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# PRODUCT QUALITY TEST OF PASOTE TEA BAGS LEAVES PASOTE (*Dysphania ambrosioides*): COMPARISON OF ANTIOXIDANT ACTIVITIES OF WATER EXTRACT WITH ACETONE EXTRACT

**Dingse Pandiangan**
Department of Biology, Faculty Mathematics and Natural Sciences, Sam Ratulangi University, Manado, North Sulawesi, Indonesia

**Paulina Veronika Yolanda Lamlean**
Study Program of Pharmacy, Faculty Mathematics and Natural Sciences, Sam Ratulangi University, Manado, North Sulawesi, Indonesia

**Nelson Nainggolan**
Department of Mathematics, Faculty Mathematics and Natural Sciences, Sam Ratulangi University, Manado, North Sulawesi, Indonesia

**ABSTRACT**

***Pasote (Dysphania ambrosioides L.) is a natural medicinal plant that is well known in Indonesia, especially the people of North Sulawesi. This plant is known to contain flavonoids, terpenes, sesquiterpenes, pigmol, xylosides, coumarin and essential oils. Pasote plants are also known to have biological activities such as antimicrobial, cytoxicity, antioxidants, larvacides, antidiabetic, antiparasitic, antiviral and molluscidal. In this study, the extraction of pasote leaves with the maceration method using water and acetone solvents was carried out in order to determine the best type of solvent that can produce pasote leaf extract with the highest antioxidant activity. Determination of antioxidant activity was carried out using DPPH on a UV Vis Spectrophotometer at a wavelength of 517 nm. The results showed that pasote leaf extract with acetone solvent had a percent inhibition (% IC) of 38.77% and pasote leaf extract with water as a solvent had an inhibition percentage of 46.96%. This shows that the best solvent that can extract antioxidant compounds in pasote leaves is water. The $IC_{50}$ acetone extract pasote leaves value 9.7 µg/mL. The water extract of pasote leaves had the most effective antioxidant activity or inhibitory power with an $IC_{50}$ value of 1.32 µg / mL. So the water extract of pasote leaves is more active than the acetone extract of pasote leaves. For further activities, including production in industry, it is better to use water extract only.***

## INTRODUCTION

Medicinal properties of natural plants have long been recognized by the Indonesian people for hundreds of years. It is estimated that Indonesian forests hold the potential for medicinal plants as many as 30,000 species, of which 940 species have been declared medicinal, of which around 78% are still obtained through direct extraction from the forest (Nurrani, 2013).

One of the natural medicinal plants which is well known in Indonesia, especially the people of North Sulawesi, is the Pasote plant (Dysphania ambrosioides L.) (Figure 1.). The genus *Dysphania* is known to contain flavonoids, terpenes, sesquiterpenes, pigmol, xylosides, coumarin and essential oils. Pasote plants are also known to have biological activities such as antimicrobials, cytoxicity, antioxidants, larvacides, antidiabetic, antiparasitic, antiviral and molluscidal (Ghareeb et al., 2016). Extraction of compounds and to determine the presence of biological activity in pasote plants can be done through an extraction process. To get a thorough extraction and obtain compounds that have pharmacological activity, the selection of the solvent used for extraction is an important factor (Arifianti et al., 2014).


**Figure 1.** Pasote (*D. ambrosioides*) 2 weeks old, used as the sample and Pasote tea bag (A) Pasote which had been harvested and dried (B).

The ideal solvent that is often used is alcohol or its mixture with water because it is the best extracting solvent for almost all low molecular weight compounds such as saponins and flavonoids (Wijesekera, 1991). In a study conducted by Maningkas et al., (2019) it was found that pasote leaves extracted using methanol solvent had a strong category of anticancer activity with an $IC_{50}$ value of 53.37 µg / mL and a strong antioxidant activity with an $IC_{50}$ value of 50.13 µg / mL.

In this study, the extraction of pasote leaves by maceration method using water and acetone as a solvent. Water is considered a solvent because it is easy to obtain, cheap, non-toxic, non-volatile and non-flammable. Meanwhile, acetone is considered a solvent because it is a well-known compound in the manufacture of pharmaceuticals and other chemical compounds (Wade, 2006). Until now, it is not known what solvent is best for extracting antioxidant compounds in pasote leaves, therefore it is necessary to to examine the anti-cholesterol test and improve the quality of *Dysphania ambrosioides* L products that have been packaged in tea bags against Wistar rats with improved taste with the addition of brown sugar and presentation.

