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Original article

Antioxidant assay of *Averrhoa bilimbi* L flower extract, chemical compound and its utilization potential

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Abstract

Belimbing wuluh (*Averrhoa bilimbi* L.) is well known in Indonesia. This plant has wide medicinal properties, ranging from lowering cholesterol, uric acid, anti diabetic, antihypertensive, antioxidant, anti-inflammatory, cough medicine, thrush, and digestive disorders to treating acne. A number of studies have been conducted on the content of its fruit and leaves, but there is still a gap in the literature on the content of the flowers. This study was conducted to determine the effectiveness of the methanol extract of *A. bilimbi* flower as a natural antioxidant, to determine the chemical compounds contained in it by using GC-MS and to determine its potential utilization. Methanol extract of *A. bilimbi* crown and petals has the ability as an antioxidant with a very low category. The antioxidant activity of the crown flower is higher than that of the petals. The methanol extract of the crown and petals was dominated by Hexanedioic acid, γ-Sitosterol and Hexadecanoic acid compounds. Based on the compounds it contains, *A. bilimbi* extract can be used as an antimicrobial, anti diabetic, anticancer, lowering cholesterol levels and for maintaining skin health.

Keywords: antimicrobial, antidiabetic, anticancer, anticholesterol

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Introduction

Averrhoa bilimbi L. known by local name *belimbing wuluh*, is well known in Indonesian society. This plant is thought to have originated from the Maluku islands (Thomas, 2007). Despite the sour taste of *A. bilimbi*, the fruit has a distinctive taste and gives a certain aroma, making *A. bilimbi* often used as a mixture in various traditional dishes.

A. bilimbi has various medicinal properties, the fruit is efficacious as lowering cholesterol, uric acid (Harjana, 2011), diabetes mellitus (Kurniawati & Lastri, 2016), cough medicine, canker sores (Winarto, 2004), as an antioxidant (Hasanuzzaman et al., 2013) and can be used to treat acne (Saputra and Anggraini, 2016). *A. bilimbi* leaves have benefits as antioxidants, anti-inflammatory (Hasim et al., 2019), anti diabetic and antihypertensive (Kurniawati & Lastri, 2016). *A. bilimbi* flowers apart from being a cough medicine can also be used as a medicine for rheumatic pain, mumps, rheumatism, thrush, acne, tinea versicolor, high blood pressure, and toothache (Ardananurdin et al., 2004). *A. bilimbi* flower has the ability as an anti-microbial which is also supported by several research results which state that the star fruit flower has the ability as an antibacterial against *Salmonella typhi*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Stapylococcus aureus* (Ardananurdin et al., 2004; Poespitaningtyas, 2004; Wibisono 2004). In addition, this plant is also reported to be able to cure stomach pain, diarrhoea, indigestion and inflammation of the rectum (Thomas, 2007).

A. bilimbi fruit extract is reported to contain alkaloids, saponins, tannins, flavonoids, phenols, and triterpenoids (Hasanuzzaman et al., 2013), oxalate compounds, essential oils and pectin (Rahayu, 2013). Meanwhile *A. bilimbi* leaves are reported to contain flavonoid compounds, phenols, alkaloids, tannins, and coumarins (Valsan and Raphael, 2016), sulfur, formic acid, peroxidase, calcium oxalate, and potassium citrate (Rahayu, 2013). Herbie (2015) stated that the stems of *A. bilimbi* contain saponins, tannins, glucosides, calcium oxalate, sulfur, formic acid, and peroxidase.

Quite a number of studies have been conducted on the content of the fruit and leaves of *A. bilimbi*, but until now there is still little literature on the content of *A. bilimbi* flower. *A. bilimbi* flower is believed to have the ability as an antioxidant. This study was conducted to determine the effectiveness of *A. bilimbi* flower methanol extract as a natural antioxidant, to determine the chemical compounds contained in it by GC-MS and to determine its potential utilization.

Methods

Tools and materials

The tools used in this study include vacuum rotary evaporator IKA RV10, UV-Vis Thermo Genesys 30 spectrophotometer, Shimadzu GCMS-QP 2010, vortex mixer, digital balance, micro pipette, erlenmeyer, measuring cup and test tube. Meanwhile, the materials used in this study include *A. bilimbi* flower, DPPH (2,2-diphenyl-1-picrylhydrazyl or free radical scavenger) and 95% methanol.

