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DETERMINATION OF ANTIOXIDANTS BY DPPH RADICAL SCAVENGING ACTIVITY OF *Limonia acidissima* L. FRUIT AND EXOCARP EXTRACTS

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ABSTRACT

Limonia acidissima is one of the medicinal plants in Indonesia with various bioactive compounds that act as an antioxidant. *L. acidissima* contains alkaloids, flavonoids, and triterpenoids that function as antioxidants. Antioxidants are compounds that help protect the body from damage to cells by free radicals. To determine the antioxidant activity of a plant, one of the most commonly used measurements is through the capture of free radicals using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals. This study has been conducted on the antioxidant activity determination of *L. acidissima* fruit and exocarp extract with 1,1 Diphenyl-2-picrylhydrazil (DPPH). This study aims to determine the antioxidant activity by observing at the IC_{50} value contained in the *L. acidissima* fruit and exocarp extract with DPPH at a wavelength of 478 nm. *L. acidissima* fruit extract has an IC_{50} value of 35.190 means antioxidant activity is very strong and *L. acidissima* exocarp extract has an IC_{50} value of 35.348 means antioxidant activity is very strong.

Keywords : *Limonia acidissima*, antioxidant, DPPH

INTRODUCTION

Free radicals are compounds that have one or more unpaired electrons. These free radicals can be generated by normal cell metabolism in the human body or can also come from external sources such as pollution, cigarette smoke, radiation and drugs that can damage cells and tissues if accumulated in the human body (Paliwal et al., 2017). Reactive oxygen species (ROS) is a type of free radical that can oxidize proteins, fats, DNA and can initiate the occurrence of chronic or degenerative diseases such as: cancer, diabetes and cardiovascular disease (Aktumsek et al., 2013).

Antioxidants are able to inactivate oxidation reactions and prevent the formation of free radicals. Antioxidants can be both synthetic and natural. Antioxidant compounds contained in plant extracts are thought to inhibit and neutralize oxidation reactions involving free radicals, both exogenous and endogenous. Oxidation reactions involving free radicals, especially OH free radicals, can damage the surrounding normal cell membrane and damage the composition of DNA so that it can cause a mutation. Mutations or damage to the composition of DNA can cause several degenerative diseases such as cancer, heart disease, cataracts, premature aging and others (Hidayati *et. al.*, 2017).

The development of natural antioxidants has received great attention in recent years (Wahdaningsi et al., 2011). *L. acidissima* fruit is a tropical fruit that has antioxidant activity. Antioxidant activity in this fruit will be influenced by differences in maturity levels. *L. acidissima* fruit has medicinal properties, namely to reduce heat, astringent and tonic, and stomach pain medicine. In Indo-China, kawista thorns and bark are used in

various traditional medicinal herbs to treat excessive menstruation, liver disorders, animal bites and stings, and to treat nausea. *L. acidissima* fruit can be used in the treatment of tumors, asthma, constipation, weak heart, and hepatitis. Research results state that *L. acidissima* Fruit contains flavonoids, glycosides, saponins, tannins, coumarins, and tyramine derivatives. Many studies have stated that ripe *L. acidissima* Fruit has potential as a medicinal plant because it has many properties, one of which is as an antioxidant. Antioxidant activity is influenced by chemical components in the fruit and these chemical components can be influenced by the maturity level of the fruit. Besides its potential as an antioxidant. *L. acidissima* fruit also has potential as an antidiabetic and kawista leaves have potential as anti-hepatoprotective. The fruit exocarp is reported to have antifungal compounds namely psoralena, xanthotoxin, 2,6-dimethoxybenzakuinone and osthenol with several alkaloid compounds, coumarins, flavones, lignans, sterols and triterpenes found to have antimicrobial activity (Dewi, 2013).

METHODS

Sample processing

L. acidissima fruit extract was maceration with ethanol (p.a.) as solvent with 1:10 ratio. The maceration process was carried out for 7 x 24 hours with occasional stirring for 5 minutes. The results of maceration were filtered using filter paper to obtain the filtrate. Then remaseration was carried out once with the same solvent. The entire filtrate obtained was concentrated with a vacuum rotary evaporator at 50˚C, then the extract was evaporated using a waterbath until a thick extract was obtained, then stored in a tightly closed glass container before being used for testing (Kusuma & Adhitya, 2021).

