC for 2 minutes. After that the base medium containing 50% FBS was added to 1.5 mL. The cell suspension was transferred in a 15 mL Falcon tube, then the cell suspension was centrifuged 1000 rpm for 5 minutes until "cell pellets" were obtained. Furthermore, a 3 mL maintenance medium is added to the "cell pellet", then suspended until homogeneous. Furthermore, cell suspension was divided into two bottles of culture and two new cell cultures were obtained. One (1) mg of methanol extract was added with 1 mL of DMSO (Dimethyl sulfoxide) until

dissolved, the stock of extract solution was used to make variations in concentration. Then the extract concentration variations were made ranging from 0.1 µg / mL, 0.3 µg / mL, 1 µg / mL, 3 µg / mL, 10 µg / mL, 30 µg / mL, and 100 µg / mL. Each extract was entered into P388 Leukemia cell culture. Extracted cells were maintained on a base medium containing 2% FBS and incubated for 24 hours so that the cells adhered to the substrate. Cell growth activity after treatment was measured by giving MTT solution. The medium was removed and given 200 µl base medium containing 2% FBS and 50 µl MTT solution for each well. Cells were given MTT to measure sample cytotoxic effects. Then the cells were incubated for 4 hours at 37ºC with dark conditions. After that, the medium was removed and given 200µl DMSO and 25µl glycine buffer. The intensity of color absorbance is measured using a microplate spectrophotometer (BioRad) at a wavelength of 540 nm. The color absorbance intensity was made to find the IC50 value of extract extract. Measurements were made three replications and each concentration was measured three times. To determine the anticancer activity of extracts of *D. nythesiana*, a calculation was made on the percentage of living cells with the following equation [13]: Percentage of live cells = (A-B) /(C-B) x 100%
Note: A = absorbance of treatment (cell + culture media + sample) B = absorbance of media controls (culture media) C = absorbance of negative control (cell + culture media)

3.3.2 Antioxidant Tests

The test of the antioxidant potential of the methanol extract of Alikokop leaves was carried out at the KOBA ITB Laboratory using the DPPH method. The method used is based on the research of Suhaling (2010), has the following stages: (1) making DPPH solution, (2) Determination of DPPH wavelength (λ) maximum, (3) Measurement of blank absorption, (4) Measurement of antioxidant activity of vitamin C and methanol extract of Alikokop leaves (*D. nythesiana*). In detail the antioxidant test stages carried out are as follows: DPPH powder as much as 40 mg is dissolved with 100 mL of methanol in a measuring flask. The solution is maintained at room temperature, protected from light for immediate use. Then the 10 mL DPPH solution is pipetted into a volumetric flask and then the volume is sufficient to reach 100 mL with methanol, homogenized then left for 30 minutes, then its absorption is measured at a wavelength of 400-800 nm using a UV-Visible spectrophotometer and the DPPH maximum wavelength is 517 nm. Then a blank absorption was measured and an absorbance value of 0.650 was obtained which was used in the antioxidant vitamin C test and the absorbance value was 0.612 to test the antioxidant activity of the methanol extract of Alikokop leaves. Furthermore, the antioxidant activity of vitamin C was carried out by means of 100 mg / 2mL of vitamin C injection dissolved in a volumetric flask with methanol to reach a volume of 50 mL, so that a stock solution with a concentration of 1000 ppm was obtained. From the stock solution each piped 0.1 mL, 0.2 mL, 0.4 mL and 0.8 mL, then added 1 mL DPPH solution and added with 4 mL methanol. So that the concentration of vitamin C 20 ppm, 38 ppm, 74 ppm and 138 ppm was obtained. The mixture is shaken and left for 30 minutes at room temperature. Each of these solutions is measured at a wavelength of 517 nm for three replications.