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Sample preparation

A. *bilimbi* flowers were obtained from Kerambitan District, Tabanan Regency, Bali (Figure 1). The sample treatment process begins by separating the flower into two separate parts, one part is the crown of the flower and the other is the other part of the flower (petals, pistils, etc.). Furthermore, each part of the plant is dried for 4-5 days.

Figure 1. Flowers of *A. bilimbi*

Maceration

The dried plant parts were weighed and soaked using 95% methanol as solvent. Maceration was performed for 4-5 days at room temperature. The filtrate was then filtered using filter paper and evaporated with a vacuum rotary evaporator to separate the crude extract from the solvent. The crude extract obtained was stored in a closed dark container for use in subsequent tests.

Antioxidant Assay Using DPPH

The antioxidant activity assay was carried out using the DPPH method, which is a reagent that reacts with antioxidants, this reagent has a purple initial colour and if it meets antioxidants it will produce a yellow colour. The yellower the sample test results indicate the greater the antioxidant content, and vice versa, if the DPPH colour remains purple it indicates the low antioxidant content of the test sample (Li et al., 2009).

Each flower part extract was dissolved in 95% methanol to various concentrations (50 ppm, 100 ppm, 150 ppm, 200 ppm, 250 ppm, and 300 ppm). Each extract solution was taken as much as 1 mL, put in a test tube and added 4 mL of 40 ppm DPPH solution (in methanol) and vortexes until homogeneous. The solution was incubated for 30 minutes in the dark and the absorbance was measured at a wavelength of 517 nm. Then, the absorbance and IC50 values were calculated. DPPH 40 ppm also calculated the absorbance value as a control. As a comparison, the absorbance value and the IC50 value were also calculated, while the concentration variations were: 1 ppm, 2 ppm, 3 ppm, 4 ppm and 5 ppm. The antioxidant activity of the sample can be determined by calculating the percentage of DPPH absorption inhibition using the formula:

$$Absorbance\ Percentage = \frac{(Absorbance\ Control - Sample\ Absorbance)}{Absorbance\ Control} x\ 100\%$$

Antioxidant activity was determined using the IC50 value (50% Inhibition Concentration). IC50 value is a strong indicator of whether or not the antioxidant power of a compound. IC50 is a number that indicates the concentration of the extract capable of inhibiting the activity of a radical by 50%. The IC50 value of each sample concentration was calculated using the linear regression equation formula, which stated the relationship between the concentrations of the antioxidant fraction expressed as the "x" axis and the percentage of inhibition expressed as the "y" axis of the measurement replication series.

GC-MS Analysis

To find out the chemical compounds contained in the extract, GC-MS analysis was carried out. The instrument used was Shimadzu GCMS-QP 2010 with a stationary phase Rtx-5MS (5% diphenyl/95% dimethyl polysiloxane) column length of 30 m, diameter of 0.25 mm. Ultra high purity helium carrier gases with a pressure of 75.2 kPa, flow rate of 1.3 mL/min, injection volume of 6. Injector temperature 230°C, ion source temperature 200°C, interface temperature 270°C, split less mode. The programmed column of 50°C was maintained for 3 min, and then increased to 150°C at an increasing rate of 5°C/min. The final column temperature was 270°C at an increasing rate of 3°C/min and maintained for 2 min.

Results and Discussion

The antioxidant assay results showed that the crown and petal methanol extract of *A. bilimbi* had IC50 values of 418.32 and 649.97, respectively (Figure 2). The antioxidant ability of these two extracts is classified as very weak. The antioxidant activity of the flower crown is higher than that of the flower petals