Phytochemical Assay

Flavonoid Test

Each 5 mL of ethanol extract of *L. acidissima* fruit and exocarp is put into a test tube plus 1 mL of 0.5 N HCl and a little Magnesium powder. If it reacts positively, it will produce an orange, pink, or red solution. (Kusuma *et. al.*, 2019)

Phenolic Test

Each 5 mL of ethanol extract of *L. acidissima* fruit and exocarp was put into a test tube plus 10 mL of water and heated for 10 minutes. then cooled, then filtered. The filtrate obtained is dripped with $FeCl_3$ solution. If it reacts positively it will produce green, purple, blue to black color (Kusuma et. al., 2019).

Antioxidant Activity Assay

Preparation of DPPH solution 40 ppm

The 40 ppm DPPH solution was made by weighing 4 mg of DPPH then dissolved with 100 mL of ethanol (p.a.) in a measuring flask.

Preparation of test solution of *L. acidissima* fruit and exocarp extract

A 100 ppm stock solution was prepared by weighing 10 mg of *L. acidissima* fruit and exocarp extracts each dissolved with 100 mL of methanol (p.a.) in a volumetric flask. Then dilutions of 20 ppm, 30 ppm, 40 ppm, 50 ppm, 60 ppm were made (Mailandari, 2012).

Standard Curve Preparation (Vitamin C)

Made 100 ppm stock solution by weighing vitamin C as much as 10 mg dissolved with 100 mL ethanol (p.a.) in a measuring flask. Then dilutions of 2 ppm, 4 ppm, 6 ppm, 8 ppm, 10 ppm were made (Mailandari, 2012).

Maximum wavelength (ƛ max) measurement

One mL of the comparison solution (6 ppm) was pipetted into a 10 mL volumetric flask and 2 mL of 40 ppm DPPH solution was added, then fullfill with methanol (p.a.), then homogenized. the absorbance was measured after 30 minutes by UV-Vis spectrophotometry at a wavelength of 400-600 nm and the maximum wavelength was determined (Mailandari, 2012).

Measurement of antioxidant activity of blank solution

DPPH 40 ppm solution was pipetted as much as 2 mL and put in a 10 mL volumetric flask, then fullfill with methanol p.a, this solution was left for 30 minutes in a dark room. Then measure the absorbance at the maximum wavelength using UV-Vis spectrophotometry (Mailandari, 2012).

Measurement of the absorbance of *L. acidissima* fruit and exocarp extract solution using UV-Vis spectrophotometry

The test was carried out by pipetting 1 mL of sample solution from various concentrations (20 ppm, 30 ppm, 40 ppm, 50 ppm and 60 ppm) into a 10 mL volumetric flask, then each added 2 mL of 40 ppm DPPH, then fullfill methanol (p.a.). Then incubated for 30 minutes at 37^{o}C, then the absorbance was measured at the maximum wavelength (Mailandari, 2012).

Measurement of the absorbance of the Vitamin C comparison solution using UV-Vis spectrophotometry

The test was carried out by pipetting 1 mL of standard curve solution from various concentrations (2 ppm, 4 ppm, 6 ppm, 8 ppm and 10 ppm) into a 10 mL volumetric flask, then each was added with 4 mL of 40 ppm DPPH, then fullfill with methanol (p.a). The mixture was then incubated for 30 minutes at 37^{o}C, then the absorbance was measured at the maximum wavelength (Mailandari, 2012).

Data processing and analysis

The data obtained was then analysed using a linear regression equation with the formula:

$$y = a + bx$$

*a = Constant, b = Slope. The values of a and b are calculated with the equation

RESULT AND DISCUSSION

L. acidissima fruit after flavanoid testing with HCl 0.5 N + MgSO4 there is a change in colour to pink and in the phenol test with FeCl3 changes colour to black, this indicates positive flavanoid compounds and phenol compounds. Furthermore, in *L. acidissima* exocarp extract after flavanoid testing with HCl 0.5 N + MgSO4 there is a change in colour to pink and in the phenol test with FeCl3 changes colour to green, this indicates that it positively contains flavanoid compounds and phenol compounds (Dewi, 2013; Disi et. al., 2024).