## METHODS

### 1. Place and time of research

This research was conducted at the Biovina Laboratory of the Biovina Herbal Sea Mitra Pineleng Minahasa North Sulawesi. The time of research from Juni 2020 to Agustus 2020.

### 2. Materials and tools

The materials used in this study were *D. ambrosioides* L. leaf samples (Figure 1), methanol p.a, acetone, water, 1,1-diphenyl-2-picrylhydrazyl (DPPH) crystals p.a (Sigma-Aldrich material code No. D9132-1G). The tools used in this research include analytical scales, ovens, glass, knives, scissors, plastic containers, blenders, rags, label paper, stirring spoons, cloth, tea bags, measuring cups, aluminum foil, erlenmeyer flasks. , stir bar, micropipette, blue and yellow tip, cuvette, label paper, small test tube, tube rack, computer, and UV-Vis spectophotometer.

### 3. Pasote leaf powder making

Pasote leaves cleaned with water to remove dirt and drained. Furthermore, the leaves of pasote are sliced into small pieces and weighed as much as 1000 grams. The leaves are then oven-dried at 40ºC until they reach a constant weight. The dried leaf sample is then mashed using a blender. The results of the blender are sifted twice until a fine powder of pasote leaves is obtained.

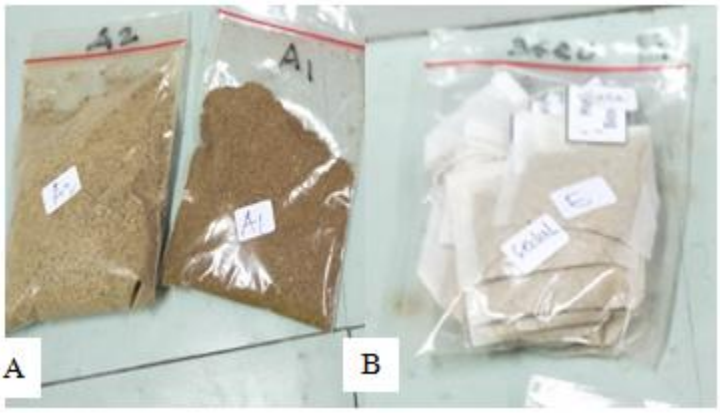


**Figure 2.** Pasote dry leaf powder (A), Pasote leaf tea in a tea bag (B).

**4. Pasote Leaf Extraction**

Extraction of the leaves begins with weighing two grams of powder each of the leaves, then put it in a tea bag and then dissolve it with 200 ml of water and acetone, then macerated for 30 minutes. The solution obtained was then analyzed for its antioxidant activity.

**5. Parameters Observed**

The parameters observed in this study were % Inhibition (% IC) and $IC_{50}$ (Inhibition Concentration 50%) which is a description of antioxidant activity using the DPPH method (Manggribeth et al., 2019; Maningkas et al., 2019; Suhaling, 2010).

**6. Antioxidant test**

Antioxidant test conducted on each of the results of the leaf extract includes:

6.1 Preparation of DPPH Solution. A total of 40 mg of DPPH powder was dissolved with 100 mL of methanol in a volumetric flask. The solution is kept at room temperature and protected from light. Determination of the Maximum Wavelength of DPPH: The DPPH solution of 10 mL is piped into a volumetric flask and then the volume is added to reach 100 mL with methanol, homogenized and then left for 30 minutes, then the absorption is measured at a wavelength of 400-800 nm using a UV-Visible spectrophotometer.

6.2 Blank Absorption Measurement. Blank absorption can be determined by taking the DPPH solution that has been prepared and put into a cuvet and then measured using a UV-Visible spectrophotomer. The absorption results obtained are then used as the absorbance value of the blank in the antioxidant activity test.

6.3 Measurement of the Antioxidant Activity of Pasote Leaf Extract. The Pasote leaf extract solution from each of the solvent variants (water and acetone) was pipette 0.1 mL, 0.2 mL, 0.4 mL, and 0.8 mL, then added 1 mL of DPPH solution and 4 mL. The mixture was shaken and left for 30 minutes at room temperature. Each solution was measured using a UV-Visible spectrophotomer with a wavelength of 517 nm with two replications. After obtaining the absorbance value of each concentration, the DPPH radical scavenging activity (% IC) is calculated using the following formula (Manggribeth et al., 2019; Maningkas et al., 2019; Sadeli, 2016) as follows:

$$\frac{(\boldsymbol{AC} - \boldsymbol{AS})}{\boldsymbol{AC}} \times \mathbf{100}\ \%$$

**Figure 3. DPPH radical scavenging activity (% IC) formula***

To determine the $IC_{50}$, a graph of the relationship between inhibitor concentration and percent inhibition was made, then a regression equation was made from the graph and the $IC_{50}$ value was calculated (Al-Ash'ary et al., 2010; Manggribeth et al., 2019; Maningkas et al., 2019).

