Antioxidant and Anticancer Activity Tests of “Pasote” Leaf Water Extracts (*Dysphania ambrosioides* L.) by In Vitro Method in Leukemia Cancer Cells

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Abstract. The purpose of this study was to obtain research information about the anticancer and antioxidant potential of Pasote leaf powder extract. Anticancer testing was carried out by the MTT test method on P388 leukemia cells. The antioxidant test was carried out by the DPPH method and determined by UV-Vis Spectro at a wavelength of 517 nm. Data analysis with the Origin Lab program for anticancer, and Excel programs for antioxidant data analysis. Pasote water extract has anticancer activity potential category as an anticancer with IC50 of 0.105 μg / mL. The results of the antioxidant testing of Pasote water included in the medium category with an IC50 value of 50.736 µg/mL. This shows that Pasote leaf water has the potential to be made antioxidant and is not different from antioxidant vitamin C. The conclusion is Pasote leaf water has the potential to be used as an anticancer and antioxidant.

1. Introduction
Indonesia has the second richest tropical forest area in the world after Brazil and is also referred to as a megabiodiversity area [1]. However, there are still many natural resources in the form of medicinal plants that have not been well managed [2]. One example of the rich diversity of medicinal plant species is *Dysphania ambrosioides* L or Pasote designation in Manado (Figure 1). This plant is believed by parents in North Sulawesi Minahasa to treat diabetes, cholesterol and cancer. Usually used as a mixture of Manado porridge "Tinutuan" in their daily lives. However, when treatment is used, use fresh boiling water. This research wants to prove the water of the dried leaves, especially the anticancer activity of leukemia cells as well as the antioxidant activity which are interrelated.

Anticancer is a drug to prevent and treat the growth of body tissue cells that are not normal. While cancer is a disease caused by the growth of body tissue cells that are not normal [3]. The cells in cancer will develop quickly, uncontrollably and continue to divide, cells will infiltrate and spread through connective tissue, blood, and attack the organs of the body [4]. Antioxidants are compounds that can inhibit or delay the oxidation of a molecule by ending the chain reaction of its initiation and...
distribution [5]. Antioxidants can protect the body from various diseases such as degenerative cancer, and help suppress the aging process [6].

Previous studies have reported that this *Dysphania* genus contains flavonoids, terpenes, pygmol sesquiterpenes, xyloside, coumarins, and essential oils. Biological activity shown from Pasote plants such as: antimicrobial, cytotoxicity, antioxidants, larvicide, antidiabetic, antiparasitic, antiviral, and molluscidal [7]. Information on the antioxidant and anticancer testing of Pasote dried leaf water extract of plants needs to be scientifically proven to be used further. Therefore, in this study, it is necessary to study the anticancer and antioxidant test of *Dysphania ambrosioides* L. *in vitro*.

![Figure 1. The appearance of pasote or sambote (*Dysphania ambrosioides*) morphology. (a) Pasote is still young and (b). Pasote start to flower, (c). Pasote has seeds and high pasote planted in pots](image)

Sulawesi is the fourth largest island in Indonesia which has a unique biodiversity of fauna and flora as a characteristic of the eastern region of the Wallacea line. North Sulawesi Province is located on the Pacific lip which also affects the biodiversity. Plant diversity or biodiversity is the biggest contributor to health and medicine. Therefore Sam ratulangi University has a Research Master Plan (RMP) which is part of its leading topic is Health and Medicine. Topic RIP Topic The element is part of the sub topic leading to the development of herbal medicines.

Minahasa Regency from direct observation has a diversity of plants that are used as medicine. One of them is "Pasote" designation for the Minahasa Kakas tribe or "Sambote" for the Minahasa Tountemboan. This plant is often used by parents in Minahasa who have experienced health problems, especially diabetes and cholesterol. From direct interviews that the plant is also used as a complement to Manado porridge ("tinutuan") as a substitute for basil. According to them the pain of sugar and cholesterol recover after using it regularly. This has become their habit and has been planted around their homes.

