WWW.IJRPR.COM

International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Determination of Total Flavonoid Content from Ethanol Extract and Various Paliasa (*KleinhoviaHospita*L.) Leaf Fractions on its Potential as an Antioxidant

***Boy Chandra* [1], *Novita Sari* [1], *Zikra Azizah* [1], *Sestry Misfadhila* [1]**

[1]School of Pharmaceutical Science Padang (STIFARM Padang), West Sumatera, Indonesia, 25147
DOI: https://doi.org/10.55248/gengpi.2023.4131

ABSTRACT

Paliasa (*Kleinhoiviahospita*L.*)* is a plant of the Sterculiaceae family which is known to have potential as an antioxidant. This study aims to determine the total flavonoid content of ethanol extract and various fractions of paliasa leaves on their potential as antioxidants. Samples were macerated with 70% ethanol for 24 hours at room temperature. The ethanol extract obtained was then evaporated at 50 ^{o}C to produce a viscous extract which was then fractionated using *n* -hexane, dichloromethane and water as solvents. The content of chemical compounds from the ethanol extract of paliasa leaves are alkaloids, flavonoids, saponins, phenols and tannins. The content of chemical compounds from the *n* -hexane fraction is tannins. The content of chemical compounds from the dichloromethane fraction are flavonoids, phenols and tannins. The content of chemical compounds from the water fraction are alkaloids, saponins, phenols and tannins. Tests for total flavonoid compounds found in the ethanol extract and dichloromethane fraction using rutin as a comparison at λ max 419 nm were 3.10% and 3.73%. Antioxidant activity testing using the *1,1-Diphenyl-2-Picrilhydrazil* (DPPH *) method* showed that the ethanol extract and fraction (*n* -hexane, dichloromethane and water) had an IC of $_{50}$ 166.55 µg/mL, 351.19 µg/mL, 85 .87 µg/mL, and 226.52 µg/mL, while gallic acid comparator has a very strong antioxidant activity with an IC50 value of $_{10.75}$ µg/mL.

Keywords : Paliasa leaves (*Kleinhoviahospita*L.) , flavonoids , antioxidants .

ABSTRACT CT

Paliasa(*Kleinhoiviahospita L* .) is a plant family of Sterculiaceae which has antioxidant potential. This research aims to determine the total levels of flavonoids of ethanol extract and various Paliasa leaf fractions toward its potential as an antioxidant. Samples were macerated by 70 % ethanol solvent for 24 hours at room temperature. Then, the ethanol extract obtained was evaporated at 50 ^{o} C to produce a thick extract which was fractionated by using some solvents such as *n* - hexane, dichloromethane, and water. The content of chemical compounds from ethanol extract of paliasa leaves are alkaloids, flavonoids, saponins, phenols, and tannins. The chemical compound of the *n* -hexane fraction is tannin. The content of chemical compounds from the dichloromethane fraction are flavonoids, phenols and tannins. The content of chemical compounds from the water fraction is alkaloids, saponins, phenols, and tannins. Flavonoid testing of the total compounds found in ethanol extract and fraction was dichloromethaned using routine as a comparison at λ max 419 nm was 3.10 % and 3.73 %. The testing of antioxidant activity used DPPH method (*1,1-Diphenyl-2-Picrilhydrazil*) and showed that ethanol extracts and fractions (n-hexane, dichloromethane and water) had IC $_{50}$ 166.55 µg/mL, 351.19 µg / mL, 85.87 µg /mL, and 226.52 µg/m. While the gallic acid comparison had a very strong antioxidant activity with its IC $_{50}$ value of 10.75 µg/mL.

Keywords: Paliasa leave (*Kleinhoviahospita*L.), flavonoids, antioxidants.

PRELIMINARY

The use of plants for traditional medicine has been carried out by the people of Indonesia. One of the plants used is paliasa leaves (*Kleinhoviahospita*L.)namely as a hepatitis drug (Regulation of the Minister of Health of the Republic of Indonesia, 2016). Paliasa leaves are empirically used to prevent the growth of gray hair on the scalp (Yunita*et al* ., 2009).