## RESULTS AND DISCUSSION

### Pasote Leaf Extraction Results

The extraction that is done is extraction by maceration method. The Pasote leaf powder that has been put into a tea bag as much as two grams per bag is soaked in water and acetone as a solvent with 200 mL of each solvent for 30 minutes, so that the extract obtained is an extract in liquid form. It is known that each extract solution has a concentration of 10000 µg / mL with a final concentration of 196 µg / mL in tube 1, 384 µg / mL in tube 2, 754 µg / mL in tube 3 and 1481 µg / mL in tube 4. Calculation of the concentration of the final solution is obtained by using the initial concentration of the solution (C1) multiplied by the initial volume of the solution (V1) and divided by the final volume of the solution (V2). The calculation of the initial concentration of each extract solution can be seen in Figure 4 and the calculation of the final concentration of the extract solution can be seen in Table 1.

$$\frac{\text{Mass}}{\text{Volume}} = \frac{2000\ \text{mg}}{200\ \text{mL}} = 10\text{mg/mL}$$

**Note**: $10\text{mg/mL} = 10000\ \mu\text{g/mL}$

**Figure 4. Formula for the initial concentration of each extract solution.**

**Table 1. Calculation of the final concentration of Pasote leaf extract in each solvent**

| Tube | V1 (mL) | C1 (µg/mL) | V2 (mL) | C2 (µg/mL) |
|---|---|---|---|---|
| *A* | 0 | 0 | 5 | 0 |
| *B* | 0,1 | 10000 | 5,1 | 196 |
| *C* | 0,2 | 10000 | 5,2 | 384 |
| *D* | 0,4 | 10000 | 5,3 | 754 |
| *E* | 0,8 | 10000 | 5,4 | 1481 |

### Results Of Determination Of Percent Inhibition Antioxidants From Leaves Of Pasote (*D. ambrosioide*s)

The macerated liquid extract of Pasote leaves obtained two types of extract solutions, namely aqueous extract solutions and acetone extracts from the Pasote leaves. From each variant of the extract solution, its antioxidant activity was tested against DPPH. The process of testing for antioxidant activity or inhibition was carried out using a visible spectrophotometer with a wavelength of 517 nm. The use of a visible spectrophotometer is because testing with this method is carried out based on the absorption of visible light against a colored solution (Al-Ash'ary et al., 2010; Manggribeth et al., 2019; Maningkas et al., 2019).

The reaction between the pasote extract of the two types of solvents against DPPH resulted in a purple to yellowish compound which degraded according to its antioxidant activity (Figure 5). With the appearance of this color reaction, this test can be carried out using a visible spectrophotometer. The darker the color, the lower the inhibitory activity of DPPH, and vice versa, the brighter to the yellowish color, the higher the inhibitory activity of DPPH, in other words, the higher the antioxidant activity.

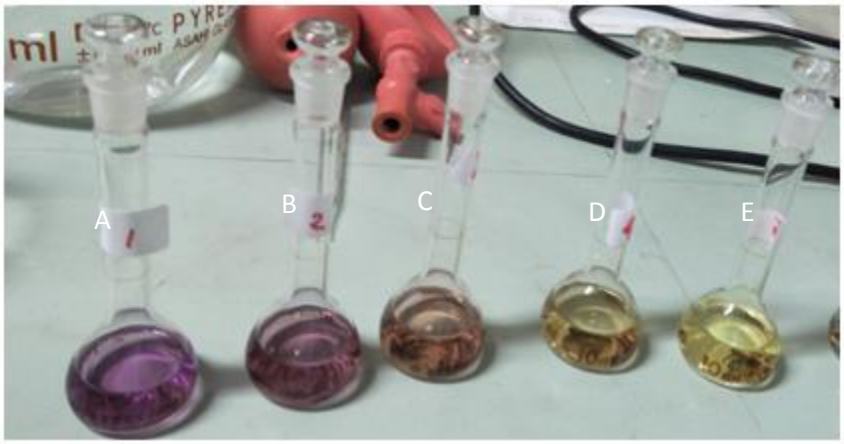


**Figure 5.** Qualitative observation of DPPH solution with sample extract as an indicator of antioxidant activity. (A) is a blank, (B) is a sample concentration of 0.1 (C) is 0.2 (D) is 0.4 (E) is 0.8 mL.