The traditional medicinal plant "Pasote or Sambote" is not yet known in general. From the results of the proposer's observation that the plant most at North Sulawesi that has not been identified and is a regional asset for the future. The Minahasa habit tends to eat a lot of meat, of course this plant is a cholesterol-lowering solution that is the main cause of heart disease. Therefore it needs to be developed into an herbal medicine for diabetes and cholesterol which is a trigger for heart disease. Besides that antioxidants are also compounds that can suppress cancer.

Degenerative diseases such as cancer and heart, which are the number one killers, are very frightening to many people, especially to areas where the "B2" and "RW" meat eaters are traditional. Danging eating habits are triggers an increase in cholesterol, fat and gout and free radicals or Ross in the blood. These diseases are triggers for cancer and narrowing of coronary arteries. One should not be surprised if many die suddenly, because they do not know that they have heart disease. Therefore this research is very urgent to be carried out in order to obtain
standardization of traditional medicines that are ready to be used which are typical in North Sulawesi.

Antioxidants are compounds that can inhibit or delay the oxidation of a molecule by ending the chain reaction of its initiation and distribution [5]. Antioxidants can protect the body from various diseases such as degenerative cancer, and help suppress the aging process [6]. Antioxidant testing used the DPPH method (1,1-diphenyl-2-picrylhydrazyl). This method is a method that is fast, simple and does not require a lot of costs [19]. To find out the potential of plants as antioxidants can be calculated using the IC50 parameter [12].

Previous studies have reported this genus to contain flavonoids, terpenes, pygmal sesquiterpenes, xyloside, coumarins, and essential oils. Biological activity shown from Pasote plants such as: antimicrobial, cytotoxicity, antioxidants, larvicide, antidiabetic, antiparasitic, antiviral, and molluscidal [12]. Information on antioxidant testing and the quality of the Pasote plant's dried leaves needs to be scientifically proven to be used further. Therefore, in this study it is necessary to study the antioxidant test and the quality of *Dysphania ambrosioides* L products that have been packaged in the form of tea bags.

The objectives of this study are (1) To determine the antioxidant potential of the Pasote (*Dysphania ambrosioides* L.) dried leaves brewing water. (2) To find out the quality of the product of Pasote (*Dysphania ambrosioides* L.) dried leaf steeping water which has been packed in the dip. The expected outcome is the discovery of new potential antioxidant compounds from pasote plants. Besides the short-term or direct results of the research are scientific articles related to the antioxidant activity of the extract of Pasote leaf steeping water (*Dysphania ambrosioides* L.) and the anticancer test.

2. Experimental

2.1 Extraction

Extraction is done by maceration, which is weighed as much as 2 grams of simplicia is stored in a tea bag to be brewed or soaked in hot water 200 mL in a glass beaker. The sample was soaked for 30 minutes in hot water while it was shaken until the water was cooled. The results of the powder immersion are then used to test anticancer and antioxidant activity.

2.2 Anticancer Activity Assay

Anticancer activity assay (for buffer and media preparation and sterilization), it is carried out in accordance with what ITB Organic Chemistry Laboratory (KOBA) has done. Anticancer test was carried out using P388 leukemia cells. Cells are kept in culture bottles on the RPMI media (Roswell Park Memorial Institute) in a multiwell plate. Cell culture is carried out in sterile conditions. Cell culture was maintained until it filled 80% of the substrate. Subcultures were carried out following the method used by Alley (1988) [8]. Cell culture that has fulfilled 80% of the substrate is associated. Dissociation was carried out by washing cell cultures with FBS (Fetal Bovine Serum) 3 times, then rinsed with EDTA 0.02% and given trypsin 0.25%. Cells were incubated at 37°C in a CO₂ incubator for 2 minutes until the cells detached from the culture bottle substrate. Cell suspension was added with a maintenance medium containing 5% FBS (Fetal Bovine Serum) with a volume ratio of 1: 1. The centrifugation supernatant is removed and the cell pellet is given a maintenance medium. Live cells are counted using an Improved Neubauer hemocytometer with the calculation formula according to Freshney [9]. One (1) mg of dried leaf water extract is added to 1 mL of DMSO (Dimethyl sulfoxide) until it dissolves as a stock of extract solution to vary the concentration. Then variations in extract concentrations start from 0.1; 0.3; 1; 3; 10; 30 and 100 μg / mL. Each extract was put into P388 leukemia cell culture. Cells that contain extracts are maintained in a medium containing 2% FBS and incubated for 24 hours so that the cells adhere to the substrate (KOBA ITB). Cell growth activity after MTT administration (3- (4,5-dimethylthiazol-2-il) 2,5-
diphenyltetrazolium bromide). The medium was removed and given 200 μL of basic medium containing 2% FBS (Fetal Bovine Serum) and 50 μL for MTT for each well. Select MTT to measure the cytotoxic effects of the sample. Cells were incubated for 4 hours at 37°C under dark conditions. After that, the medium is removed and given 200 μL DMSO (Dimethyl sulfoxide) and 25 μL glycine buffer. The intensity of the color absorbance was replaced using a microplate spectrophotometer (Bio Rad) at a wavelength of 540 nm. The intensity of the color absorbance was made to find the inhibitory concentration value of 50% (IC$_{50}$) of the Pasote leaf extract. Measurements were made 3 times from each concentration, each concentration was repeated three times (KOBA ITB).

