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Antioxidant Activity on The Extract Combination of *Averrhoa bilimbi L.* and *Phaleria macrocarpa*

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ABSTRAK

Latar Belakang: *Berbagai penyakit degenerative seperti DM erat kaitannya dengan stres oksidatif dan abnormalitas lipid akibat radikal bebas yang tidak terkendali. Salah satu penangkal radikal bebas yaitu antioksidan, Averrhoa bilimbi L. dan Phaleria macrocarpa merupakan tanaman yang memiliki banyak khasiat yang bermanfaat terutama senyawa antioksidannya.*

Tujuan: *Penelitian ini bermaksud untuk melihat aktivitas antioksidan dari kombinasi ekstrak Averrhoa bilimbi L. dan Phaleria macrocarpa berdasarkan pada nilai IC_{50} sehingga dapat dilihat potensinya sebagai alternatif pengobatan DMT2.*

Metode: *Ekstrak diolah menggunakan metode maserasi dengan etanol 96% kemudian dilakukan remaserasi sebanyak dua kali, lalu dikombinasikan dengan konsentrasi 50% Averrhoa bilimbi L dan 50% Phaleria macrocarpa. Pengujian aktivitas antioksidan dilakukan dengan metode DPPH berdasarkan perhitungan nilai IC_{50}.*

Hasil: *Hasil penelitian menunjukkan bahwa asam askorbat memiliki nilai IC_{50} sebesar 10,65 ppm, Averrhoa bilimbi L. memiliki nilai IC50 sebesar 8,29 ppm, Phaleria macrocarpa memiliki nilai IC_{50} sebesar 6,43 ppm, dan kombinasi ekstrak Averrhoa bilimbi L. dan Phaleria macrocarpa memiliki nilai IC_{50} sebesar 6,43 ppm. Nilai IC_{50} sebesar 5,26 ppm. Semakin kecil nilai IC_{50} maka semakin kuat aktivitas antioksidannya.*

Kesimpulan: *Berdasarkan hasil tersebut dapat disimpulkan bahwa kombinasi ekstrak Averrhoa bilimbi L. dan Phaleria macrocarpa memiliki aktivitas antioksidan yang lebih tinggi dibandingkan dengan kedua ekstrak tunggal sehingga kombinasi tersebut berpotensi menjadi alternatif pengobatan DMT2.*

KATA KUNCI*: Averrhoa bilimbi L.; Phaleria macrocarpa; DPPH; IC_{50}*

ABSTRACT

Background: *Various degenerative diseases such as DM are closely related to oxidative stress and lipid abnormalities due to uncontrolled free radicals. One of the free radical scavengers, namely antioxidants, Averrhoa bilimbi L. and Phaleria macrocarpa are plants that have many useful properties, especially their antioxidant compounds.*

Objectives: *This study aims to examine the antioxidant activity of the combination of extracts of Averrhoa bilimbi L. and Phaleria macrocarpa based on the IC50 value so that it can be seen its potential as an alternative treatment for DMT2.*

Methods: *The extract was processed using the maceration method with 96% ethanol then remaceration twice, then combined with a concentration of 50% Averrhoa bilimbi L and 50% Phaleria macrocarpa. Testing of antioxidant activity was carried out using the DPPH method based on the calculation of the IC50 value.*

Results: *The results showed that ascorbic acid had an IC_{50} value of 10.65 ppm, Averrhoa bilimbi L. had an IC_{50} value of 8.29 ppm, Phaleria macrocarpa had an IC_{50} value of 6.43 ppm, and a combination of Averrhoa bilimbi L. and Phaleria macrocarpa extracts have an IC_{50} value of 5.26 ppm. The smaller the IC_{50} value, the stronger the antioxidant activity.*

Conclusion: *Based on these results, it can be concluded that the combination of extracts of Averrhoa bilimbi L. and Phaleria macrocarpa has higher antioxidant activity than the two single extracts so the combination has the potential to be an alternative treatment for DMT2.*

KEYWORDS*: Averrhoa bilimbi L.; Phaleria macrocarpa; DPPH; IC_{50}*

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INTRODUCTION

Natural ingredients are often used as traditional medicine by the people of Indonesia. These drugs are usually used for prevention, cure, and recovery efforts. The use of natural ingredients in traditional medicine is believed to be safer and has relatively low side effects compared to synthetic drugs. One of the natural ingredients often used is natural ingredients with high antioxidant content. Antioxidants have a role in reducing the capacity of free radicals so that they can inhibit cell damage. Antioxidants have oxidation-reduction reactions that play a role in neutralizing free radicals, reducing singlet and triplet oxygen as well as peroxide decomposition (1).