The chemical compounds contained in paliasa leaves include quercetin, kaemferol, tannins, rutin, tirterpenes, prusidicacid , saponins, cardenolin, bufadienol and anthraquinones (Regulation of the Minister of Health of the Republic of Indonesia, 2016). According to Yunita*et al* ., (2009) qualitative and quantitative analysis of paliasa leaves was positive for the presence of alkaloids, flavonoids and saponins with alkaloid levels of 2.83%, flavonoids 19.78%, saponins 14.23%. Besides that, paliasa leaves contain flavonoid compounds (Ministry of Health of the Republic of Indonesia, 2010).

Paliasa is a plant that functions to protect the body from free radical attacks. This property is also known as an antioxidant which is an electron donor or reductant. This compound has a small molecular weight but is able to inactivate the development of oxidation reactions, by preventing the formation of radicals. Antioxidants are also compounds that can inhibit oxidation reactions, by binding to free radicals and highly reactive molecules. As a result, cell damage will be inhibited (Winarsi, 2007).

The main function of antioxidants is used as an effort to minimize the occurrence of the oxidation process of fats and oils, reduce the occurrence of spoilage processes in food and extend the shelf life in the food industry . In addition, antioxidants can also increase the stability of the fat contained in food, and prevent loss of qualitysensory and nutrition .Free radicals can be defined as molecules or compounds that are in a free state and have one or more unpaired free electrons(Hernani & Mono, 2005) .

Research conducted by Hasanuddin&Andini (2017) showed that the ethanol extract and ethyl acetate fraction from paliasa leaves had free radical activity with an $IC_{50\ value}$ of 4.556 mg/mL and 3.113 mg/mL respectively, while the $IC_{50\ value}$ of the comparison sample was vitamin C 0.106 mg/mL. The ethanol extract of paliasa leaves has antioxidant activity with an IC value of $_{123.70}$ µg/mL (Suryani*et al* ., 2017). IC_{50} is the level of a compound that can inhibit free radicals by 50%. The smaller the $IC_{50\ value}$, the greater the antioxidant potential.

Based on the description above, a study was conducted to determine the total flavonoid content of ethanol extract and various fractions of paliasa(*Kleinhoviahospita*L.) leaves on their potential as antioxidants.

RESEARCH METHODS

Tools and materials

The tools used include: UV-Vis spectrophotometer (T70), *rotary evaporator* (Ika), *moisture balance* (Ohaus), analytical balance (Precisa), blender (Miyako), Mesh 60 sieve, desiccator, and other glassware tools.

The materials used in this study were Paliasa(*Kleinhoviahospita*L.) leaves, Mg powder, acetic anhydrous acid, lead (II) acetate, sulfuric acid, chloroform, hydrochloric acid, iron (III) chloride, bromine, lead acetate, sodium acetate. , aluminum chloride, n-hexane, ethyl acetate, 70% ethanol, DPPH *(1,1-Diphenyl-2-Picrilhydrazil* , methanol pa, gallic acid, rutin, and distilled water.

Procedure

Implicit setup

Paliasa leaf samples (*Kleinhoviahospita L.*)taken in Babeko Village, MuaraBungo District, Jambi.

Simple standardization paliasa leaves

It is carried out based on the Indonesian Herbal Pharmacopoeia as follows

(Ministry of Health of the Republic of Indonesia, 20 10):

Simplicia Standardization Examination

1. Examination of simplicia was tested macroscopically.
2. Microscopic examination
3. Determination of drying shrinkage
4. Determination of total ash content
5. Determination of acid insoluble ash content
6. Determination of water soluble essence content
7. Determination of ethanol soluble essence content

Extract Manufacturing

An extract of 200 grams of paliasa leaves was prepared by maceration using 2000 mL of 70% ethanol solvent. Soak for the first 6 hours while stirring occasionally, then let stand for 18 hours. The macerate is separated by filtration, this extraction process is repeated 2 times, using the same type and amount of solvent. All macerate is collected, then evaporated using a rotary evaporator at a temperature below ± 50 ºC to obtain extracts (Ministry of Health of the Republic of Indonesia, 2000).

