NASKAH PUBLIKASI

AKTIVITAS ANTIOKSIDAN KOMBINASI EKSTRAK DAUN *Averrhoa bilimbi* L. DAN MADU LEBAH KELULUT

ANTIOXIDANT ACTIVITY OF EXTRACT COMBINATION FROM Averrhoa bilimbi L. LEAVES AND STINGLESS BEE HONEY

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Aktivitas Antioksidan Kombinasi Ekstrak Daun *Averrhoa Bilimbi* L. dan Madu Lebah Kelulut

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ANTIOXIDANT ACTIVITY OF EXTRACT COMBINATION FROM *Averrhoa bilimbi* L. LEAVES AND STINGLESS BEE HONEY

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Abstract
Belimbing wuluh (*Averrhoa bilimbi* L.) leaves and stingless bee honey contains phenolic compounds that can act as antioxidants. This study aims to find out the antioxidant activity of extract combination from stingless bee honey (Trigona spp.) and Belimbing Wuluh (*Averrhoa bilimbi* L.) leaves. This research was used DPPH (*2,2-diphenyl-1-picrilhidrazyl*) method. Then measured by UV-VIS spectrophotometry. The results showed the greatest IC_{50} of extract combination from stingless bee honey and *Averrhoa bilimbi* L. leaves was 2:1 (90,36 ppm) and was classified as strong activity. This activity was influenced by the synergistic effect between the combination of secondary metabolites contained therein.

Key words: antioxidants, stingless bee, honey, *Averrhoa bilimbi* L.

AKTIVITAS ANTIOKSIDAN KOMBINASI EKSTRAK DAUN *Averrhoa bilimbi* L. DAN MADU LEBAH KELULUT

Abstrak
Daun belimbing wuluh (*Averrhoa bilimbi* L.) dan madu lebah kelulut mengandung senyawa fenolik yang dapat berperan sebagai antioksidan. Penelitian ini bertujuan untuk mengetahui aktivitas antioksidan dari kombinasi ekstrak madu lebah kelulut (*Trigona* spp.) dan daun belimbing wuluh (*Averrhoa bilimbi* L.). Penelitian ini menggunakan metode DPPH (*2,2-diphenyl-1-picrilhidrazyl*). Kemudian diukur dengan spektrofotometri UV-VIS. Hasil penelitian menunjukan IC_{50} terbesar dari kombinasi 2:1 ektrak madu lebah kelulut dan daun *Averrhoa bilimbi* L. adalah (90,36 ppm) dan tergolong dalam aktivitas yang kuat. Aktivitas ini dipengaruhi oleh efek sinergis antara kombinasi metabolit sekunder yang terkandung di dalamnya.

Kata kunci: antioksidan, lebah kelulut, madu, *Averrhoa bilimbi* L.

Introduction

Indonesia is a tropical country characterized by high temperatures and ultraviolet radiation[1]. Excessive exposure to UV rays can lead to the formation of free radicals in the body that can cause a number of skin problems, including skin redness, pigmentation, and long-term cancer risk[2]. Human health will be affected by skin damage so maintaining and protecting the skin is necessary for health. Therefore, it is necessary to defend against the dangers of free radicals and premature aging that can harm the skin[3]. Premature aging can be prevented through 2 ways, namely internally and externally. Internal prevention is done by increasing the consumption of fruits and vegetables that are high in antioxidants. While externally, one of them is by using cosmetics that have active substances as antioxidants[1]. Antioxidants are very beneficial for health because they can prevent

premature aging and degenerative disorders[4]. Antioxidants can fight free radicals found in the body produced by the body's metabolism, air pollution, contaminated food, and sunlight[5].

