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Antioxidant Activity Test and Determination Of Total Flavonoids Levels of Kedondong Laut (*Nothopanax fructicosum* (L.) Miq) Leaf Extract

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ABSTRACT

Kedondong *laut (Nothopanax fruticosum (L.) Miq.)* is a plant from the family *Araliaceae*. Leaves of kedondong laut useful for diuretik, analgesics, as an antidiarrheal and arthritis. The characteristics of sea kedondong are shrubs, yellowish green, the size of small flowers are green and the fruit is greenish purple and the height of the tree reaches up to 3 meters. Extracted by stratified extraction using solvents n-Hexane, ethyl acetate, and 96% ethanol with a percent rendender for n-Hexane solvent 1.504% ethyl acetate 2.784%, ethanol 3.698%. The results showed that each extract of kedondong laut leaves (*Nothopanax fructicosum* (L.) Miq has antioxidant activity with IC50 value of n-hexane extract of 33.839μg/mL, ethyl acetate extract of 12.604μg/mL and ethanol extract of 2.222 μg/mL ethanol extract of kedondong laut leaves (*Nothopanax fructicosum* (L.) Miq) has higher antioxidant power than n-hexane extract and ethyl acetate, the total flavonoid compound content is 0.09902 gQE/g extract or 9.902%, ethyl acetate extract is equal to 0.13253 gQE /g extract or 13.253%, and 96% ethanol extract at 0.09345 gQE / g extract or 9.345%, ethyl acetate extract has a greater flavonoid content than n-hexane extract and 96% ethanol.

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INTRODUCTION

Degenerative disease is a disease caused by a decrease in body cell function (Hasanah *et al.*, 2017), generally caused by free radicals (Banjarnahor and Artanti, 2015). This situation will cause oxidative stress which is closely related to the causes of degenerative diseases such as osteoarthritis, cancer, diabetes, cardiovascular disease (Banjarnahor and Artanti, 2015). Antioxidants are compounds that have the ability to inhibit *reactive oxygen species* (ROS), *reactive nitrogen species* (RNS), and free radicals such as H_2O_2 (Handayani *et al.*, 2014). Many studies mention that antioxidants have important role in preventing the occurrence of degenerative diseases (Jatmika *et al.*, 2015). One of the antioxidant compounds that is often studied is flavonoids, (Banjarnahor and Artanti, 2015) and plants that contain flavonoids have the efficacy of treating various degenerative diseases such as stroke, rheumatism, heart disease, and cancer (Handayani *et al.*, 2016).

The use of plants in the treatment of degenerative diseases is increasing in popularity. Kedondong laut leaves (*Nothopanax fruticosum* (L.) Miq) is one of a variety of plants that has this potential. Kedondong laut leaves (*Nothopanax fruticosum* (L.) Miq) contain flavonoid compounds, and identification results

show that the crystals resulting from isolation from the leaves of kedondong laut (*Nothopanax fruticosum* (L.) Miq) are flavonoid compounds (Handayani *et al.*, 2016). Flavonoids that have antioxidant activity include flavones, flavonoids, isoflavones, catechins, and chalcons (Handayani *et al.*, 2016).

The magnitude of the potential of kedondong laut of plants (*Nothopanax fruticosum* (L.) Miq) to be utilized properly and to add scientific data to these plants, research needs to be carried out to determine the antioxidant activity and levels of flavonoid kedondong laut leaves extract (*Nothopanax fruticosum* (L.) Miq)

RESEARCH METHOD

Sampling

The research sample is kedondong laut leaves (*Nothopanax fruticosum* (L.) Miq) obtained from Wajo District, South Sulawesi.

Samples of kedondong laut leaves (*Nothopanax fruticosum* (L.) Miq) collected, then wet-sorted by washing to remove impurities that are still attached to the sample. Then dry-sorted and reshaped by cutting the sample into small pieces. After that, the sample is dried in a drying cabinet with temperature of $\pm 50^{o}$C. Then it is pollinated and ready for extraction (Handayani *et al.*, 2016).