Examination of Extract Standardization

1. Determination of total ash content
2. Determination of acid insoluble ash content
3. Determination of water content

Extract Fractionation

The viscous extract of paliasa leaves obtained was fractionated using *n* - hexane, dichloromethane and water as solvents. The condensed extract of paliasa leaves was diluted first using 60 mL of distilled water which had been heated, stir until homogeneous in a separatory funnel, add 60 mL of *n* - *hexane solvent, shake homogeneously and then leave to stand until a boundary was seen between the two solvents, after which the n* -fraction was separated. hexane was removed from the separatory funnel. The remaining fraction was added with 60 mL of dichloromethane solution and 60 mL of heated distilled water, then shaken homogeneously and allowed to stand until a separation boundary was seen between the two solvents, then removed from the separatory funnel. Then the distilled water fraction will remain. The extraction of each fraction was repeated three times using 60 mL of solvent for each extraction. The first, second and third saris are collected. The fractionated extract was thickened using *a rotary evaporator* .

Reactant Manufacturing

1. Mayer's reagent

A total of 1.35 g of mercury (II) chloride was dissolved in 100 mL of 5% KI solution (Ministry of Health of the Republic of Indonesia, 1989).

2. Wagner's reagent

Potassium iodide is dissolved in a little water, adding iodine little by little (Ministry of Health of the Republic of Indonesia, 1989).

3. Bouchardat fixer

Dissolve 2 g of iodine P and 4 g of potassium iodide P in enough water to make up to 100 mL (Ministry of Health of the Republic of Indonesia, 1989).

Qualitative Analysis of Paliasa Leaves

A qualitative analysis of the ethanol extract and various fractions of hexane, dichloromethane, water from paliasa leaves was carried out as follows:

1. Alkaloids

To 5 mL of extract add 1 mL of 2 N hydrochloric acid and 9 mL of water, heat over a water bath for 2 minutes, cool and filter. Transfer 3 drops of filtrate to a test tube, add 2 drops of Bouchardat LP. If no precipitate occurs in the experiment, then it does not contain alkaloids.

If with Mayer LP a white or yellow precipitate forms which dissolves in methanol P and with Bouchardat LP a brown to black precipitate forms, then there is a possibility that alkaloids are present. (Ministry of Health of the Republic of Indonesia, 1995).

2. Flavonoids

The 5 mL extract examined was added with 10 mL methanol P, using a back-cooling device for 10 minutes. Filter hot through a small folded filter paper. Dilute the filtrate with 10 mL of water. After cold add 5 mL of kerosene ether P, Shake carefully, let stand. Take the methanol layer, vaporize it at 40 ◦C under pressure. The remainder is dissolved in 5 mL of ethyl acetate P, filtered.

Evaporate to dryness 1 mL of the experimental solution, the remainder is dissolved in 1 mL to 2 mL of 95% P ethanol, add 0.5 gram zinc powder P and 2 mL of 2 N hydrochloric acid, let stand for 1 minute. Add 10 drops of concentrated hydrochloric acid P, if within 2 to 5 minutes it becomes an intense red color, it indicates the presence of flavonoids (Ministry of Health of the Republic of Indonesia, 1995)

3. Saponin test

Foaming reaction:

Put 5 mL of the extract being examined into a test tube, add 10 mL of hot water, cool and then shake vigorously for 10 seconds. If the substance being examined is in the form of a liquid preparation, dilute 1 mL of the preparation under investigation with 10 mL of water and shake vigorously for 10 minutes. On the addition of 1 drop of 2 N hydrochloric acid, the foam did not disappear (Ministry of Health of the Republic of Indonesia, 1995).

1 mL of plant extract is added 5 drops of iron (III) chloride, a phenolic compound that gives a green to black blue color (Hanani, 2015).

4. Phenol test

1 mL of plant extract is added 5 drops of iron (III) chloride, a phenolic compound that gives a green to black blue color (Hanani, 2015).

5. Tannin test

To 1 mL of the extract, 3-4 drops of diluted iron (III) ammonium sulfate were added. If a green to blue-black color is formed, the extract contains tannins (Ministry of Health of the Republic of Indonesia, 1995).

6. Terpenoid test

As much as 1 mL of the extract was added 2 drops of anhydrous acetic acid and 1 drop of concentrated sulfuric acid, a change in purple or red to blue green indicated the presence of terpenoids (Ministry of Health of the Republic of Indonesia, 1995).