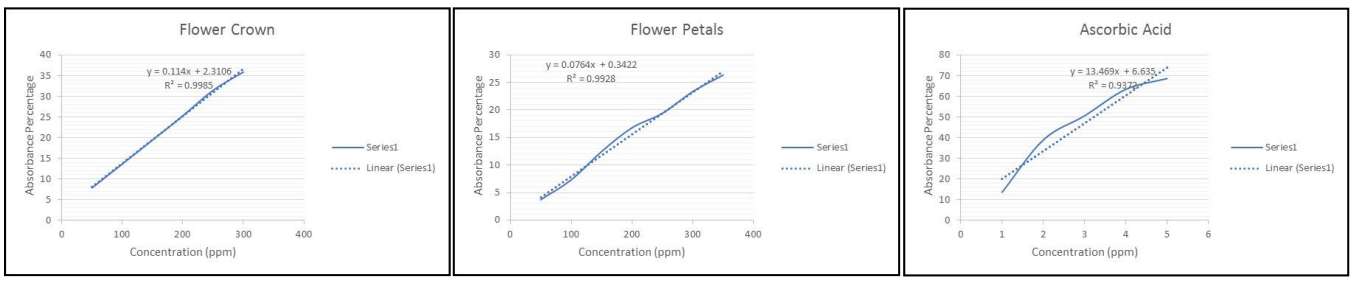

Figure 2. Antioxidant assay regression graph

The antioxidant activity of a compound can be divided into several categories: very strong, strong, moderate, weak, and very weak. Antioxidants are said to be very strong if they have an IC50 value of less than 50 ppm, a strong antioxidant has an IC50 value in the range of 50 ppm to 100 ppm, moderate antioxidants have an IC50 value ranging from 100 ppm to 150 ppm, weak antioxidants have a range of 150 ppm to 200 ppm, and IC50 of more than 200 ppm is a very weak antioxidant category (Blois, 1985). When compared with the IC50 value of ascorbic acid which is 5.50, the crude extract of the crown and petals has a much lower antioxidant activity, this is presumably because the extract of the crown and petals did not contain compounds that act as antioxidants, or extraction using methanol as a solvent could not dissolve compounds that were antioxidants. of flowers. Another possibility is that the two extracts are crude extracts (not pure compounds) which contain many other compounds that are not antioxidants, in contrast to ascorbic acid which is used as a comparison, which are pure compounds that do have antioxidant properties.

The results of the GC-MS analysis (Figure 3 & Figure 4) showed that the methanol extract of the crown and petals were both dominated by Hexanedioic acid, bis(2-ethylhexyl) ester compounds. Compounds of γ - Sitosterol and Hexadecanoic acid, ethyl ester were also contained in both extracts in quite high amounts (Table 1 & Table 2).

Table 1. Chemical compounds contained in the methanol extract of the flower crown

No.	Ret. Time	Area	Area (%)	Name	Similarity (%)
1	37.384	5481521	5.14	Hexadecanoic acid, ethyl ester	92
2	48.879	87325774	81.88	Hexanedioic acid, bis(2-ethylhexyl) ester	97
3	61.732	13846801	12.98	γ-Sitosterol	89

Table 2. Chemical compounds contained of flower petals

No.	Ret. Time	Area	Area (%)	Name	Similarity (%)
1	37.390	10430919	6.88	Hexadecanoic acid, ethyl ester	92
2	40.855	2742160	1.81	Phytol	93
3	42.247	2715221	1.79	Butyl 9,12-octadecadienoate	91
4	42.441	4275562	2.82	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl ester	80
5	46.072	3293008	2.17	Tetracosane	91
6	48.879	87325774	81.88	Hexanedioic acid, bis(2-ethylhexyl) ester	97
7	50.413	3531726	2.33	Hexatriacontane	90
8	51.382	6708987	4.34	Hexatriacontane	90
9	55.430	2868927	1.89	Hexatriacontane	90
10	56.332	4660616	3.08	Hexatriacontane	92
11	59.362	2919886	1.93	Squalene	93
12	60.994	18217489	12.02	Tetratriacontane	93
13	61.699	13981165	9.23	γ-Sitosterol	86

Compounds of hexadecanoic acid and hexanedioic acid are compounds that are also found in the microalgae *Haematococcus pluvialis* which are reported to have antioxidant and antimicrobial abilities (Rodríguez-Meizosoetal., 2010). Hexadecanoic acid, also known as palmitic acid, is a saturated fatty acid which is the main component of the ethanolic extract of the microalgae *Nannochloropsis* sp. (Agustini et al., 2014). According to Agoramoorthy et al., (2007) fatty acids such as palmitic acid can function as antibacterial and antioxidant.