The measurement of the antioxidant activity of the methanol extract of Alikokop leaves was carried out by means of 50 mg of the sample extract dissolved in a measuring flask with methanol to reach a volume of 50 mL, a stock solution with a concentration of 1000 ppm was obtained. Then from the stock solution each piped 0.1 mL, 0.2 mL, 0.4 mL and 0.8 mL and added 1 mL DPPH solution and 4 mL methanol. So that the concentration of 20 ppm, 38 ppm, 74 ppm and 138 ppm was obtained. The mixture is shaken and left for 30 minutes at room temperature. Each of these solutions is measured at a wavelength of 517 nm as many as 3 replications. After obtaining the absorbance value from each concentration, then the DPPH radical capture activity (% IC) is calculated by the following formula [8]: (Absorbance of the control solution - Absorbance of the test solution)/Absorbance of the control solution X 100%

3.4 Data Analysis

The results of the observation data were tabulated and the IC50 values were then determined to determine the antioxidant and anticancer potential by using regression analysis through the Microsoft Excel application program.

4. Results and Discussion

4.1 Results of Extraction of Leaves of *Dischidia nythesiana*

Making the extract begins with measuring the water content of the sample. Samples of *D. nythesiana* leaves as much as 1000 grams were dried and obtained 86 grams of dry weight. The water content of the samples obtained was 91.4%. Measurement of sample water content is important because it will affect the extraction process, which is to ensure that the active compounds in the cell are mixed with the solvent used [9]. In this study, the extraction method used was the maceration extraction method, by immersing the sample with methanol as much as 1000mL for seven times 24 hours accompanied by a stirring process with a duration of one stirring every six hours. Stirring is done with the aim to flatten the concentration of the solution so that the degree of concentration is maintained [14]. The solvent used is methanol. Besides because methanol is known to be able to attract maximum secondary metabolites in plants, it is also known that methanol is a good solvent for use in antioxidant tests because this solvent does not interfere with DPPH reactions and absorbance measurements [8]. The soaked sample is then filtered and a liquid extract is obtained (Figure 2a), then evaporation is carried out to vaporize the solvent, and then stirring using a waterbath is obtained so that thick maserate is obtained (Figure 2b). The extraction results were then used for antioxidant tests and anticancer tests.

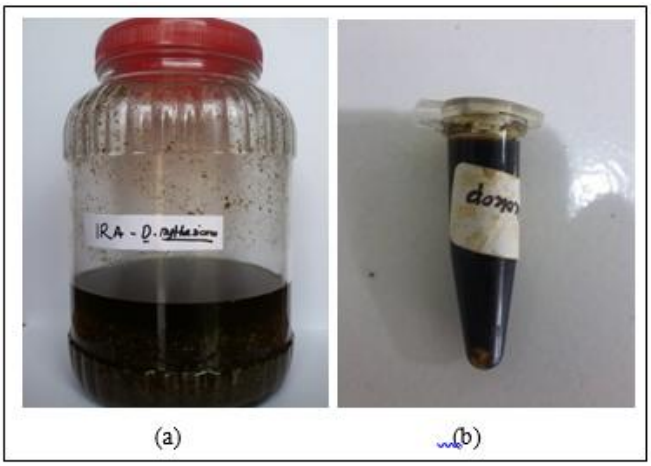
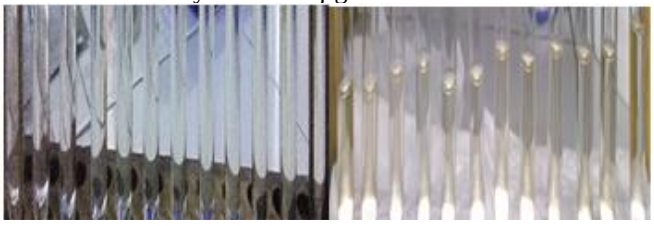


Figure 2: Results of liquid extract (a) and thick extract (b) samples of *D.nythesiana*