Quantitative Analysis of Paliasa Leaves

Quantitative analysis is used to calculate the amount or amount of a component contained in a particular compound or material.

Quantitative analysis of various fractions of hexane, dichloromethane and water from the ethanol extract of paliasa leaves as follows:

1. Determination of total flavonoid content of paliasa leaves

a. Preparation of mother liquor routine

Weigh routine as much as 10 mg, then dissolve it with 80% ethanol in a 10 mL volumetric flask, add 80% ethanol to the mark, so that a mother liquor is obtained with a concentration of 1000 µg/mL.

b. Routine wavelength determination

Pipette 0.7 mL of the mother liquor into a 10 mL volumetric flask, add 80% ethanol to the mark, to obtain a concentration of 70 µg/mL, then pipette 0.5 mL of the test sample, add 1.5 mL of ethanol P, 0.1 ml of 10% aluminum (III) chloride, 0.1 ml of 1 M sodium acetate and 2.8 ml of distilled water, shake until homogeneous. After 30 minutes of incubation. The absorbance was measured using a visible spectrophotometer at a wavelength of 419 nm.

c. Creation of routine calibration curves

From the main solution, concentrations of 60, 70, 80, 90 and 100 µg/mL were made. By pipetting as much as 0.6; 0.7; 0.8; 0.9 and 1 mL, then put into a 10 mL volumetric flask added with 80% ethanol up to the mark, then pipetted as much as 0.5 mL of the test sample added with 1.5 mL of ethanol P, 0.1 mL of aluminum (III) 10% chloride, 0.1 ml of 1 M sodium acetate and 2.8 ml of distilled water, shake until homogeneous. After 30 minutes of incubation. The absorbance was measured using a visible spectrophotometer at a wavelength of 419 nm.

d. Determination of the levels of flavonoid compounds in the ethanol extract and dichloromethane fraction of paliasa leaves.

Weigh 0.5 gram of sample into a 10 mL volumetric flask, then pipet as much as 0.5 mL of test sample added with 1.5 mL of ethanol P, 0.1 ml of 10% aluminum (III) chloride, 0.1 ml of sodium acetate 1 M and 2.8 ml of distilled water, shake until homogeneous. After 30 minutes of incubation. The absorbance was measured using a visible spectrophotometer at a wavelength of 419 nm. (Ministry of Health of the Republic of Indonesia, 2008).

2. Antioxidant activity test

a. Preparation of 30 µg/mL DPPH solution

DPPH was weighed as much as 10 mg then dissolved with methanol pa in a 100 mL volumetric flask, so that a solution with a concentration of 100 µg/mL was obtained. Then diluted by means of a pipette 30 mL of 100 µg/mL DPPH solution, put it into a 100 mL volumetric flask plus pa methanol up to the boundary mark, so that a solution with a concentration of 30 µg/mL was obtained (Molyneux, 2004).

b. Preparation of Control Solution and Optimization of the Maximum Wavelength of DPPH

Pipette 3.8 mL of DPPH solution (30 µg/mL) into the vial, then add 0.2 mL of methanol pa and homogenize and cover the vial with *aluminum foil* , then incubate in the dark for 30 minutes. Determine the absorption spectrum and maximum wavelength using a UV-Visible spectrophotometer at a wavelength of 400-800 nm (Andayani*et al* ., 2012).

c. Preparation of Gallic Acid Comparison Solution

A total of 10 mg of gallic acid was added to sufficient methanol pa, then the final volume was made up with methanol pa to 100 mL (100 µg/mL). Then, from this solution, a series of concentrations of 4 µg/mL, 6 µg/mL, 8 µg/mL, 10 µg/mL, and 12 µg/mL were made. Pipetted 0.4 mL, 0.6 mL, 0.8 mL, 1 mL, and 1.2 mL of each test solution, then put into a 10 mL volumetric flask and then the volume was made up with methanol pa up to the mark line. To determine the antioxidant activity of each concentration, pipette 0.2 mL of sample solution with a micropipette and put it in a vial and cover with *aluminum foil* , then add 3.8 mL of 30 µg/mL DPPH solution. The solution was then homogenized and allowed to stand for 30 minutes, then the absorbance was measured by UV-Visible spectrophotometry at the maximum wavelength of DPPH. All work is done in a room that is protected from sunlight (Andayani*et al* ., 2012).