The darker the color, the lower the inhibitory activity of DPPH, and vice versa, the brighter to the yellowish color, the higher the inhibitory activity of DPPH, in other words, the higher the antioxidant activity. From the two variants tested, it was seen that at the same concentration the type of water solvent had better inhibitory power than the acetone solvent. With an initial sample concentration of 10000 µg/mL, acetone solution had percent inhibition (% IC) of 38.77% and water solution had percent inhibition of 46.96%. The percentage of inhibition of water is greater than the percentage of inhibition of acetone. This means that the water-soluble pasote extract contains bioactive compounds that can inhibit DPPH better than the acetone-solvent extract of pasote. This shows that the water compounds have polarity properties that are relatively the same as the compounds found in Pasote leaves. So it can be concluded that the antioxidant compounds that act as DPPH inhibitors from Pasote leaves can be extracted well if using a water solvent.

**$IC_{50}$ Results of Pasote Leaf Water Extract in Inhibiting DPPH**

After it was known that the water extract of Pasote leaves had the best inhibitory ability, then the $IC_{50}$ was determined from the extract. $IC_{50}$ or 50% Inhibitor Concentration is a concentration of inhibitors that can inhibit enzyme activity by as much as 50%. A compound is known to be a very strong antioxidant compound if the $IC_{50}$ value is <10 µg / mL, strong if the $IC_{50}$ value ranges from 10-50 µg / mL, while if the $IC_{50}$ value ranges from 50-100 µg / mL, it is weak if the $IC_{50}$ value ranges between 100-250 µg / mL and is inactive when the $IC_{50}$ value is above 250 µg / mL[7]. To obtain the $IC_{50}$ value from the water extract of Pasote leaves, linear regression calculations were carried out at several concentrations of 196 µg / mL, 384 µg / mL, 754 µg / mL and 1481 µg / mL against the percent inhibition. The results obtained can be seen in Table 2 and Fugure 5.

**Tabel 2. Test results of pasote leaf brew water extract**

| Replication | Inhibitor Concentration (µg/mL) | Percent Inhibition |
|---|---|---|
| *I* | 196 | 45,62 |
| | 384 | 46,23 |
| | 754 | 47,00 |
| | 1481 | 51,15 |
| | 196 | 42,38 |

| | | |
|---|---|---|
| *II* | 384<br>754<br>1481 | 43,00<br>46,23<br>51,00 |

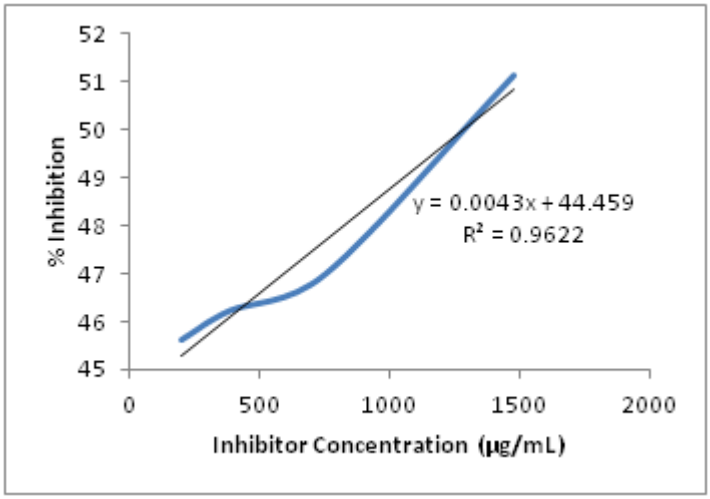


**Figure 6.** Graph of the relationship between concentration of inhibitor and percent of inhibition in replication I leaves water extract

The data in Table 2 is converted into a graph of the relationship between inhibitor concentration and percent inhibition of leaves Pasote water extract. This graph can be seen in Figure 6 and Figure 7. From the graph of the relationship between inhibitor concentration and percent inhibition obtained at each replication, the calculation is then carried out using the regression equation formula $IC_{50} = Y + bX$, $Y = IC_{50} = 50$. The results of the calculation of $IC_{50}$ for Pasote leaf water extract based on the regression equation formula can be seen in Table 3.