Table 1. Phytochemical test results of *L. acidissima* Fruit and exocarp extracts

Extract	Test	Reagents	Test Result	Description
Fruit	Flavanoids	HCl 0,5 N + $MgSO_4$	Pink	+
	Phenol	$FeCl_3$	Black	+
Exocarp	Flavanoids	HCl 0,5 N + $MgSO_4$	Pink	+
	Phenol	$FeCl_3$	Green	+

The results of antioxidant activity determination of *L. acidissima* fruit extract can be seen in table 2.

Table 2. Antioxidant activity measurements of *L. acidissima* fruit extract

Sample	Concentration (ppm)	Absorbance	% Inhibition	IC_{50}
L. acidissima fruit extract	20 ppm	0,592	39,714	35,190
	30 ppm	0,547	44,399	
	40 ppm	0,471	52,036	
	50 ppm	0,369	62,423	
	60 ppm	0,293	70, 162	

In this treatment, the absorbance results were entered into the curve obtained y=0.7984x + 21.904 and R2 = 0.9849. These results are in accordance with those listed in the Lambert-Beer law, the range of Y values must be in the range of 0.2-0.8 (Disi *et. al.*, 2024). The linear regression graph of Antioxidant activity measurements of *L. acidissima* fruit extract can be seen in figure 1.

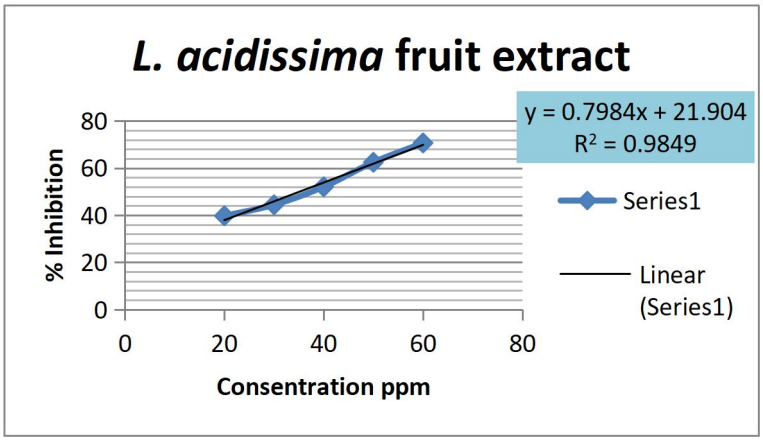

Figure 1. Linear Regression Equation *L. acidissima* fruit extract

The results of antioxidant activity determination of *L. acidissima* exocarp extract can be seen in table 3.

Table 3. Antioxidant activity measurements of *L. acidissima* exocarp extract

Sample	Concentration (ppm)	Absorbance	% Inhibition	IC_{50}
L. acidissima exocarp extract	20 ppm	0,602	38,696	35,1348
	30 ppm	0,584	40,529	
	40 ppm	0,442	54,989	
	50 ppm	0,326	66,802	
	60 ppm	0,301	69,348	

In this treatment, the absorbance results were entered into the curve obtained y=0,8758x + 19,042 and R2 = 0.9398. These results are in accordance with those listed in the Lambert-Beer law, the range of Y values must be in the range of 0.2-0.8 (Disi *et. al.*, 2024). The linear regression graph of Antioxidant activity measurements of *L. acidissima* exocarp extract can be seen in figure 2.

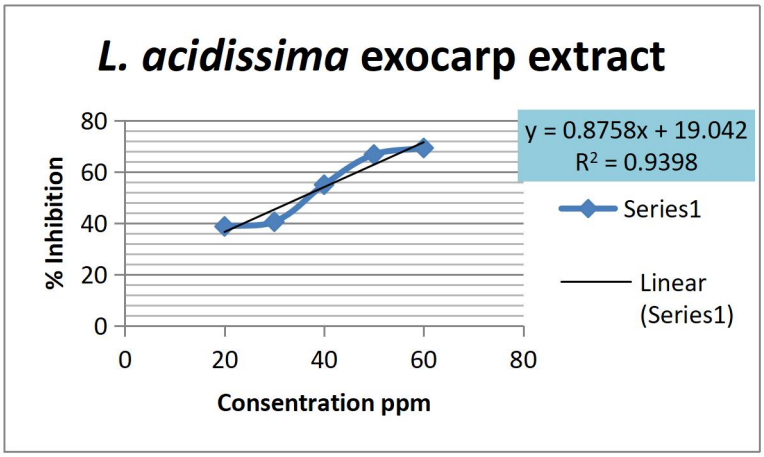

Figure 2. Linear Regression Equation *L. acidissima* exocarp extract

The results of antioxidant activity determination of Vitamin C as positive control can be seen in table 4.