d. Paliasa Leaf Antioxidant Activity Test

Testing of the ethanol extract of paliasa leaves was carried out by weighing 25 mg of the extract, then putting it into a volumetric flask of 25 mL and dissolving it with methanol pa up to the boundary mark, so that a concentration of 1000 µg/mL was obtained. Make dilutions with concentrations of 120, 140, 160, 180 and 200 µg/mL, by pipetting 1.2 mL, 1.4 mL, 1.6 mL, 1.8 mL and 2 mL respectively from the main solution of 1000 µg /mL. Then put it into a 10 mL flask then add methanol pa up to the mark mark.

Testing of the *n -hexane fraction of* paliasa leaves was carried out by weighing 25 mg of the *n -hexane fraction* , then putting it into a volumetric flask of 25 mL and dissolving it with methanol pa to the boundary mark, so that a concentration of 1000 µg/mL was obtained. Make dilutions with concentrations of 200, 250, 300, 350 and 400 µg/mL, by pipetting 2, 2.5, 3, 3.5 and 4 mL of the mother liquor 1000 µg/mL respectively. Then put it into a 10 mL flask then add methanol pa up to the mark mark.

Testing of the dichloromethane fraction of paliasa leaves was carried out by weighing 25 mg of the dichloromethane fraction, then putting it into a volumetric flask of 25 mL and dissolving it with methanol pa to the mark, so that a concentration of 1000 µg/mL was obtained. Perform dilutions with concentrations of 40, 60, 80, 100 and 120 µg/mL, by pipetting 0.4, 0.6, 0.8, 1 and 1.2 mL of the mother liquor 1000 µg/mL respectively. Then put it into a 10 mL flask then add methanol pa up to the mark mark.

Testing of the water fraction of paliasa leaves was carried out by weighing 25 mg of the water fraction, then putting it into a volumetric flask of 25 mL and dissolving it with methanol pa up to the boundary mark, so that a concentration of 1000 µg/mL was obtained. Make dilutions with concentrations of 180, 200, 220, 240 and 260 µg/mL, by means of pipetting 1.8, 2, 2.2, 2.4 and 2.6 mL respectively then put into a 10 mL flask then add methanol pa to the limit mark. To determine the antioxidant activity of each concentration, 0.2 mL of sample solution was pipetted using a micropipette ,then added 3.8 mL of 30 µg/mL DPPH solution, then put into a vial and covered with *aluminum foil* . The mixture was homogenized and incubated for 30 minutes in the dark, the absorption was measured with a UV-Visible spectrophotometer at the maximum wavelength of DPPH (Ismayanti, *et al.*, 2013).

RESULTS AND DISCUSSION

Standardization test results

a. From the results of the simplicia characterization it can be seen that **:**
   - Macroscopic testing
   - Microscopic testing
   - The average drying shrinkage of paliasa leaves is 7.6653 % ± 0.3503
   - The average total ash content of paliasa leaf simplicia is 8.6901 % ± 0.1650
   - The average acid insoluble ash content of paliasa leaf simplia was 0.8960% ± 0.0328
   - The average water-soluble essence content of paliasa leaf simplicia is 22.8312 % ± 0.12441
   - The average content of soluble extract in ethanol from paliasa leaf simplia is 17.2683 % ± 0.2045

b. Thin Layer Chromatography Test Results

Thin layer chromatography pattern of paliasa leaves R *f*1 =0, 11 ,*Rf*2 = 0.24 , *Rf*3 =0, 32,*Rf*4 = 0.47, R *f* 5 = 0.61, R *f* 6 = 0.93, comparator R *f* 2 = 0.24

c. From the characterization of the extract it can be seen that:
   - The average total ash content of paliasa leaf extract is 8.5050 % ± 0.3058
   - The average acid insoluble ash content of paliasa leaf extract was 0.9731% ± 0.0206
   - The average water content of paliasa leaf extract is 8.2133 % ± 0.6035