The compound γ-sitosterol is an important plant sterol, first reported to be found in *Girardinia heterophylla*. γ-sitosterol is reported to be able to reduce hyperglycaemia in STZ-induced diabetic rats due to increased insulin secretion and inhibition of glucogenesis (Balamurugan et al. 2011). Docking studies of γ-sitosterol ligand with four different target proteins showed that this compound is a good molecule, which is compatible with various targets associated with diabetes mellitus, so that γ-sitosterol can be considered to be developed into an antidiabetic protein (Balamurugan et al. 2012).). Sundarraj et al. (2012) reported that γ-sitosterol contained in *Acacia nilotica* has anticancer activity in vitro, γ-sitosterol has potential anticancer activity through growth inhibition, cell cycle arrest and apoptosis in cancer cells.

Phytol is an organic compound that is also used in the synthetic manufacture of vitamins E and K_1. Phytol is also a compound that acts as an effective adjuvant with little toxicity in antibacterial immunity testing (Lim et al, 2006). Pratiwi & Rivai (2015) stated that terpenoid compounds such as phytol have antibacterial activity.

Squalene is an organic compound commonly found in plants, animals and humans. Squalene is a triterpene type organic compound that is vital in the synthesis of hormones, vitamin D, cholesterol and other substances in the body. Squalene is also often used as an ingredient in moisturizers or antioxidant supplements. Naturally, squalene can be formed through the breakdown of cholesterol in the liver and circulates in the blood. Besides being able to maintain healthy skin, squalene is also believed to reduce bad cholesterol (LDL) levels, prevent cardiovascular disease, and have antitumor and anticancer effects (Gunes, 2013).

9,12,15-Octadecatrienoic acid or α-linolenic acid is a type of essential fatty acid that is often found in various types of grains such as walnuts, chia, hemp, soybeans and other vegetable oils (Ghanaim, 2012). These fatty acids cannot be formed in the body, so they must be consumed through food (Harrison, 2006). α-linolenic acid (ALA), is an omega-3 fatty acid that is known to have more properties than other fatty acids, especially in preventing damage to cell membranes. Besides being

useful for normal growth, omega 3 also has a critical role in the formation and growth of brain function. More in-depth research has also found that omega 3 fatty acids can reduce swelling and help prevent several chronic diseases such as heart disease, arthritis and alzheimer's (Pratiwi, 2012).