Tabel 4. Antioxidant activity measurements of Vitamin C

Sample	Concentration (ppm)	Absorbance	% Inhibition	IC_{50}
Vitamin C	2 ppm	0,864	12,016	12,184
	4 ppm	0,702	28,513	
	6 ppm	0,695	29,226	
	8 ppm	0,629	35,947	
	10 ppm	0,574	41,547	

In this treatment, the absorbance results were entered into the curve obtained y=3,3248x + 9,501 and R2 = 0,8961. These results are in accordance with those listed in the Lambert-Beer law, the range of Y values must be in the range of 0.2-0.8 (Disi *et. al.*, 2024). The linear regression graph of Antioxidant activity measurements of *L. acidissima* exocarp extract can be seen in figure 2.

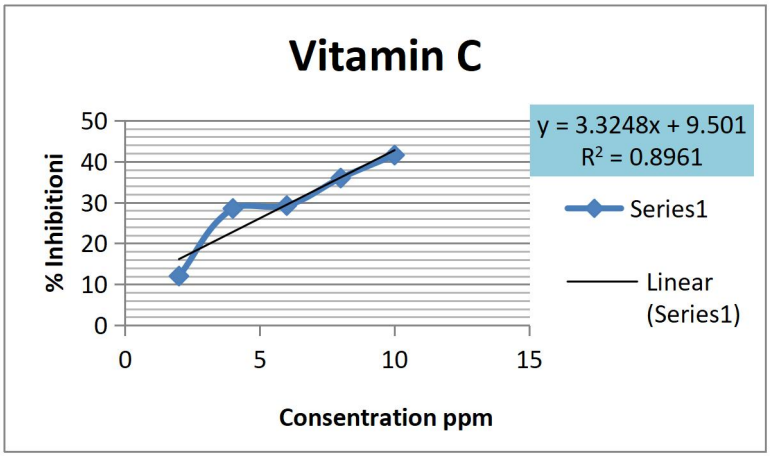

Figure 3. Linear Regression Equation Vitamin

Antioxidant activity test using DPPH radicals scavenging is to see the inhibitory ability of a plant extract against DPPH radicals whose absorbance is measured on a visible light spectrophotometer with maximum wavelength. The strength of a sample to soak DPPH radicals is indicated by the decrease in the intensity of the purple colour of the DPPH solution so that the absorbance also decreases. So the higher the concentration of the test solution added to the DPPH solution, the lower the absorbance of DPPH. This indicates the immersion reaction of DPPH radicals by the sample extract. So the IC50

value is inversely proportional to the antioxidant ability of a compound. The smaller the IC50 value, the stronger the ability of a compound as an antioxidant (Kusuma et. al., 2019).

The mechanism of antioxidant reaction with DPPH by electron transfer or hydrogen radicals on DPPH neutralises the free radical properties of DPPH. DPPH is an organic compound containing unstable nitrogen with strong absorbance at a wavelength of 417 nm. If all electrons on DPPH are paired, there is a decrease in the intensity of the DPPH colour. In spectrophotometric measurements, the absorbance value read is the value of the remaining purple colour of DPPH. Differences in IC_{50} values occur because there are components of compound variations of conssentration of the extract (Kusuma et. al., 2019.

The parameter used to determine the antioxidant activity of an extract is IC_{50}. The IC_{50} value is defined as the amount of concentration of the test compound that can immerse free radicals by 50%. The IC_{50} value is obtained by using a linear regression equation that states the relationship between the sample concentration with symbol x and the average radical capture activity with symbol y. The linear regression equation at a wavelength of 478 nm obtained in the measurement of vitamin C comparator is y = 3.3248x + 9.501 with IC_{50} 12.184, in *L. acidissima* fruit extract is y = 0.7984x + 21.904 with IC_{50} 35.190 and for *L. acidissima* exocarp extract is y = 0.8758x + 19.042 with IC_{50} 35.348, while the results. The IC_{50} value of vitamin C comparator is almost comparable to the IC_{50} value of *L. acidissima* fruit and exocarp extracts. Based on the level of antioxidant strength of the test compounds using DPPH vitamin C reagent, *L. acidissima* fruit and exocarp extract have very strong potential, namely IC_{50} value <50, because the positive sample contains flavonoid and phenol compounds. The exocarp often contains a higher concentration of phenolic compounds and flavonoids, which are potent antioxidants, as a protective mechanism against environmental stressors like UV radiation, pathogens, and herbivores (Ghasemzadeh & Ghasemzadeh, 2011). In contrast, the fruit pulp may have a lower concentration of these compounds, as its primary role is more related to seed dispersal and storage of sugars and nutrients (Nadjib, 2018).