Extraction

The dried samples of kedondong laut leaves (*Nothopanax fruticosum* (L.) Miq) were weighed as much as 300 grams and put into maceration containers and added as much as 2200 mL solvent until the simplicia was soaked. Extraction was done by soaking the sample with n-hexane, ethyl acetate, and ethanol 96% solvent separately for 3 days, stirred occasionally, then filtered using filter paper (Handayani *et al.*, 2016).

Antioxidant Test

Qualitative test

n-Hexane, ethyl acetate, and ethanol extract were diluted using methanol, then dotted on F254 silica gel plate then eluted with mobile phase n-Hexane:ethyl acetate 8:2. The plate then was sprayed with *1,1- diphenyl-2-picrylhydrazyl* (DPPH) and allowed to dry until dried and it was showing spot changing colour to yellow with purple background (Syarif *et al.*, 2016).

Quantitative Test

The making of 1,1- diphenyl-2-picrylhydrazyl (DPPH)

5 mg of DPPH was diluted with 100 mL of Methanol Pro Analysis in a volumetric flask to obtain 500 ppm of DPPH solution (Handayani *et al.*, 2016).

The Making of Sample Solution

1000 ppm stock solution was made by weighing n-hexane, ethyl acetate, and ethanol 96% extracts 10 mg each and dissolved with methanol p.a while stirred and homogenized then added to 10 mL. Then each stock solution was diluted by pipetting 0.05 mL; 0.1 mL; 0.15 mL ; 0.2 mL dan 0.25 mL to make 10 ppm, 20 ppm, 30 ppm, 40 ppm, dan 50 ppm then added with Methanol p.a up to 5 mL final volume (Brand-Williams *et al.*, 1995).

The Making of Quercetin as Standard

1000 ppm stock solution was made by weighing 10 mg of Quercetin and dissolved with Methanol p.a while stirred and homogenized then add the volume up to 10 mL. Then each stock solution was diluted by pipetting 0.2 mL; 0.4 mL; 0.6 mL; 0.8 mL and 1.0 mL to make 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm then added with Methanol p.a up to 5 mL final volume (Brand-Williams *et al.*, 1995).

Maximum Wavelength Determination

DPPH stock solution 50 ppm was pipetted 4 mL then measured its absorbance at 516 nm wavelength (Handayani *et al.*, 2016).

Antioxidant Activity Measurement

2 mL sample solution was pipetted from each concentration, then add 2 mL of DPPH 50 ppm, incubated for 30 minutes at 37ºC, measure its absorbance at 516 nm wavelength.

The same measurement was done to Quercetin as standard (Handayani, Malik & Rumata 2016, h. 161). The percentage of DPPH inhibition was calculated by the equation (Maisuthisakul *et al.*, 2008).

Quantitative Flavonoid Test

Determination of the maximum wavelength (λ max) quercetin

Determination of the maximum wavelength of quercetin was carried out by running quercetin solutions in the wavelength range of 400-800 nm. The maximum absorbance obtained at a given wavelength is the maximum wavelength of quercetin (Fawwaz *et al.*, 2017).

Making a standard quercetin solution

Weighed as much as 10 mg of standard quercetin standard and dissolved in 10 mL p.a ethanol for 1000 ppm. From the standard solution of quarsetin 1000 ppm, pipetted as much as 1 mL and sufficient volume to 10 mL with p.a ethanol for 100 ppm. From

a standard solution of 100 ppm quercetin, several concentrations were made, namely 6 ppm, 8 ppm, 10 ppm, 12 ppm, 14 ppm, and 16 ppm. From each concentration of the standard quercetin solution was added 3 mL ethanol, 0.2 mL $AlCl_3$, 0.2 mL potassium acetate 1 M, and mixed with aquabidestillate to 10 mL. Then incubated for 30 minutes at room temperature and absorbance was measured with a UV-Visible spectrophotometer at a wavelength of 429 nm (Dahlia *et al.*, 2016).