Phytochemical test

Results of phytochemical tests performed on ethanol extract, *n* -hexane fraction, dichloromethane fraction and water fraction.Chemical compound from ethanol extract paliasa leaves are alkaloids, flavonoids, saponins, phenols and tannins.The chemical compound of the hexane fraction is tannin. The chemical compounds of the dichloromethane fraction are flavonoids, phenols and tannins . The chemical compounds of the water fraction are alkaloids, saponins, phenols and tannins

Test results for determining total flavonoid content in ethanol extract and dichloromethane fraction of paliasa leaves

The results of determining the routine maximum absorption wavelength of 70 µg/mL show the maximum absorption at a wavelength of 419 nm with an absorption of 0.307. The total flavonoid content of the ethanol extract and dichloromethane fraction were 3.10% and 3.73%.

Test results of antioxidant activity of various hexane, dichloromethane, and water fractions from the ethanol extract of paliasa leaves

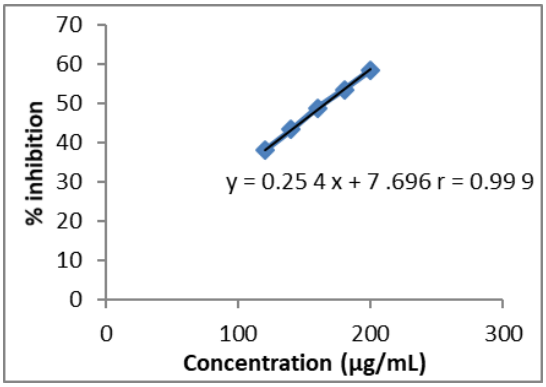
a) Testing the antioxidant activity of the ethanol extract of paliasa leaves at concentrations of 120 µg/mL , 140 µg/mL , 160 µg/mL , 180 µg/mL and 200 µg/mL. The linear regression equation for the ethanol extract of paliasa leaves obtained is Y = 0.254x + 7.696 with an IC value of 50 from theethanol extract ofpaliasa leaves, namely 166.55 µg/mL (Fig .a).

b) Testing the antioxidant activity of the hexane fraction of paliasa leaves at concentrations of 200 µg/mL , 250 µg/mL 300 µg/mL, 350 µg/mL and 400 µg/mL. The linear regression equation for the hexane fraction of paliasa leaves obtained was Y = 0.114x + 9.964 with an IC50 value of 351.19 µg/mL (Figure b).

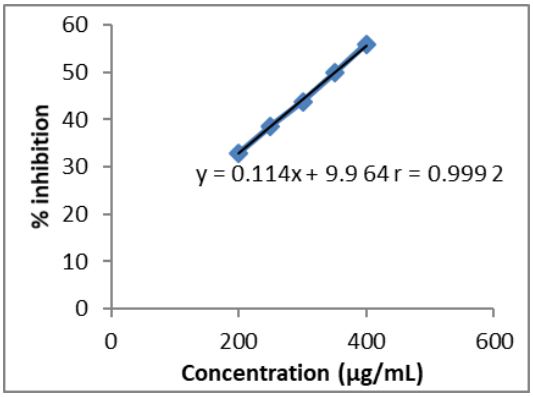
c) Testing the antioxidant activity of the dichloromethane fraction of paliasa leaves at concentrations of 40 µg/mL , 60 µg/mL , 80 µg/mL , 100 µg/mL , and 120 µg/mL . The linear regression equation for the ethyl acetate fraction of paliasa leaves obtained is Y = 0.294x + 24.752 with an IC value of $_{50}$ which is 85.877µg /mL(Figure c).

d) Testing the antioxidant activity of the water fraction of paliasa leaves at concentrations of 180 µg/mL , 200 µg/mL, 220 µg/mL, 240 µg/mL , and 260 µg/mL. The linear regression equation for the water fraction of paliasa leaves obtained is Y = 0.363x – 32.227 with an IC value of $_{50}$ which is 226.520µg /mL(Figure d).