CONCLUSION

Based on the results of the research conducted, it can be concluded that the vitamin C comparator has an IC_{50} value of 12.184 means antioxidant activity is very strong, *L. acidissima* fruit extract has an IC_{50} value of 35.190 means antioxidant activity is very strong and *L. acidissima* exocarp extract has an IC_{50} value of 35.348 means antioxidant activity is very strong. So that the samples of *L. acidissima* fruit and exocarp extract have very strong antioxidant potential.

REFERENCES

Aktumsek, A., Zengin, G., Guler, G. O., Cakmak, Y. S., Duran, A., 2013. *Antioxidant Potentials and Anticholinesterase Activities of Methanolic and Aqueous Extracts of Three Endemic Centaurea L. species. Food and Chemical Toxicology*. 55, 290-296.

Dewi, R,. 2013. Bioktivitas Buah *L. acidissima* (*Limonia acidissima*) Bima dan Penentuan Sidik Jarinya menggunakan Kromatografi Lapis Tipis. *Skripsi*. Institut Pertanian Bogor: Bogor, 65-69

Disi, M. Z. A., Yusuf, M. F. R. H., Rahman, I. 2024. Aktivitas Antioksidan dan Penentuan Kadar Total Flavonoid Fraksi Daging Buah Pala (*Myristica fragrans* Houtt). *Journal Syifa Sciences and Clinical Research*. 6(3): 287-300. https://doi.org/10.37311/jsscr.v6i3.28740.

Ghasemzadeh, A., & Ghasemzadeh, N. (2011). *Flavonoids and phenolic acids: Role and*

biochemical activity in plants and human. Journal of Medicinal Plants Research, 5(31), 6697–6703.

Hidayati, M. D., Ersam, T., Shimizu, K., Fatmawati, S., 2017. *Antioxidant Activity of Syzygium polyanthum Extracts. Indonesian Journal of Chemistry*. 17 (1), 49-53.

Kusuma, I. M., Veryanti, P. R., Saragih, E. T. D. 2019. Pemanfaatan Ekstrak Kulit Buah Kawista (*Limonia acidissima*) Sebagai Anti Asam Urat Secara In Vivo pada Mencit Jantan. Institut Sains dan Tekhnologi Nasional: Jakarta Selatan, 65-69

Mailandari, M. 2012. Uji Aktivitas Antioksidan Ekstrak Daun *Garcinia kydia* Roxb dengan Metode DPPH dan Identifikasi Senyawa Kimia Fraksi yang Aktif. *Skripsi*. Jakarta. FMIPA. Universitas Indonesia, 1-39

Mulyati, S. 2021. Uji Aktivitas Antioksidan Ekstrak Buah dan Kulit Buah Kawista (*Ferronia limonia* (L) Swingle) dengan Metode DPPH (1,1 Diphenyl-2-picrylhydrazil). *Skripsi*. Universitas Pancasakti. Makassar.

Najib, 2018. *Ekstraksi Senyawa Bahan Alam*. Deepublish: Yogyakarta, 37-39-41.

Paliwal, S. K., Sati, B., Faujdar, S., and Sharma, S., 2017. Antioxidant and Antibacterial Activities of Various Extracts of Inula cuspidata CB Clarke stem. Beni-Suef University Journal of Basic and Applied Sciences. 6 (2), 97-105.

Siagian, P. 2012. *Keajaiban antioksidan*. Jakarta: PT Gramedia Pustaka Utama

Suratmo, 2009. Potensi Ekstrak daun Sirih Merah (*Piper crocatum*) sebagai Antioksidan. *Skripsi*. Jurusan Kimia, Fakultas MIPA, Universitas Brawijaya Malang, Indonesia.

Wahdaningsih, S., Setyowati, E. P., Wahyuono, S. 2011. Aktivitas Penangkap Radikal Bebas dari Batang Pakis (Alsophila glauca). *Majalah Obat Tradisional*, 16 (3): 156 – 160.