2.3 Antioxidant Activity Test with DPPH Method

Determination of IC$_{50}$ from Pasote (sample) and vitamin C (standard) water extracts was carried out by the free radical reduction method using DPPH (1,1-diphenyl-2-picrylhydrazyl) with UV-Visible spectrophotometry. The procedure performed in testing antioxidant activity with the DPPH method is as follows: 1. Making DPPH solution DPPH powder of 0.4 mg was dissolved with 150 mL methanol in Erlenmeyer at room temperature and protected from light. 2. Placement of wavelength (λ) maximum DPPH, DPPH 10 mL solution pipetted into a measuring flask. Methanol is added until the volume reaches 100 mL is homogenized and allowed to stand for 30 minutes then the absorption is measured at a wavelength of 400-800 nm using UV-Visible spectrophotometry and the maximum wavelength of DPPH is 517 nm. 3. Measurement of the antioxidant activity of vitamin C This study as a standard that is, vitamin C (Ascorbic acid) 100 mg in 2 mL. Vitamin C is dissolved with methanol until the volume is 50 mL obtained stock solution with a concentration of 1000 ppm. Some volumes made of 0.1, 0.2, 0.4 and 0.8 mL are added to the volumetric flask. Next 1 mL DPPH was added to the volumetric flask containing vitamin C. Methanol was added until the volume reached 5 mL beaten and allowed to stand for 30 minutes at room temperature. Each of these solutions was measured at absorption at a wavelength of 517 nm, carried out by three replications. 4. Measurement of Pasote antioxidant activity Pasote extract weighed as much as 50 mg and then dissolved with methanol as much as 50 mL obtained stock solution with a concentration of 1000 mg / mL. Pipettes of 0.1, 0.2, 0.4 and 0.8 mL were added to the volumetric flask. Next 1 mL DPPH was added to the volumetric flask containing the extract added with methanol until the volume reached 5 mL. The solution was shaken until homogeneous and allowed to stand for 30 minutes at room temperature and then measured its absorption at a wavelength of 517 nm, replicated three times [10].

2.4 Data analysis

The potential of anticancer and antioxidant activity of Pasote leaf extract can be determined by conducting IC$_{50}$ (Inhibition Concentration 50) assay to test the potential of anticancer and antioxidants using regression analysis with the Origin Lab application program. Analysis of antioxidant data using the Excel program in Windows Program.

