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Phytochemical Screening And Activity Test of Antioxidant Ethanol Extract of Buni Leaves (*Antidesma bunius* L. Spreng) Using DPPH Method

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Received: February 28th, 2021 Revised: April 18th, 2021 Accepted: April 22th, 2021 **Abstract:** Research on phytochemical screening and antioxidant activity testing of the ethanol extract of buni leaves (Antidesma bunius L. Spreng) has been carried out. This study aims to identify secondary metabolite compounds contained in buni leaf plants to see the activity of the ethanol extract of buni leaves using the DPPH method. Buni leaves were extracted in 96% ethanol by maceration method, then evaporated using a rotary evaporator. The results of thick extract were screened for phytochemicals such as phenolics, tannins, flavonoids, saponins, alkaloids, steroids and terpenoids using a qualitative color test method and carrying out antioxidant activity using a UV-Vis spectrophotometer with Vitamin C as a comparison. The results showed that the ethanol extract of buni leaves contained phenolic compounds, tannins, flavonoids, saponins, alkaloids, steroids and terpenoids as well as IC_{50} Vitamin C of 5.22 ppm which is classified as a very strong antioxidant, while the IC_{50} of buni leaves ethanol extract is lower at 61, 8 ppm which is classified as a strong antioxidant.

Keywords: Antidesma bunius L. Spreng, Phytochemical Screening, Antioxidants

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Introduction

Free radicals are atoms or molecules that contain one or more unpaired electrons so that they become unstable and very reactive (Phaniendra & Babu, 2015). Free radicals can cause oxidative stress which accelerates the premature aging process (Sofwan, 2013). The bad effects of free radicals can be overcome with reducing compounds known as antioxidants. Exogenous antioxidants can be synthetic or natural. It's just that the use of synthetic antioxidants is very limited by the government because it can provide dangerous side effects for the body, one of which is carcinogenic. Therefore, antioxidants that come from nature are preferred because they are safer (Wulansari, 2018). According to Selamoglu, Amin, Ugur, & Ozgen (2018),

the presence of certain secondary metabolites in plants plays an important role in providing antioxidant properties, one of which is phenolic compounds. In addition, according to Ramadenti, Sundaryono, & Handayani (2017), the presence of secondary metabolites such as alkaloids, saponins, tannins, flavonoids, steroids and terpenoids can also act as antioxidants. Some of these secondary metabolites are also found in the leaves of buni plants such as terpenoids (Elya et al., 2011) and flavonoids, saponins, tannins, steroids, and alkaloids (Islam et al., 2018). The existence of this content indicates that this plant has the potential to be efficacious as an antioxidant. Therefore, in this study, a qualitative phytochemical screening

was carried out using color testing of several secondary metabolites such as phenolics, tannins, flavonoids, saponins, alkaloids, steroids and terpenoids as well as testing the antioxidant activity of the ethanol extract of buni leaves using the DPPH method. The DPPH method was chosen because it is simple, sensitive, easy, fast and requires a small number of samples. The results of the antioxidant test will be expressed in the form of the IC₅₀ value which is calculated based on the regression equation.

Method

1. The tools and materials

Aluminum foil, analytical balance, aquadest, blue tip, buni leaves (Antidesma bunius l.), centrifuge, chloroform, concentrated H₂SO₄ 97%, concentrated HCl 37%, DPPH, dragendorf reagent, ethanol 96%, ethanol pa, FeCl₃ 1%, filter paper, glass tools, HCl 2N, lieberman burchard reagent, mayer reagent, mg powder, rotary evaporator, rubberbulb, stopwatch, UV-Vis spectrophotometer, Ascorbic acid, Vortex, wagner reagent, water bath and yellow tip.

2. Preparation of buni leaf extract

The fresh buni leaves obtained are washed, dried by air drying and made into powder. 500 grams of simplicia powder were immersed in 3 liters of 96% ethanol in a container for 2x24 hours and then remacerated. The resulting filtrate is evaporated using a rotary evaporator at 60°C.

3. Phytochemical Screening

- a. *Preparation of a phytochemical test solution*. 500 mg extract dissolved into 50 mL of 96% ethanol (Susanti, I.N.A., & N.K, 2014).
- b. *Test for Phenolics*. 1 ml of the test solution is added with a few drops of 1% FeCl₃ solution. If green, red, purple, blue, dark blue, blackish blue or blackish green colors are formed, it indicates the presence of phenolics (Harborne, 1987).
- c. *Test for Flavonoid*. 2 ml of the test solution was heated for 5 minutes, after which 1 mL of concentrated HCl and 0.05 mg of Mg powder were added. If a red, yellow or orange color is formed, it indicates the presence of flavonoids (Harborne, 1987).
- d. *Test for tannins*. 1 ml of the test solution is added to 2-3 drops of 1% FeCl₃ solution. If a dark blue or blackish green color is formed, it indicates the presence of tannins (Harborne, 1987).
- e. *Test for Saponin*. 2 ml of the test solution is added with 10 ml of hot water, after that it is cooled and shaken for 10 seconds, if there is foam for about 10 minutes with a height of 1 to 10 cm and when

HCl 2N is added, the foam does not disappear, indicating the presence of saponins (Rabima & Marshall, 2017).