There are two ways to ward off free radicals: from within and outside the body(2). The way from within the body is from metabolism while the way from outside the body is by consuming foods and drinks high in antioxidants such as vitamins A, C, E, and beta-carotene(3). However, antioxidants in the body cannot neutralize free radicals when the amount exceeds the maximum capacity of the enzyme. So it takes antioxidants from outside the body to help ward off free radicals (4).

Free radicals are molecules that have one or more unpaired electrons. These free radicals can cause various degenerative diseases such as diabetes mellitus, hypertension, stroke, and heart disease. In addition, free radicals can also cause premature aging (5). In patients with DMT2 free radicals especially reactive oxygen species (ROS) in all tissues are formed due to hyperglycemia conditions that damage the natural enzymatic antioxidant defenses(6). Due to the high free radicals, lipid peroxidation also increases in cell membranes and produces the final product, namely malondialdehyde (MDA) (7). The research of Sunita et al., (2020) revealed that DMT2 patients experienced a significant increase in MDA by 6.77 times compared to the control group (healthy people) and found a strong relationship between GDP and MDA levels (8). MDA is one of the secondary products that is considered the best biomarker of oxidative stress on lipids (9). Therefore, in patients with DMT2 the need for antioxidant compounds to ward off free radicals. One of the natural ingredients that have lots of antioxidants are *Averrhoa bilimbi L*. and *Phaleria macrocarpa*. *Averrhoa bilimbi L*. and *Phaleria macrocarpa* are fruits that are rarely used properly, so optimization of the benefits of these fruits can be done by making them a new alternative therapy for people with DMT2.

Averrhoa bilimbi L. is known as a year-round flowering garden plant that can grow up to 5-10 meters with the characteristics of having a short stem with a diameter of 30 cm, and low and wavy branches. The leaves are arranged in a double with a small, oval shape. The flowers are compound flowers arranged in panicles 5-20 cm long in groups and the fruit is oval (6). *Averrhoa bilimbi L*. contains flavonoid compounds, saponins, tannins, and triterpenoids. In addition, *Averrhoa bilimbi L* also contains a lot of vitamin C. A large amount of content in *Averrhoa bilimbi L*. can be used as a medicine for coughs, rheumatism, mumps, canker sores, diabetic diarrhea, and hypertension (10). The high level of antioxidants in *Averrhoa bilimbi L*. is proven by research conducted by Yanti and Saputri (2019) which states that *Averrhoa bilimbi L*. has high antioxidant levels and the more extracts tested, the higher the antioxidants (3).

In addition to *Averrhoa bilimbi L*., a fruit that is rarely used properly is *Phaleria macrocarpa*. *Phaleria macrocarpa* is a shrub from the Thymelaceae tribe that thrives in the lowlands to an altitude of 1200 meters above sea level. *Phaleria macrocarpa* has a green color before ripe and maroon when ripe (11). *Phaleria macrocarpa* has a high flavonoid content. These flavonoids act as antioxidants, cardioprotective, antidiabetic, anticancer, and anti-inflammatory. In addition to flavonoids, *Phaleria macrocarpa* also contains saponins, tannins, alkaloids, and phalerin (12). The high level of antioxidants in *Phaleria macrocarpa* is also proven by research conducted by Putri, et al (2020) which states that *Phaleria macrocarpa* has a very strong antioxidant activity when viewed from the IC_{50} value (13). Both fruits are scientifically proven to have good health benefits. However, there is still no scientific evidence regarding the combination of the two fruits, so this study aims to determine the antioxidant activity of the combination of *Averrhoa bilimbi L*. and *Phaleria macrocarpa* extracts against DPPH radicals expressed in IC50 values so that they can see their potential for DMT2 patients.

MATERIALS AND METHODS

The materials used in this study were simplicia *Averrhoa bilimbi L*. and *Phaleria macrocarpa*, 96% ethanol, aquadest, ascorbic acid, and DPPH. The tools used in this study were analytical balance, and spectrophotometry.

Sample preparation

The sample used in this study was simplicia of *Averrhoa bilimbi L*. obtained from the Laboratory of Herbal Materia Medica Batu and *Phaleria macrocarpa* obtained from Merapi Farma Yogyakarta plantation. These fruits have met the standard before being made into simplicia such as not easily brittle, odorless, no bacteria growing, not moldy, light green in *Averrhoa bilimbi L*. and red in *Phaleria macrocarpa*, oval in *Averrhoa bilimbi L*., and round in shape. and smooth on the *Phaleria macrocarpa*. Then the two simplicia are mashed until each is in the form of a powder.

Extraction

Both simplicia were extracted using 96% ethanol solvent for 24 hours by maceration method and remaceration was performed twice. The maceration results obtained were then filtered using filter paper and evaporated using a water bath to obtain a thick extract. The extract was then combined with a concentration of 50% *phaleria macrocarpa* fruit extract and 50% *Averrhoa bilimbi L* fruit extract to test their antioxidant activity.