Indonesia is rich in natural resources that can be utilized as a source of natural antioxidants, one of which is *Averrhoa bilimbi* L. and stingless bee honey. Stingless bee honey and *Averrhoa bilimbi* L. leaves have been widely used as traditional medicine, not only traditional medicine, the efficacy of *Averrhoa bilimbi* l. leaves and stingless bee honey has been scientifically tested[6]. These plants contain secondary metabolite compounds such as flavonoids and phenolics and *Averrhoa bilimbi* L. leaves have activities as antibacterial, antioxidant, antimicrobial and anti-inflammatory (reduce and suppress inflammation)[7,8,9]. Stingless bees are characterized by having no sting and being small in size[10]. Meanwhile, stingless bee honey has pharmacological effects as an antidiabetic, antioxidant, and antibacterial[11,12]. The purpose of this study was to determine the antioxidant activity of extract from *Averrhoa bilimbi* L. leaves and stingless bee honey

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Metode

Instrument

The tools used extraction include a set of Rotary evaporator, spektrofotometer UV-Vis (*Genesys 10s UV-Vis*) *Genesys 10s UV-Vis*), Volumetric flask (Iwaki), vortex (*Scilogex MX-S*), Measuring cup (Iwaki), Spatula, Micropipettes (Scilogex), and Pasteur pipette.

Material

The materials used for this study include extract combination from stingless bee honey (*Trigona* spp.) and Belimbing Wuluh (*Averrhoa bilimbi* L.) leaves, DPPH, etanol 95%, methanol, and aquadest.

Procedure

a. Preparation Extract from *Averrhoa bilimbi* L. Leaves

Averrhoa bilimbi L. leaves extract that have been obtained are oven dried and mashed, then macerated using 95% ethanol solvent. The resulting macerate was concentrated using a rotary evaporator at 50°C with 200 rpm[13]. The extracts were then made into comparisons as shown in table 1. Each comparison was tested for antioxidant activity.

b. Preparation of 0.1 mM DPPH Solution

DPPH powder weighed as much as 1,98 mg was dissolved using methanol p.a and put into a 50 mL volumetric flask until the volume was sufficient with methanol p.a to the limit mark, then the volumetric flask was given aluminum foil and placed in a dark room[14].

(a) (b)

Figure 1 (a) Stinglees bee honey (b) *Averrhoa bilimbi* L.

Table 1 Comparison of antioxidant test of the combination of stingless bee honey and *Averrhoa bilimbi* L. leaves extract[15]

Ratio	Antioxidant Activity	
	Stingless bee honey (mg)	***Averrhoa bilimbi* L. leaves extract (mg)**
1 : 0	100	0
2 : 1	50	25
1 : 1	50	50
1 : 2	25	50
0 : 1	0	100

c. Antioxidant Test Combination of Stinglees Bee Honey and Extract from *Averrhoa bilimbi* L.

Extract from *Averrhoa bilimbi* L. leaves and stingless bee honey and their combination were weighed as much as 100 mg, then dissolved with methanol in a 10 ml volumetric flask. then dilution with methanol was carried out so that several concentrations of test solutions were obtained at 10, 30, 50, 70, 90 ppm. The results were homogenized using a vortex and added 0,1 mM DPPH as much as 3 mL and incubated for 30 minutes at room temperature under dark conditions, then measured the absorbance at a wavelength of 517 nm. The IC_{50} value was calculated using a linear regression equation with the relationship between concentration and % inhibition[16].

d. Preparation of Vitamin C Solution

Vitamin C weighed as much as 10 mg was put into a 50 ml volumetric flask and dissolved with methanol to obtain a concentration of 200 ppm. This solution was further diluted with methanol until the concentration of vitamin C solution was obtained, namely 2, 3, 4, 5, 6 ppm. The results of vitamin C dilution were added with 3 mL of 0,1 mM DPPH and incubated for 30 minutes at room temperature under dark conditions, then measured the absorbance at a wavelength of 517 nm. The IC_{50} value was calculated using a linear regression equation with the relationship between concentration and % inhibition[16].

Result

The result of thick extract *Averrhoa bilimbi* L. leaves in the study was 13 grams. Maceration results are said to be good if the yield is> 10%[13], in the results of the study, the yield of the extract was 13% so that these results met the standard requirements. Then extract combination from *Averrhoa bilimbi* L. leaves and stingless bee honey was made in a ratio of (1:0), (2:1), (1:1), (1:2), and (0:1). Then tested for antiokxidant activity of extract combination from *Averrhoa bilimbi* L. leaves and stingless bee honey which has the highest antioxidant activity in the 2:1 ratio with an IC_{50} value of 90,36 ppm. The measurement results of antioxidant activity of the combination of 1:1, the combination 1:2, and stingless bee honey are (IC_{50} 106,18 ppm), (IC_{50} 102,10 ppm), (IC_{50} 132,76 ppm) respectively. That the highest activity is possessed by *Averrhoa bilimbi* L. leaves extract with an IC_{50} value of 18,05 ppm.