3. Results and Discussion

3.1 The Result of simpilisia and extraction of pasote leaves (Dysphania ambrosioides L.)

The results of extraction of Pasote leaves obtained fresh weight of 1500 grams, the leaves obtained were dried using an oven for 1 day and obtained 117 grams of dry weight with 91.6% moisture content. Leaf samples mixed with water were evaporated to produce 21.77 grams of dried leaf extract. The results obtained in this study were crude extracts, with a water yield value of 17.34%.
3.2 Anticancer activity assay

This study uses leukemia cancer cells (P388) which can be used in research to determine the toxicity of an extract against leukemia cancer cells *in vitro*. Anticancer testing using the MTT method (3-(4,5-dimethylthiazol-2-il)-2,5-diphenyltetrazolium bromide) which is purple. According to Meiyanto [11], the more concentrated purple in an experiment, the higher the absorbance value and is directly proportional to the number of living cells. Conversely, the fading purple (yellow) in an experiment, the lower the absorbance value and is directly proportional to the number of dead cells. This shows the effect of giving pasote water extract to P388 cells. Anticancer testing has six concentrations of 100, 30, 10, 3, 1, 0.3 and 0.1 ppm (Table 1). Anticancer testing was conducted to determine the anticancer activity of pasote extract on cancer cells using the IC\(_{50}\) parameter, it can be seen the potential of plants as anticancer [12]. The results of anticancer testing of Pasote water extract in this study obtained IC\(_{50}\) values of 0.105 μg/mL (Fig. 2). Previous research by [13] stated that the IC\(_{50}\) value of the *P. vittata* methanol extract was 82.81 μm/mL. These results can be said that Pasote water extract has more potential anticancer as an anticancer. According to [14], the cytotoxicity of an extract based on its IC\(_{50}\) value was classified into 3 namely: Potential cytotoxicity (IC\(_{50}\) <100 μg/L), moderate cytotoxicity (IC\(_{50}\) <1000 μg/L) and low (IC\(_{50}\) > 1000 μg/L).

| Concentration (ppm) | P1     | P2     | P3     | Average |
|---------------------|--------|--------|--------|---------|
| 100                 | 2.992  | 1.712  | 2.845  | 2.516   |
| 30                  | 0.446  | 0.469  | 0.939  | 0.618   |
| 10                  | 0.324  | 0.21   | 0.390  | 0.308   |
| 3                   | 0.285  | 0.06   | 0.449  | 0.265   |
| 1                   | 0.395  | 0.339  | 0.383  | 0.372   |
| 0.3                 | 0.523  | 0.433  | 0.736  | 0.564   |
| 0.1                 | 0.497  | 0.608  | 0.608  | 0.571   |

*Figure 2.* Anticancer testing, Determination of the concentration of 50% (IC\(_{50}\)) P388 anticancer by MTT test with Regression analysis uses Origin Lab of 0.105 μg/mL.
3.3 Antioxidant Testing

The results of the antioxidant testing of Pasote leaf extract in each concentration of color changes gradually where purple turns yellow. The change in purple to yellow in the antioxidant activity test indicates 50% inhibition of free radical activity. The interaction of antioxidants with DPPH by electron transfer or hydrogen radical in DPPH, will neutralize the free radical character of DPPH and form a reduced PPH. If all of the electrons in the DPPH free radical become pairs, the color of the solution changes from dark purple to bright yellow and the absorbance at the 517 nm wavelength will be lost [15]. The absorbance measurement data was analyzed by the percentage of inhibitory activity using the following equation:

\[
\frac{A_c - A_s}{A_c} \times 100\% \quad \text{........................................... (1)}
\]

Where \(A_c\) is absorbance control, and \(A_s\) is absorbance sample. Replication 1 is obtained by the linear regression equation \(Y = 0.0468X + 44.338 \quad R^2 = 0.9521\). Replication 2 obtained the linear regression equation \(Y = 0.0755X + 40.56 \quad R^2 = 0.9938\). Replication 3 obtained the linear regression equation which is \(Y = 0.0432X + 44.815 \quad R^2 = 0.7782\) (Table 1). R value approaching +1 (positive value) illustrates that with increasing concentration of extract, the greater antioxidant activity can be seen from the curve of the relationship of concentration of pasote leaf water extract to percent inhibition or percent antioxidant [5].