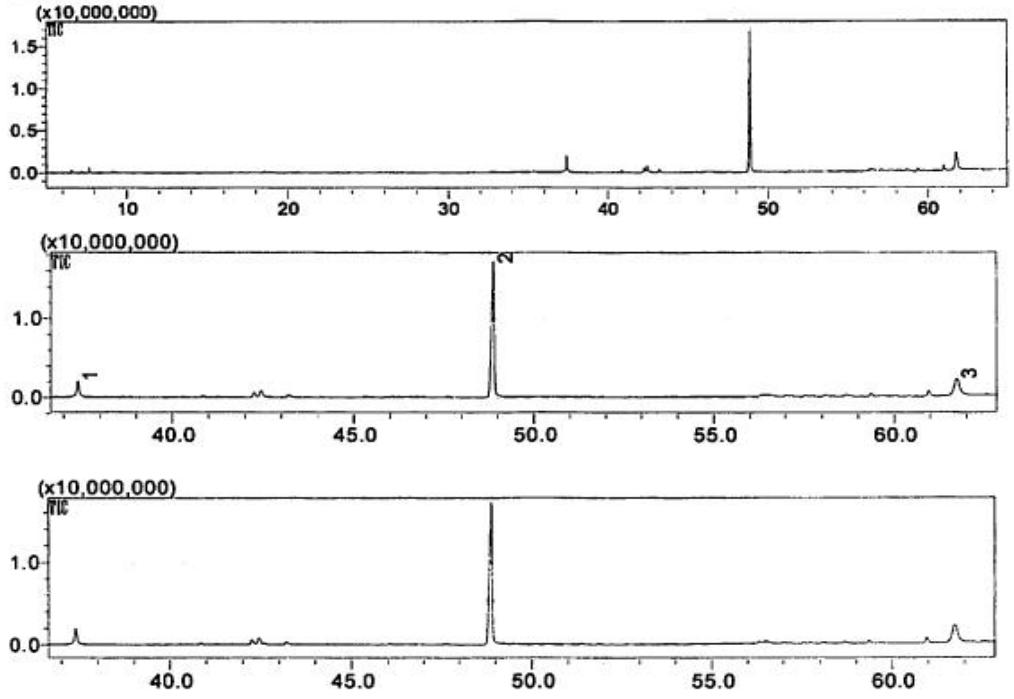

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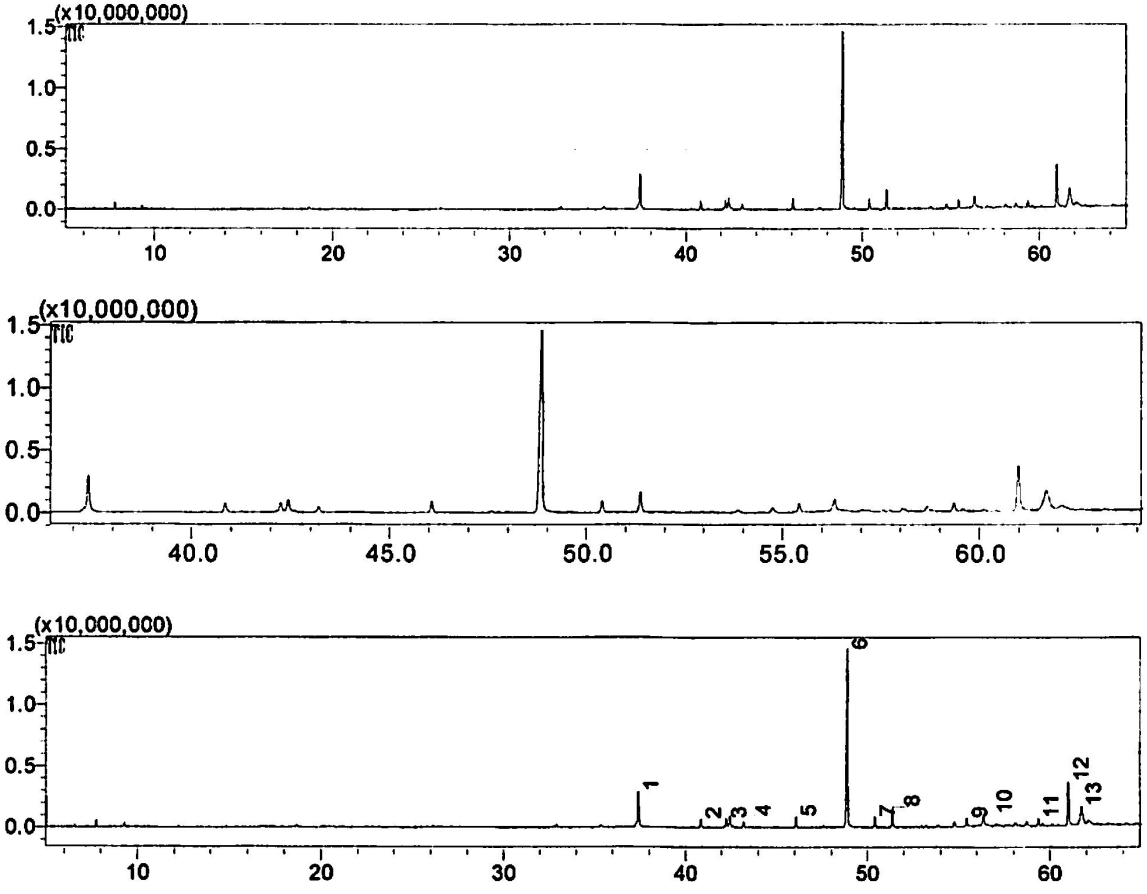
Figure 3. Results of GC-MS analysis of methanol extract of *A. bilimbi* flower crown