Discussion

a. Extract Yield Results

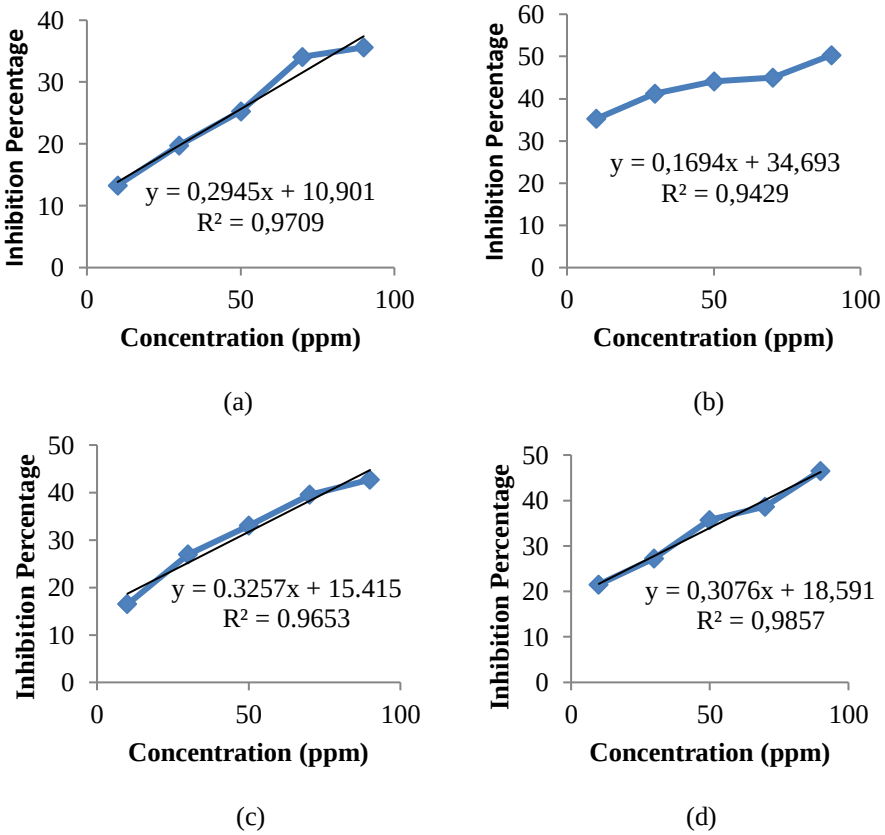
This research uses the maceration method using 95% ethanol solvent. Ethanol 95% is preferred because it can extract more antioxidant compounds than water[17]. The extraction process in the study by soaking 100 grams of fine powder of *Averrhoa bilimbi* L. leaves in a mixture of 1000 mL of 95% ethanol for 3 days, doing 1 solvent change to increase the yield of compounds and yields and stirring occasionally. The purpose of stirring is to create a more even concentration of active compounds in the liquid and also achieve a rapid balance[3]. Then concentrate the macerate using a rotary evaporator at 50°C with 200 rpm to maintain the stability of flavonoid compounds. The concentration was continued using a waterbath at 50°C until a thick extract was obtained. The result of thick extract in the study was 13 grams. Maceration results are said to be good if the yield is> 10%[13], in the results of the study, the yield of the extract was 13% so that these results met the standard requirements.