4.2 Test the Potential of Antioxidants

Testing of antioxidant activity was carried out using DPPH. The DPPH method was chosen because it is simple, easy, fast and requires only a few samples. This research refers to the theory [15] which states that antioxidant compounds will react with DPPH radicals through the mechanism of donating hydrogen atoms and causing color changes from purple to pale yellow. The absorbance measurements of the samples using UV-Vis spectra with a wavelength of 517 nm with a sample volume of 0.5 mg and DPPH of 0.4 mg. Where the concentration of samples and comparative concentrations used were 20, 38, 74, and 138 ppm. The comparison used as a positive control was vitamin C as much as 200 mg / 2mL. Vitamin C is used as a comparison because it has been shown to have antioxidant activity against DPPH radicals and is commonly used for the determination of antioxidant activity qualitatively and quantitatively [16]. A compound expressed as an antioxidant compound is very strong if the IC50 value <10 µg / mL, is strong if the IC50 value is between 10-50 µg / mL, medium if the IC50 value ranges from 50-100 µg / mL, it is weak if the IC50 value ranges from 100- 250 µg / mL and is not active if IC50 is above 250 µg / mL [17]. The tests performed showed positive results for samples and comparators which can be seen through the change of color from purple to pale yellow (Figure 3a, 3b), but the resulting color is not too yellow. This shows that the sample of Alikokop leaf methanol extract contains compounds that have antioxidant activity, but at the concentrations used, vitamin C is known to have strong antioxidant activity which is 48.552 µg / mL, and methanol extract of Alikokop leaves is known to have weak antioxidant activity ie 67.136 µg / mL.



(a) (b)

Figure 3: Color change of sample extract *D.nythesiana* (a) and comparison solution of vitamin C (b)

The parameters used to determine the magnitude of the ability of compounds as antioxidants are IC50. IC50 value is the concentration of antioxidant compounds needed to reduce DPPH radicals by 50%. IC50 value is obtained from linear regression equation which states the relationship between extract concentration or test fraction as X axis and% radical capture as Y axis. The smaller the IC50 value the more active the extract or fraction as DPPH radical capture compound or antioxidant compound [8]. Measurement of antioxidant activity of methanol extract of Alikokop leaves and vitamin C with DPPH was carried out with three replications. The greater the concentration used, the lower the absorbance value, and the lower the absorbance value obtained, the higher the percentage of inhibition. This proves that the greater the decrease in DPPH absorbance, the stronger the antioxidant activity found in the solution. After the percent inhibition value was obtained, the IC50 calculation was performed using a linear equation on the concentration value with percent inhibition. The best linear regression equation is found in the methanol extract of Alikokop leaves in the second replication which has a confidence level of 99.29%, with a value of Y = 0.0205 and X = 48,623. Similarly, the comparison solution of the linear regression equation is best found in the second replication which has a confidence level of 96.76%, with a value of Y = 0.0347 and X = 48.309. In this study obtained a blank with an absorbance value of 0.650 used in the treatment of vitamin C and 0.612 for Alikokop methanol extract. Blanks are solutions that get the same treatment with samples and comparators but do not contain analates. The purpose of measuring blank absorbance is to know the amount of absorption by non-analytic substances [18] obtained in the methanol extract of Alikokop leaves was lower when compared to the antioxidant activity found in vitamin C. This can be caused by various factors, including because the methanol extract of Alikokop leaves is still a mixture of compounds and it is not known which compounds are antioxidants where the presence of compounds that are not antioxidant is likely to affect the antioxidant activity of the methanol extract of the Alikocop leaf itself. The results of the absorbance measurements, percent inhibition and linear regression equation values of the comparative solution and the test solution for the methanol extract of Alikokop leaves can be seen in Table 1 and Table 2.

Table 1: Results of measurements of absorbance, percent inhibition and linear regression equation values of methanol extract of alikopkop leaves (*Dischidia nythesiana*) and Vitamin C

Sample	Concentration (µg/mL)	Absorbance	%IC	Linier Regression Equation
Blank	-	0,612	-	-
Methanol Extract Replication I	20	0,075	48,945	y=0,0198x + 48,673 (R^2=0,9851)
	38	0,071	49,599	
	74	0,068	50,089	
	138	0,062	51,396	
Extract Methanol Replication II	20	0,075	48,945	y=0,0205x + 48,623 (R^2=0,9929)
	38	0,072	49,435	
	74	0,067	50,252	
	138	0,060	51,396	
Extract Methanol Replication III	20	0,077	48,618	y=0,0212x + 48,575 (R^2=0,9213)
	38	0,070	49,762	
	74	0,067	50,252	
	138	0,060	51,396	