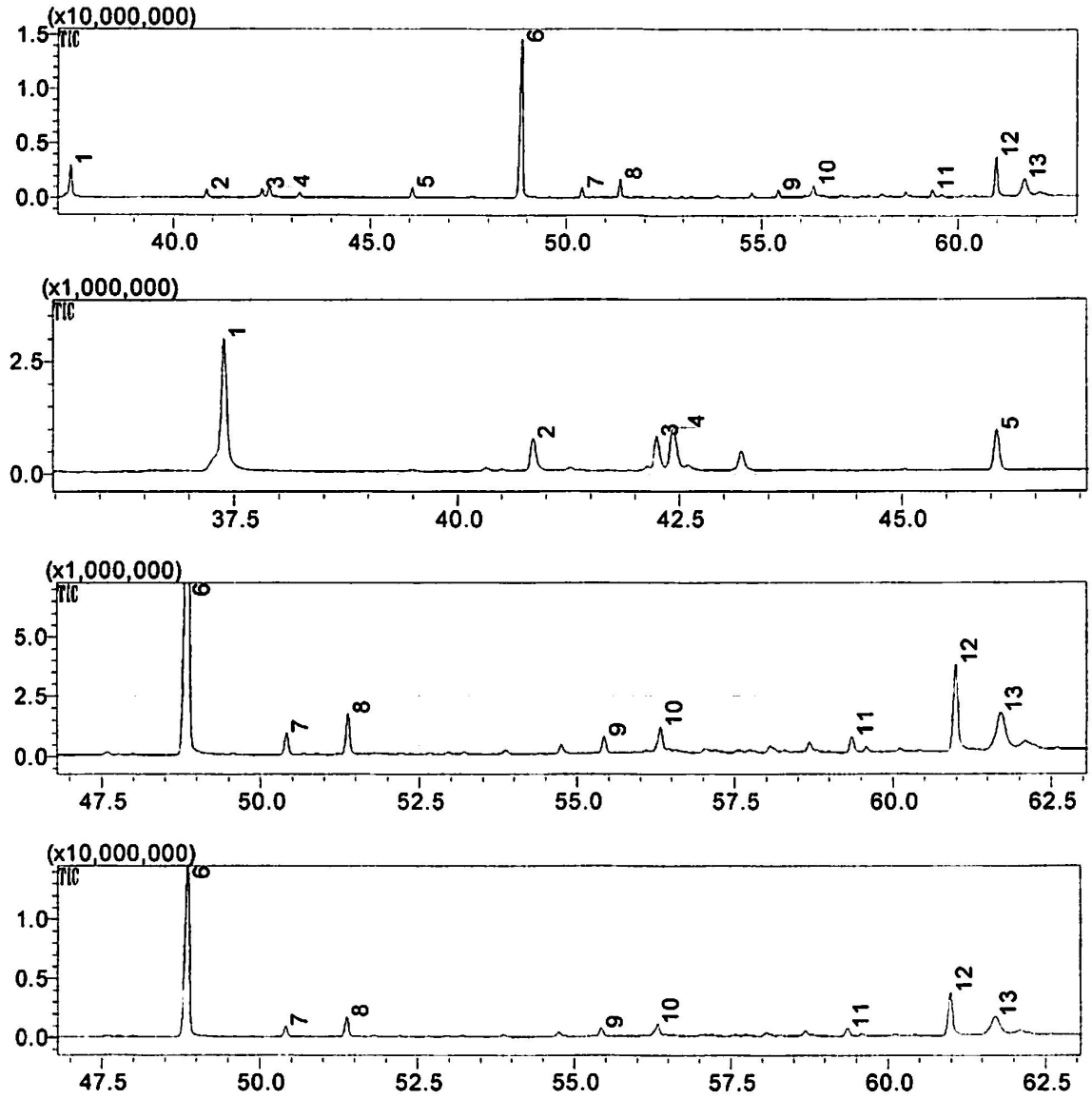


Figure 4. Results of GC-MS analysis of methanol extract of *A. bilimbi* flower petals

In this present investigation, the crown and petal methanol extract of *A. bilimbi* exhibited antioxidant potency. The antioxidant activity of the flower crown is higher than that of the flower petals. It seems the presence of hexanedioic acid, γ-sitosterol and hexadecanoic acid in the methanol extracts responsible for antioxidant activity. In addition, the presence of those compounds suggest that *A. bilimbi* flower extract can be used as an antimicrobial, antidiabetic, anticancer, antihyperlipidemia, and anti ageing agent.

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