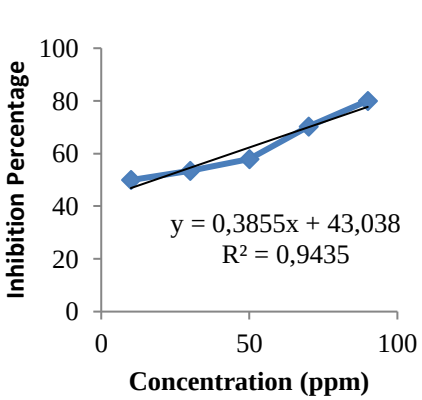
b. Antioxidant Activity Measurement

Antioxidant test was conducted using DPPH free radical immersion method. DPPH method was used because this method is simple, easy, fast, and only requires a small amount of sample[19]. The absorbance of the extract and DPPH can be measured using a spectrophotometer at a wavelength of 517 nm. Antioxidant testing using the DPPH method, the results can be seen in the IC_{50} value. If the IC_{50} value in the test <50 ppm. Then the antioxidant activity is very strong. 50-100 ppm then the antioxidant activity is strong, 100-150 ppm then the antioxidant activity is moderate, 150-200 ppm then the antioxidant activity is weak, and >200 ppm then the antioxidant activity is very weak[20]. Measurement of antioxidant activity of the extract combination from stingless bee honey and *Averrhoa bilimbi* L. leaves using 100 mg of each sample dissolved with methanol to form a mother solution of 10.000 ppm. Then extract combination from *Averrhoa bilimbi* L. leaves and stingless bee honey was made in a ratio of (1:0), (2:1), (1:1), (1:2), and (0:1) so as to obtain a sample solution with a concentration of (10, 30, 50, 70, and

90 ppm)[16]. Measurement of antioxidant activity of each concentration was pipetted each required volume of 0,01; 0,03; 0,05; 0,07; 0,09 mL. Added methanol p.a as much as 10 mL. then added DPPH solution by pipetting as much as 2 mL into the measuring flask of each comparison. Shaken the mixture until homogeneous and allowed to stand for 30 minutes in a dark place then measured the absorbance at a wavelength of 517 nm using Uv-Vis spectrophotometry.

Figure 2 Preparation of Antioxidant Test Combination of Stinglees Bee Honey and Extract from *Averrhoa bilimbi* L.





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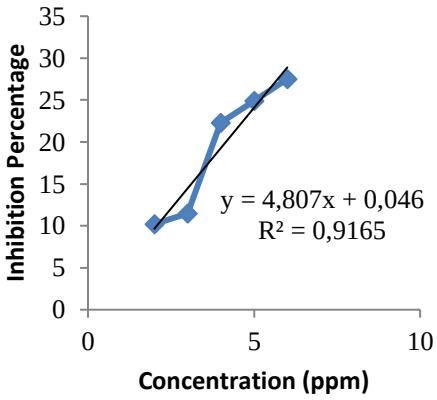

(f)

Based on figure 3, it shows that the highest activity is possessed by *Averrhoa bilimbi* L. leaves extract with the a linear regression equation y= 0.3855x + 43.038 and R^2 = 0.9435 which shows the IC_{50} value of 18,05 ppm, while of the three extract combination from *Averrhoa bilimbi* L. leaves and stingless bee honey which has the highest antioxidant activity in the 2:1 ratio with the a linear regression equation y = 0,1694x + 34,693 and R^2 = 0,9429 which shows the IC_{50} value of 90,36 ppm. The measurement results of antioxidant activity of the combination of 1:1 with the a linear regression equation y = 0,3257x + 15,415 and R^2 = 0,9653 which shows the IC_{50} value of 106,18 ppm, the combination 1:2 with the a linear regression equation y = 0,3076x + 18,591 and R^2 = 0,9857 which shows the IC_{50} value of 102,10 ppm, and 1:0 with persamaan regresi linear madu lebah kelulut yaitu y = 0,2945x + 10,901 dan R^2 = 0,9709 which shows the IC_{50} value of 132,76 ppm. The IC_{50} value of the three extract combination from *Averrhoa bilimbi* L. leaves and stingless bee honey has moderate antioxidant activity.

In the antioxidant test using the DPPH test using vitamin C as a comparison, because vitamin C is a natural antioxidant compound that can be used as a comparison in the antioxidant activity test[20]. The IC_{50} value of vitamin C in table 3 is used to compare the results of the IC_{50} value of extract combination from *Averrhoa bilimbi* L. leaves and stingless bee honey. The IC_{50} value of vitamin C is 10,39 ppm with the a linear regression equation y= 4.807x + 0.046 dan R^2 = 0.9165. The IC_{50} value of vitamin C shows that vitamin C has very strong antioxidant activity.

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LAMPIRAN

NP 1 : ANTIOXIDANT ACTIVITY OF EXTRACT COMBINATION FROM Averrhoa bilimbi L. LEAVES AND STINGLESS BEE HONEY

by Bunga Putri Sari

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