**Table 3. $IC_{50}$ calculation results based on the regression equation**

| Replication | Linear Equation | $IC_{50}$ | SD | X |
|---|---|---|---|---|
| *I* | Y=0.0043X+44.459<br>(r=0.96) | 1,29 µg/mL | 0,04 | 1,32 µg/mL |
| *II* | Y=0.0069X+40.782<br>(r=0.99) | 1,34 µg/mL | 0,04 | 1,32 µg/mL |

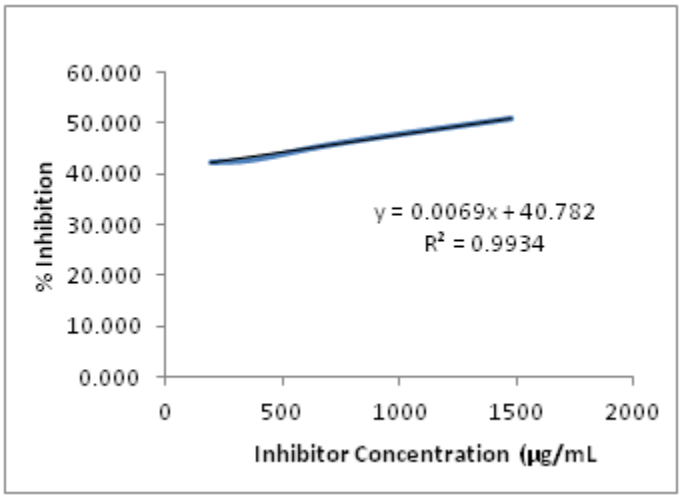


**Figure 7.** Graph of the Relationship Between Concentration of Inhibitor and Percent of Inhibition in Replication II of Pasote leaves water extract

The results of the calculation of the linear regression equation showed that the $IC_{50}$ value of the water extract of the leaves was 1.32 µg/mL. Knowing that the $IC_{50}$ value in the water extract of Pasote leaves is 1.32 µg/mL, indicating that the antioxidant compounds in the water extract of Pasote leaves have a very strong inhibitory power against DPPH. Based on the results of the research that has been obtained, it can be concluded that the best solvent that can extract antioxidant compounds in pasote leaves is water, and water extract of pasote leaves has the most effective antioxidant activity or inhibitory power with an $IC_{50}$ value of 1.32 µg/mL.

**$IC_{50}$ Results of Pasote Leaf Acetone Extract in Inhibiting DPPH**

The data in Table 4 is converted into a graph of the relationship between inhibitor concentration and percent inhibition of leaves Pasote acetone extract. From the graph of the relationship between inhibitor concentration and percent inhibition obtained at each replication, the calculation is then carried out using the regression equation formula $IC_{50} = Y + bX$, $Y = IC_{50} = 50$. The results of the calculation of $IC_{50}$ for Pasote leaf acetone extract based on the regression equation formula can be seen in Table 5.

**Tabel 4. Test results of pasote leaf acetone extract**

| Replication | Inhibitor Concentration (µg/mL) | Percent Inhibition |
|---|---|---|
| *I* | 196 | 31.31 |
| | 384 | 38.69 |
| | 754 | 38.54 |
| | 1481 | 44.39 |
| *II* | 196 | 35.46 |
| | 384 | 36.39 |
| | 754 | 40.08 |
| | 1481 | 44.23 |

**Table 5. $IC_{50}$ calculation results based on the regression equation acetone extract**

| Replication | Linear Equation | $IC_{50}$ | SD | X |
|---|---|---|---|---|
| *I* | Y=0.0084X+32.286 (r=0.90) | 17.27 µg/mL | 1.05 | 9.77 µg/mL |
| *II* | Y=0.007X+34.123 (r=0.99) | 2.27 µg/mL | 0.77 | 9.77 µg/mL |

The results of the calculation of the linear regression equation showed that the $IC_{50}$ value of the water extract of the leaves was 9.77 µg/mL. Knowing that the $IC_{50}$ value in the aceton extract of Pasote leaves is 9.77 µg/mL, indicating that the antioxidant compounds in the aceton extract of Pasote leaves have a very strong inhibitory power against DPPH. Based on the results of the research that has been obtained, it can be concluded that the best solvent that can extract antioxidant compounds in pasote leaves is water, and water extract of Pasote leaves has the most effective antioxidant activity or inhibitory power with an $IC_{50}$ value of 9.77 µg/mL more than water extract.

The discussion of these results indicates that the antioxidant activity of water extract and acetone leaves of pasote is different. The smaller $IC_{50}$ is water extract than acetone. Therefore, for the next experiment is to use the extract of water from the leaves of the pasote, either for production purposes or for future use. Based on the results of the research that has been obtained, it can be concluded that the best solvent that can extract antioxidant compounds in pasote leaves is water. The water extract of pasote leaves had the most effective antioxidant activity or inhibitory power with an IC50 value of 1.32 ug / mL.

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