Determination of total flavonoid levels

Determination of total flavonoid levels refers to the procedure of Chang *et al.* (2002) using quercetin as a standard. Weighed kedondong laut leaf extract (*Nothopanax fruticosum* (L.) Miq.) As much as 10 mg and dissolved in 10 mL ethanol p.a. The solution was piped as much as 1 mL and added 3 mL ethanol, 0.2 mL AlCl3, 0.2 mL potassium acetate 1 M, and 5.6 mL aquabidestillata. Then incubated for 30 minutes at room temperature and absorbance was measured with a UV-Visible spectrophotometer at a wavelength of 429 nm. Sample solutions were made in three replications (Dahlia *et al.*, 2016).

RESULTS AND DISCUSSION

Making of kedondong laut leaves (*Nothopanax fructicosum* (L.) Miq) extract was done by using the maceration method. The selection of extraction methods in this study was based on the sensitivity of antioxidant compounds to high temperatures, therefore the maceration method was chosen, where the extraction method was carried out without heating and carried out at room temperature (Pratiwi *et al.*, 2016). The results obtained from extracting kedondong laut (*Nothopanax fructicosum* (L.) Miq). Table 1 shows,

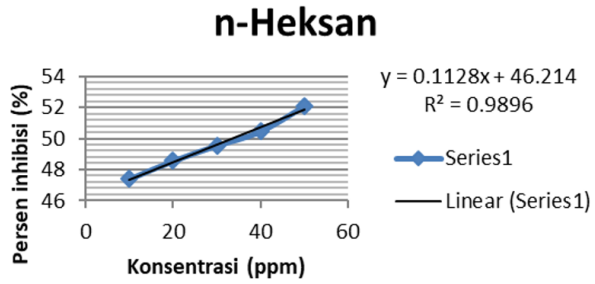

Figure 1: Graph between the concentration of n-hexane extract of kedondong laut leaves (*Nothopanax fructicosum* (L.) Miq) with % inhibition.

Based on the results of the research conducted qualitatively, it was shown that n-hexane extract, ethyl acetate, and 96% ethanol contained flavonoids, phenolics, and saponins. Based on the Handayani study, (2016) the same results were obtained because the three solvents had been able to attract compounds that have good antioxidant activity.

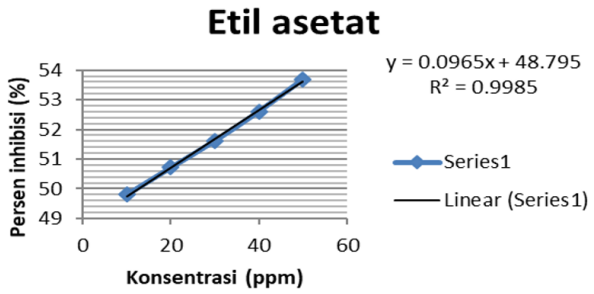

Figure 2: The graph between the concentration of ethyl acetate extract of kedondong laut leaves (*Nothopanax fructicosum* (L.) Miq) with % inhibition.

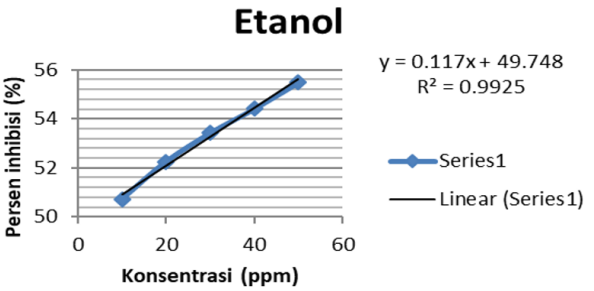

Figure 3: Graph between the concentration of ethanol extract of kedondong laut leaves (*Nothopanax fructicosum* (L.) Miq) with % inhibition.

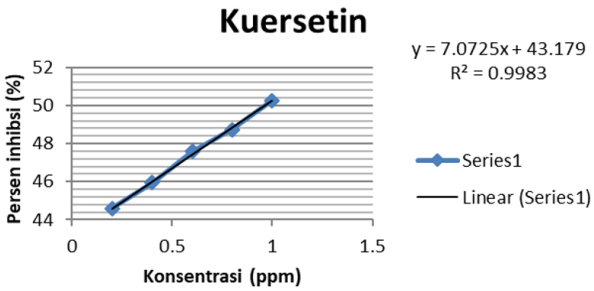

Figure 4: The graph between the Quercetin concentration with % inhibition

According to Phongpaichit *et al.* (2007), an antioxidant is very strong if the IC50 value is less than 10 μg / mL, strong if the IC_{50} value is between 10-50 μg / mL, medium if the IC_{50} value ranges from 50-100 μg / mL, weak if the IC_{50} value ranges from 100-250 μg / mL and is not active if the IC_{50} is above 250 μg / mL.