e) Testing the antioxidant activity of gallic acid at concentrations of 4 µg/mL , 6 µg/mL , 8 µg/mL , 10 µg/mL and 12 µg/mL each had a percent inhibition value of 13.69%, 24.43%, 35.87 %, 45.40 %, and 56.84 %. The linear regression equation for gallic acid obtained is Y = 5.363x – 7.662 with an IC50 value of gallic acid of 10.75 µg/mL (Figure e)



a



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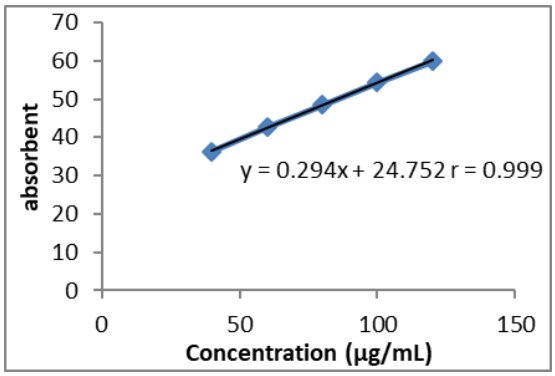

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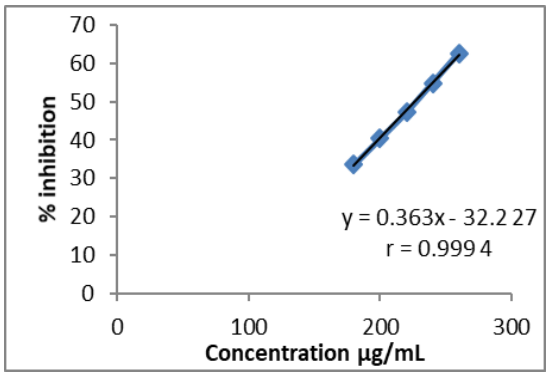

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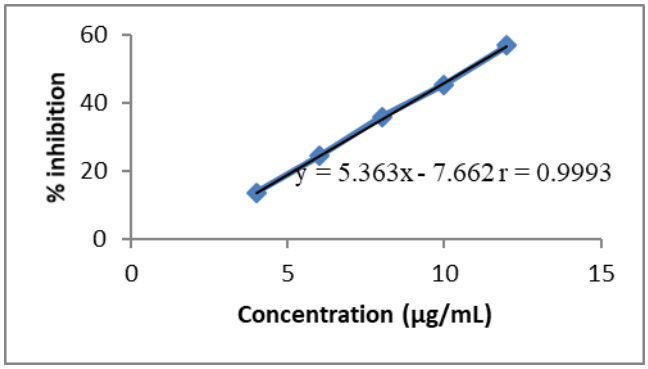

e

Description: Correlation Curve Between % Inhibition and Concentration a. Eethanol extract of paliasa leaves, b. The *n -hexane* fraction , c. Dichloromethane fraction, d. Water Fraction, e. Gallic Acid

Discussion

Simplisia from kpaliasa leaves is then made into an extract using the maceration method. Maceration is a simplex extraction method by immersing it in a solvent at room temperature so that damage or degradation of metabolites can be minimized. The solvent used in the manufacture of the extract is ethanol. Ethanol was chosen because it is easier to penetrate cellular membranes to extract intracellular material from plant material.

Testing total flavonoid levels in the ethanol extract and dichloromethane fraction of paliasa leaves based on the results of the calibration curve in Figure 10 obtained the regression equation y = 0.014x – 0.689. The correlation coefficient is r = 0.998, this figure is close to 1, which means that there is a very high correlation between absorbance and compound content and shows a relationship between the two. Obtained total flavonoid levels in the ethanol extract and dichloromethane fraction of 3.10% and 3.73%.

The antioxidant activity of the ethanol extract of paliasa leaves was obtained with an IC value of 166.55 µg/mL, the n-hexane fraction of paliasa leaves was shown with an IC value of $_{50}$ 351.192 µg/mL, the dichloromethane fraction of paliasa leaves with an IC value of $_{50\ 85.877}$ µg/mL and the water fraction paliasa leaves with an IC value of $_{226.52}$ µg/mL. Gallic acid as a comparison has antioxidant activity as indicated by an IC50 value of $_{10.75}$ µg/mL. The results showed that the antioxidant activity of the ethanol extract, *n -hexane fraction* , dichloromethane and paliasa leaf water was lower than the antioxidant activity of gallic acid.