Antioxidant Activity test

One of the tests to determine the antioxidant activity of radical scavengers is the DPPH method (1,1 Diphenyl-2-picrylhydrazyl). Initially, the extract from *Averrhoa bilimbi L*., *Phaleria macrocarpa*, and their combination was weighed as much as 10 mg, then dissolved in 10 mL of ethanol p.a (1000 g/mL), this solution was the mother liquor. Then several volume variations of the sample mother liquor were pipetted (6 different volumes to obtain 6 variations of sample concentration for the sample antioxidant activity curve) and each was added to a 5 mL volumetric flask, then added ethanol p.a. to the mark and homogenized. In each solution, 1 mL of sample was pipetted and 1 mL of DPPH solution was added, then homogenized and incubated at an operating time range of 0-60 minutes at room temperature, and the absorbance was measured at a length of 516 nm.

The DPPH method provides information about the rate at which they are tested and their reactivity with radicals. DPPH provides strong absorption at a wavelength of 516 nm in dark purple. The oxidizer consumes electrons and loses color in relation to the number of electrons captured. Based on research conducted by Maesaroh, et al (2018) which compared various methods of testing antioxidant activity, it was found that the DPPH method was the most effective and efficient compared to the FRAP and FIC methods (14).

IC_{50} Value

The percentage of inhibition was calculated using the formula:

$$\% \text{ Inhibition} = \frac{(\text{Absorbance of blank} - \text{absorbance of sample})}{\text{Absorbance of blank}} \times 100\%$$

Percent inhibition is the inhibition value of free radicals. Meanwhile, to calculate the antioxidant concentration needed to reduce DPPH by 50%, the IC_{50} value was calculated. The IC_{50} value is calculated by entering the 50% value into the standard curve equation as y and then calculating the x value as the IC_{50} concentration. The following is the IC50. calculation formula; (15).

$$y = ax + b$$
$$50 = ax + b$$
$$(x)\ IC_{50} = \frac{50 - b}{a}$$

The smaller the IC_{50} value, the higher the antioxidant activity. IC_{50} classification is: Very strong if <50 ppm, strong if 51-100 ppm, moderate if 101-150 ppm, weak if 150-200 ppm, and very weak if > 200 ppm (16).

RESULTS AND DISCUSSIONS

In this study, the samples used were *Averrhoa bilimbi L*. and *Phaleria macrocarpa*. The fruits were cleaned and then dried using the oven method at 50°C for one hour. This drying process is intended so that the sample can last a long time without reducing its content during storage and shipping. The dry sample in the form of simplicia was then extracted using the maceration method in 96% ethanol with two remaceration processes. The maceration method was chosen because maceration is a cold extraction method that cannot damage the active substance in the sample due to heating. The choice of ethanol solution as a solvent is based on its polarity, which can dissolve semi-polar to polar secondary metabolites, including the active substances found in *Averrhoa bilimbi L*. and *Phaleria macrocarpa*. Ethanol as a solvent also has safe properties that are not toxic, prevents the growth of mold at a concentration of more than 20%, is safe and harmless to the environment, and has a low boiling point so that it is easily evaporated. The properties of ethanol make the absorption process better (17). The maceration results obtained are then filtered and evaporated using a water bath so that a thick extract is obtained.

After obtaining the extract from each sample, the antioxidant activity test was measured using the DPPH method using UV-Vis spectrophotometry at a maximum absorption wavelength of 516 nm with an absorbance value of 0.611. Furthermore, the results of the measurement of the absorbance of the standard solution are then made into a standard curve between the absorbance and concentration of each sample to obtain a linear regression equation. The results of the linear regression equation can then determine the value of an antioxidant activity based on the IC_{50} value.

Based on the results of data analysis in table 1, it can be seen that testing the antioxidant activity of ascorbic acid (vitamin C) obtained an IC_{50} value of 10.65 ppm which means the antioxidant activity is very strong, this is the result of calculations from the linear regression equation, namely y = 6.9139x – 23,631. Ascorbic acid is one of the natural antioxidants that is the comparison sample in this study, the IC_{50} value of ascorbic acid is in line with research conducted by Safrida et al (2020) which states that the IC50 value of ascorbic acid is also

Table 1. Results of Determination of Antioxidant Activity of Ascorbic Acid

Concentration (ppm)	Absorbance	Inhibition (%)	Linear Regression Equation	IC_{50} (ppm)
1.000	0.713	-16.69	y = 6.9139x - 23.631	10.65 (Very strong)
2.000	0.671	-9.81		
3.000	0.631	-3.27		
4.000	0.583	4.58		
5.000	0.546	10.64		
6.000	0.5	18.17		
7.000	0.461	24.55		

Table 2. Results of Determination of Antioxidant Activity of *Averrhoa bilimbi L.* Extract

Concentration (ppm)	Absorbance	Inhibition (%)	Linear Regression Equation	IC_{50} (ppm)
200	0.58	4.58	y = 6.21x - 1.5367	8.29 (Very strong)
400	0.543	11.07		
600	0.502	17.83		
800	0.473	22.47		
1000	0.448	28.69		
1200	0.338	36.55		

included in the very strong category, which is 6.35 ppm (18).