Table 2: Results of measurements of absorbance, percent inhibition and IC50 of the comparative solution and blank

Sample	Concentration (µg/mL)	Absorbance	%IC	Linier Regression Equation
Blank	-	0,650	-	-
Vitamin C Replication I	20	0,102	49,308	Y=0,349x + 48,297 (R^2=0,9181)
	38	0,099	49,769	
	74	0,097	50,077	
	138	0,075	53,462	
Vitamin C Replication II	20	0,103	49,154	Y=0,0347x + 48,309 (R^2=0,9676)
	38	0,099	49,769	
	74	0,095	50,385	
	138	0,076	53,308	
Vitamin C Replication III	20	0,102	49,308	Y=0,0337x + 48,378 (R^2=0,9425)
	38	0,099	49,769	
	74	0,096	50,231	
	138	0,076	53,308	

Based on the results of three replications carried out for each solution with different concentrations, the results showed that the average IC50 value of vitamin C was 48.552 µg / mL with SD = 0.300. The average IC50 value of Alikokop leaf methanol extract was 67.136 µg / mL with SD = 0.084. The smaller the IC50 value of a compound, the stronger the antioxidant activity of the compound because with a small concentration it can have an effect on DPPH [8]. The results of measurement of IC50 vitamin C and methanol extract of Alikokop leaves can be seen in Table 3.

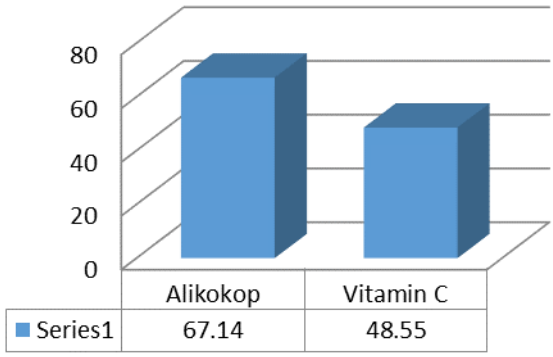
Table 3: Results of IC50 vitamin C and methanol extract of Alikokop leaves

Vitamin C				
Replicassion	IC_{50} (µg/mL)	SD	$\bar{X}$(µg/mL)	$\bar{X}$ ± SD
1	48,796			
2	48,731	0,300	48,552	48,552 ± 0,300
3	48,130			
Methanol extract of alikokop leaves				
Replicassion	IC_{50} (µg/mL)	SD	$\bar{X}$(µg/mL)	$\bar{X}$ ± SD
1	67,020			
2	67,170	0,084	67,136	67,136 ± 0,084
3	67,217			

The results of testing the antioxidant activity carried out at various concentrations showed that the highest concentration of antioxidant activity of vitamin C was still higher when compared with samples of methanol extract of Alikokop leaves (Figure 4). Further research is needed regarding the identification of compounds that act as antioxidant agents in Alikokop leaf extract.

4.3 Anticancer Activity Test with MTT (Micro tetrazolium) Method

The results of the anticancer test in this study aimed to determine the anticancer potential of the methanol extract of Alikokop leaves using the MTT method. The MTT method is a colorimetric method based on changes in tetrazolium salts to formazam in mitochondria which are active in living cells [19]. MTT will be broken down into purple formazan, the number of formazan crystals formed correlates with cell viability [20]. In vitro anticancer tests have been carried out on Leukemia P388 cancer cells [21] and on MmT06054 Mouse Mammary Cancer cells [19]. The anticancer test results of Alikokop leaf methanol extract were determined by calculating the IC50 value, the concentration value which was able to suppress the growth of 50% of the P388 leukemia cell population. Anticancer test results and IC50 values of methanol extract of Alikokop leaves can be seen in Table 4.



4.4 Anticancer Activity Assay with MTT (Microtetrazolium) Method

The results of the anticancer test in this study aimed to determine the anticancer potential of the methanol extract of Alikokop leaves using the MTT method. The MTT method is a colorimetric method based on changes in tetrazolium salts to formazam in mitochondria which are active in living cells (Pandiangan et al., 2008). MTT will be broken down

into purple formazan, the number of formazan crystals formed correlates with cell viability [20]. In vitro anticancer tests have been carried out on Leukemia P388 cancer cells [9,10] and on MmT06054 Mouse Mammary Cancer cells [19]. The anticancer test results of Alikokop leaf methanol extract were determined by calculating the IC50 value, the concentration value which was able to suppress the growth of 50% of the P388 leukemia cell population. Anticancer test results and IC50 values of methanol extract of Alikokop leaves can be seen in Table 4.