From the results of the research that has been done, it can be concluded that the antioxidant activity obtained from the ethanol extract of paliasa leaves is classified as weak with an IC value of $_{50}$ 166.551 µg/mL, the hexane fraction of paliasa leaves is classified as very weak with an IC value of $_{50}$ 351.192 µg/mL, the dichloromethane fraction of paliasa leaves is classified as strong with an IC50 value of $_{85.877}$ µg/mL and the water fraction of paliasa leaves was classified as very weak with an IC50 value of $_{226.520}$ µg/mL. Gallic acid as a comparison has a very strong antioxidant activity with an IC50 value of $_{10.751}$ µg/mL. This shows that the antioxidant activity of the ethanol extract, hexane fraction, dichloromethane and water is lower than the antioxidant activity of gallic acid.

From the results of the research that has been done, it can be concluded that research on ethanol extract is classified as weak in its antioxidant power with IC $_{50}$ 166.551 µg/mL. In Hasanuddin&Andini's (2017) study, the ethanol extract was classified as very weak with an IC value of $_{4.556}$ mg/mL. A compound is said to have the ability as a very strong antioxidant if the IC $_{50\ value}$ is less than 50 ppm (category 1), strong if the IC $_{50\ value}$ is 50-100 ppm (category 2), while if the IC $_{50\ value\ is}$ 100-150 ppm (category 3), weak if the IC $_{50\ value\ is}$ between 150-200 ppm (category 4), and very weak if the IC $_{50\ value}$ is more than 200 ppm (category 5) (Molyneux 2004).

The antioxidant test on the dichloromethane fraction had a lower IC $_{50}$ value with an IC $_{50}$ of 85.877 µg/mL compared to other solvents. This is presumably because the chemical compounds that are antioxidants such as phenols and flavonoids are more abundant in the dichloromethane fraction than in the ethanol extract. The results of the total flavonoid content determination showed that the dichloromethane fraction had a higher flavonoid content of 3.73% compared to the 3.10% ethanol extract.

The difference in the IC $_{50\ value\ of\ the}$ extract and each fraction is caused by the distribution of secondary metabolite compounds that act as antioxidants (flavonoids, phenols and tannins) based on the polarity of the solvent used. The results showed that the semi-polar dichloromethane fraction showed stronger antioxidant properties with an IC50 value of $_{85.877}$ µg/mL because the compounds responsible as antioxidants were more extracted in the dichloromethane fraction, when compared to the ethanol extract, hexane fraction, and water fraction. . Compounds extracted in the dichloromethane fraction are suspected to be flavonoid compounds. According to Gusrav*et al* ., (2007) flavonoid compounds act as antioxidants because they have hydroxyl groups that can release protons in the form of hydrogen ions. The hydrogen ion has only one proton and has no electrons, so that the radical electrons present in the nitrogen atoms in the DPPH compound bind to hydrogen ions to produce reduced DPPH (Gusrav*et al* ., 2007).

In addition, one of the factors that affect IC $_{50}$ is concentration. Where the concentrations used of gallic acid are each of a small value when compared to the extracts and various fractions. The small concentration used gives a smaller value of % inhibition, so the antioxidant activity (IC $_{50}$) is low, which means the antioxidant content is very strong (IC $_{50}$).

CONCLUSION

From the data obtained in this study, it can be concluded that:

1. The content of chemical compounds from the ethanol extract of paliasa leaves are alkaloids, flavonoids, saponins, phenols and tannins. The content of chemical compounds from the hexane fraction is tannin. The content of chemical compounds from the dichloromethane fraction are flavonoids, phenols and tannins. The content of chemical compounds from the water fraction are alkaloids, saponins, phenols and tannins.
2. The total flavonoid content of the ethanol extract of paliasa leaves was 3.10% and the dichloromethane fraction was 3.73%.
3. The ethanol extract of paliasa leaves has a weak antioxidant activity with an IC50 value of $_{166.551\ µg}$ /mL , the hexane fraction has a very weak antioxidant activity with an IC50 value 351.192 µg/mL , the dichloromethane fraction has a strong antioxidant activity with an IC value of $_{50}$

85.877 µg/mL , the water fraction has a very weak antioxidant activity with an IC value of $_{50}$ 226,520 µg/mL. Gallic acid as a comparison has a very strong antioxidant activity with an IC50 value of 10.751 $_{\mu g}$/mL .

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