Based on the results of data analysis in table 2, it can be seen that testing the antioxidant activity of *Averrhoa bilimbi L*. obtained an IC_{50} value of 8.29 ppm, which means that the antioxidant activity is very strong, this is in line with research conducted by Chowdhury et al (2012) which stated that the antioxidant activity based on the IC_{50} value of *Averrhoa bilimbi L*. is also included in the very strong category, which is 20.35 ppm (19). The IC50 value is the result of calculations from the linear regression equation, namely y = 6.21x – 1.5367.

Based on the results of data analysis in table 3, it can be seen that the antioxidant activity test on the extract of *Phaleria macrocarpa* fruit obtained an IC_{50} value of 6.43 ppm, which means that the antioxidant activity is very strong. This is in line with research conducted by Putri et al (2020) which stated that the extract of *Phaleria macrocarpa* fruit has a very strong antioxidant activity with an IC50 value of 28.24 ppm (13). The IC_{50} value is the result of the calculation of the linear regression equation, namely y = 5.5686x + 14,213.

Table 3. Determination of the Antioxidant Activity of *Phaleria macrocarpa* Extract

Concentration (ppm)	Absorbance	Inhibition (%)	Linear Regression Equation	IC_{50} (ppm)
10	0.48	20.40	y= 5.5686x + 14.213	6.43 (Very strong)
20	0.45	24.77		
30	0.42	31.26		
40	0.39	35.62		
50	0.35	42		
60	0.31	48.17		

Based on the results of data analysis in table 4, it can be seen that testing the antioxidant activity of the combined extract (*Averrhoa bilimbi L*. and *Phaleria macrocarpa*) obtained an IC_{50} value of 5.26 ppm, which means that the antioxidant activity is very strong, this is the result of calculations from linear regression, namely y = 10,811x - 6,842.

Based on the results of the IC_{50} value in each of these samples, it can be seen that the value is <50 ppm, which means that the extract of

Table 4. Results of Determination of Antioxidant Activity of Combination Extracts of *Averrhoa bilimbi L*. and *Phaleria macrocarpa*

Concentration (ppm)	Absorbance	Inhibition (%)	Linear Regression Equation	IC_{50} Value (ppm)
100	0.57	6.05	y= 10.811x - 6.842	5.26 (Very Strong)
200	0.53	13.2		
400	0.46	24.82		
600	0.40	35.30		
800	0.32	47.63		
1000	0.25	58.97		

activity of the combined extract. The content contained in both fruits can help treat people with DMT2 using saponins that work as inhibitors of the -glucosidase enzyme so that it can inhibit the breakdown of carbohydrates into glucose, besides that saponins also reduce glucose absorption in the intestine, inhibit the glucose transporter GLUT-1, glycogen storage. and increased insulin receptor sensitivity in tissues (Dede et al. 2019). The tannins contained in the fruit also have an important role in reducing blood glucose levels. Tannins act as astringents that can precipitate intestinal mucous membrane proteins and form a layer that protects the intestines, thereby inhibiting glucose absorption (Mulyaningsih 2019). The very strong antioxidant activity in the combination of these extracts can indicate that the combined extract of *Averrhoa bilimbi L* and *Phaleria macrocarpa* has a synergistic relationship and can inhibit oxidation reactions. The antioxidants in both fruits, especially flavonoids, can suppress the production of free radicals and their chain reactions by capturing oxidants, inhibiting the production of inflammatory mediators, repairing damaged molecules, and initiating and increasing endogenous antioxidants as the body's defense system to reduce oxidation reactions and reduce MDA levels in people with DMT2. (Elsayed Azab et al. 2019).

CONCLUSIONS AND RECOMMENDATIONS

Based on the results of the study, it can be concluded that the combination of extracts of *Averrhoa bilimbi L.* and *Phaleria macrocarpa* has very strong antioxidant activity. This is evidenced by the IC_{50} result, which is 5.26 ppm. The high antioxidant activity in the combination of the two fruits can potentially be used as a new alternative therapy for DMT2 patients, so it is hoped that the results of this study can be a basic reference for further research.

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