Based on the results obtained IC_{50} values of n-hexane extract of kedondong laut leaves (*Nothopanax fruticosum* (L.) Miq) included in

Table 1: Results in yield value of n-Hexane, Ethyl Acetate, and Ethanol 96% extracts of Kedondong laut leaves *(Nothopanax fructicosum* (L.) Miq)

Solvent	Sample weight (g)	Solvent weight (mL)	Extract weight (g)	Yield value (%) (b/b)
n-Hexane	300	2200	4,513	1,504
Ethyl acetate	300	2200	8,352	2,784
Ethanol 96%	300	2200	11,096	3,698

strong antioxidants because IC_{50} values range from 10-50 μg / mL 33.839 μg / mL, ethyl acetate extract kedondong laut leaves (*Nothopanax fruticosum* (L.) Miq) included in strong antioxidants because IC_{50} values range from 10-50 μg / mL is 12.604 μg / mL while ethanol 96% extract of kedondong laut leaves (*Nothopanax fruticosum* (L.) Miq) included in very strong antioxidant because the IC_{50} value obtained <10 μg / mL is 33.839 μg / mL, and for quercetin as the comparative standard IC_{50} <10 μg / mL is 0.965 μg / mL.

Graph of the relationship between each n-hexane extract, ethyl acetate extract, ethanol extract of kedondong laut leaves (*Nothopanax fruticosum* (L.) Miq) and comparison of quercetin with% inhibition (Phongpaichit *et al.*, 2007), can be seen in the following Figures 1, 2, 3 and 4,

Testing of quantitative analysis with UV-Vis spectrophotometry was used as a control solution that functions as a compound that does not need to be analyzed. The results of running a quercetin standard solution obtained a maximum wavelength of 429 nm, used to measure standard uptake and samples. The absorbance results from the recorded concentration series obtained linear line equations namely y = 0.0575x – 0.0987 with R^2 value of 0.998.

The results of the absorbance analysis of n-hexane extract, ethyl acetate and ethanol 96% of kedondong laut leaves. Sample solutions were made in three replications. Replication is done for data accuracy so that the levels of flavonoids obtained are calculated as equivalent quercetin (QE).

Determination of total flavonoid levels in ethyl acetate extract of kedondong laut has the greatest flavonoid content, which is 0.13253 gQE/g extract (13.253%).

This shows that ethyl acetate solvents are effective in attracting flavonoid compounds on the leaves of kedondong laut. Flavonoid compounds are generally polar because they have bonds with sugar to form glycosides. The effect of glycosides causes flavonoids to be less reactive and tend to be more soluble in water solvents. However, flavonoids also contain two or more hydroxyl groups, causing them to dissolve easily in semi-polar solvents, such as ethyl acetate (Handayani *et al.*, 2016).

CONCLUSIONS

Based on the results of the research that has been done, it can be concluded. Antioxidant activity of kedondong laut leaves (*Nothopanax fruticosum* (L.) Miq) n-hexane extract has strong antioxidant activity with IC_{50} value of 33.839 μg / mL, ethyl acetate extract has strong antioxidant activity with IC_{50} value of ethyl acetate extract of 12.604 μg / mL , and 96% ethanol extract has very strong antioxidant activity with an IC_{50} value of 2.222 μg / mL. Whereas quercetin as the comparison standard has IC_{50} value <10 μg / mL which is 0.965 μg / mL.

A total flavonoid compound of 0.09902 gQE / g extract or 9.902%, ethyl acetate extract of 0.13253 gQE / g extract or 13.253%, and ethanol extract 96% of 0.09345 gQE / g extract or 9.345%.

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