Table 4: Anticancer test results and IC50 values of methanol extract of Alikokop leaves against Leukemia P388 cells

Concentration (µg/mL)	*Replication*			*Everage*	*IC_{50} (µg/mL)*
	1	2	3		
100	0,151	0,146	0,147	0,148	
30	0,862	0,665	0,701	0,743	
10	1,697	1,533	1,524	1,585	24,88
3	2,237	1,627	1,541	1,802	
1	1,731	1,659	1,536	1,642	
0,3	2,010	2,341	2,103	2,151	
0,1	1,666	1,576	2,304	1,849	

The results above indicate that the IC50 value of the methanol extract of Alikokop leaves has an anticancer effect on P388 leukemia cells with a barrier concentration value of 24.88 µg / mL (Figure 5). An extract is said to be potential as a chemopreventive agent if the IC50 value is less than 100 ppm [20] and according to [21] the strength of anticancer activity is stated as follows: 1. IC50 5 µg / mL = very active; 2. IC50 5-10 µg / mL = active; 3. IC50 11-30 µg / mL = medium; and 4. IC50 50> 30 µg / mL = inactive.

Based on the provisions of Cho et al., (2009) it can be stated that Alikokop leaf extract has the potential as an anticancer agent with strong IC50 value activity ranging from 11-30 µg / mL which is at a moderate level. Previous research from Ngama et al. (2015) stated that IC50 values of methanol extract of *P.vittata* leaves against P388 leukemia cells were 82.81 µg / mL and were classified as less active extracts. This corroborates the results of the analysis of Alikokop leaf extract which has an IC50 value of 24.88 µg / mL. The greater the IC50 value, the more the anticancer potential decreases [9]. Anticancer activity found in Alikokop leaf extract is probably caused by the presence of secondary metabolites contained in Alikokop plants namely flavonoids, saponins, tannins and steroids which are likely to play an anticancer role [10].

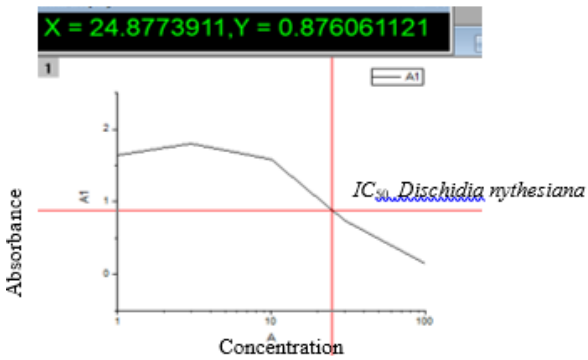


Figure 5. Graph of the logarithmic equation between the average absorbance and the concentration of extract in determining IC50 of Alikokop leaf methanol extract of 24.88 µg / mL

The data generated through this study adds data on the efficacy of natural ingredients that have anticancer effects on leukemia cells. Further research is needed to explain the mechanism of the anticancer effect of the methanol extract of Alikokop leaves on P388 leukemia cells, trace and isolate the active compounds in the methanol extract of Alikokop leaves. Likewise, the implementation in the community still needs further study. Based on the results of testing of antioxidant and anticancer activities that have been carried out, conclusions can be drawn that (1). The methanol extract of Alikokop leaves (D. nythesiana) has the potential as an antioxidant with a moderate concentration of inhibition values, with IC50 of 67.136 µg / mL. (2). Methanol extract of Alikokop leaves (*D. nythesiana*) has the potential as an anticancer agent for P388 leukemia cells with a moderate concentration of inhibition values, with IC50 of 24.88 µg / mL. Further research is needed to find out the active compounds in Alikokop leaf extract which acts as an antioxidant and anticancer, as well as deeper studies for further application in society.

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