| Replication | Absorbance Value | % Inhibition | Average of Inhibition | Linear Regression | IC 50  |
|-------------|------------------|--------------|----------------------|-------------------|-------|
| (µg/mL)     |                  |              |                      |                   |       |
| 0           | 0.650            | 0            | 0                    | -                 | -     |
| 1           | 0.126            | 45.615       |                      | y = 0.0468x + 44.338 \(R^2 = 0.9521\) | 53.37 |
|             | 0.122            | 46.231       | 47.500               | y = 0.0755x + 40.56 \(R^2 = 0.9938\) | 50.736|
|             | 0.117            | 47.000       |                      |                   |       |
|             | 0.090            | 51.154       |                      |                   |       |
| 2           | 0.147            | 51.154       |                      |                   |       |
|             | 0.143            | 42.385       | 45.654               |                   |       |
|             | 0.122            | 43.000       |                      |                   |       |
|             | 0.091            | 51.000       |                      |                   |       |
| 3           | 0.134            | 46.231       |                      |                   | 50.013|
|             | 0.116            | 46.231       |                      |                   |       |
|             | 0.102            | 44.385       | 47.731               | y = 0.0432x + 44.815 \(R^2 = 0.7782\) |       |
|             | 0.097            | 50.077       |                      |                   |       |

This study uses vitamin C injections of 100 mg/2 mL as a comparison compound. Vitamin C is a water-soluble antioxidant, the use of vitamin C as a comparison in testing the antioxidant activity is to find out how strong the antioxidant potential is in the extract of pasote leaf water when compared with vitamin C.
The calculation results obtained IC\textsubscript{50} value of pasote water extract was 122.013 μg/mL including the moderate category as an antioxidant, for IC\textsubscript{50} value of vitamin C 48.552 μg/mL was included in the very strong category because <50 μg/mL. Previous research from [16], antioxidant activity test and phytochemical screening of pokoasi and kluwih leaf extracts as a source of natural antioxidants with IC\textsubscript{50} vitamin C values of 46.74 μg/mL and IC\textsubscript{50} values of pokoasi leaf extract and kluwih respectively also 89,659 μg/mL and 54,719 μg/mL where vitamin C used has higher antioxidant activity compared to extracts. The results of this study can be seen from the results of IC\textsubscript{50} active pasote leaves and potential as antioxidants, from the IC\textsubscript{50} value where the level of antioxidant strength of the compound is very strong if IC\textsubscript{50} <50 μg/mL, IC\textsubscript{50} strength is 50-100 μg/mL, while IC\textsubscript{50} is 101-150 μg/mL, weak IC\textsubscript{50} > 150 μg/mL. The smaller the IC\textsubscript{50} value, the higher its antioxidant activity [17]. The cause of the antioxidant activity of pasote water extract is lower than vitamin C is still in pasote water extract in the form of impure extract.

| Replication | Concentration (µg/mL) | Absorban Value | % Inhibition | Linear Regretion | % Equally |
|-------------|-----------------------|----------------|--------------|-----------------|-----------|
| Blanko      | 0                     | 0,650          | 0            |                 | 0         |
|             | 20                    | 0,102          | 49,308       |                 |           |
|             | 38                    | 0,099          | 49,769       | Y = 0,0349X + 48,297 |           |
|             | 74                    | 0,097          | 50,077       | R\textsuperscript{2} = 0,9181   |           |
|             | 138                   | 0,075          | 53,462       |                 |           |
|             | 20                    | 0,103          | 49,154       |                 |           |
| 1           | 38                    | 0,099          | 49,769       | Y = 0,0347X + 48,309 |           |
|             | 74                    | 0,095          | 50,385       | R\textsuperscript{2} = 0,9676   |           |
|             | 138                   | 0,076          | 53,308       |                 |           |
|             | 20                    | 0,102          | 49,308       |                 |           |
| 2           | 38                    | 0,099          | 49,769       | Y = 0,0337X + 48,378 |           |
|             | 74                    | 0,096          | 50,231       | R\textsuperscript{2} = 0,9425   |           |
|             | 138                   | 0,076          | 53,308       |                 |           |

4. Conclusion

The water extract of Pasote (D. ambrosioides) leaves has an IC\textsubscript{50} value of 50.736 µg/mL with the DPPH method which has potential as an antioxidant. P388 leukemia anticancer activity test results of water extract Pasote using the MTT Assay method obtained IC\textsubscript{50} of 0.105 µg/mL has the potential to be very strong as an anticancer.

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