- f. *Test for Alkaloid.* 0.5 grams of viscous extract were dissolved with 1 ml of HCl 2N and 9 ml of aquadest, then put into a test tube and then heated over a water bath for 2 minutes, then cooled and filtered. The resulting filtrate is then divided into 3 test tubes where each mayer, Wagner and dragendorf reagent is added. If a white precipitate is formed in the mayer reagent, an orange precipitate in the Dragendorf reagent and a brown precipitate in the Wagner reagent, indicating the presence of alkaloids (Departemen Kesehatan Republik Indonesia, 1977).
- g. *Test for Terpenoid*. Extract was dissolved with chloroform and then filtered. The filtrate is then added with a few drops of concentrated sulfuric acid. If a brownish red color is formed at the boundary between the two phases, it indicates that there are terpenoids (Onuekwusi, Akanya, & Evans, 2014).
- h. *Test for Steroid*. Extract was dissolved into chloroform, filtered and the filtrate was added with Lieberman burchard reagent. If a green blue ring is formed, it indicates the presence of steroids (Habibi, Firmansyah, & Setyawati, 2018).

4. Antioxidant Activity Test with DPPH

solution was prepared DPPH with а concentration of 0.1 mM and a standard solution of ascorbic acid was prepared by dissolving 50 mg of ascorbic acid powder in ethanol pa up to 50 mL to obtain a ascorbic acid solution with a concentration of 1000 ppm. The solution was then diluted to obtain six different concentrations (1, 2, 3, 4, 5, and 6 ppm) to create a calibration curve. The sample solution was prepared by dissolving 50 mg extract with ethanol pa in a 50 mL volumetric flask (1000 ppm). The sample solution was diluted into six concentrations (5, 10, 20, 40, 50 and 80 ppm). Each standard or sample solution was pipetted and 0,1mM DPPH solution was added with a ratio of 2:2. The mixture was placed into dark vial and shaken homogeneously. All solutions then were being incubated in a dark place at room temperature for 30 minutes, then the absorption was measured using a UV-VIS spectrophotometer at a wavelength of 517 nm. Free radical scavenging activity was calculated using the following formula

DPPH Scavenging effect :
$$\left[\frac{AB - AA}{AB}\right] x 100 \%$$

where, AB = absorbance of DPPH solution; AA = absorbance of standard or extract solution. The

concentration of extract or standard which exhibited 50% radical scavenging (IC_{50} value) was deduced from the linear regression of concentration versus the percentage of inhibition.

Result and Discussion

1. Phytochemical Screening.

The results of phytochemical screening can be seen in Table 1.

| Table 1. | The results | of Phy | ztochemica | Screening |
|-----------|-------------|----------|------------------|-----------|
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| Facendery The | | |
|-------------------------|--------------------------|--------|
| Secondary Motobolito | Ethanolic Extract | The |
| Metabolite | | result |
| Phenolic | | + |
| Flavonoid | | + |
| Tannin | | + |
| Saponin | | + |
| Alkaloid | | |
| | (a) Hasil uji mayer | + |
| | (b) Hasil uji wagner | + |
| TT • 1 | (c) Hasil uji dragendorf | + |
| Terpenoid | | + |
| Steroid | | + |
| | | |

Phenolic compounds can be identified due to the reaction between the aromatic -OH group on 1% FeCl₃ and phenolic compounds. This reaction forms a colored complex thought to be iron (III) hexafenolate (Habibi et al., 2018).

Flavonoid compounds can be identified by the addition of magnesium powder and hydrochloric acid which is marked by a change in color to red due to the reduction of flavonoid compounds (Sangi, Momuat, & Kumaunang, 2012). Mg and HCl metal powder functions to reduce the benzopyrone core contained in the flavonoid structure and form flavilium salts which are red (Prayoga, Nocianitri, & Puspawati, 2019).

Tannin compounds can be identified due to the formation of complex compounds between tannins and FeCl₃. FeCl₃ compound will be ionized to give Fe³⁺ and Cl⁻. The Fe³⁺ cation will form a coordination covalent bond with the O atom of the hydroxy group, resulting in a colored complex compound (Tjitda & Nitbani, 2019).

Saponin compounds can be identified because saponin compounds have hydrophilic groups that bind to water while hydrophobic groups, namely aglycones (sapogenins) will bind to air. when shaking it, this causes hydrolysis and can form micelles (Tandi, Melinda, Purwantari, & Widodo, 2020).

Alkaloid compounds can be identified due to ligand replacement. Nitrogen atoms that have lone pairs on alkaloids can replace iodo ions in these reagents (Sangi et al., 2012). Alkaloid compounds were identified in the addition of major reagents due to the reaction between mercury (II) chloride and potassium iodide to form a complex compound of potassium tetraiodomerkurate (II). These compounds will form coordination covalent bonds with nitrogen atoms derived from alkaloid components and produce a potassium-alkaloid complex (Tjitda & Nitbani, 2019); (Nugrahani, Andayani, & Hakim, 2016).

Alkaloid compounds were also identified in the addition of dragendorf reagents due to the bismuth nitrate from the reagent reacting with potassium iodide to form bismuth (III) iodide deposits which then dissolved in excess potassium iodide to form potassium tetraiodobismuth (Permadi, Sutanto, & Wardatun, 2015). Alkaloid compounds were also identified when the addition of Wagner's reagent was indicated by the formation of a brown precipitate. These deposits are potassium-alkaloids. Wagner reagent contains I3- ion which is produced as a result of the reaction between iodine and I- from potassium iodide. K+ metal ions will form coordinate covalent bonds with nitrogen in alkaloids and form potassium-alkaloid complexes which precipitate (Mustarichie, Musfiroh, & Levita, 2011).

Terpenoid and steroid compounds to form color by H_2SO_4 (Habibi et al., 2018). Steroid compounds can be identified due to reactions that occur between steroids and acetic acid anhydrous is the acetylation reaction of the –OH group on steroids (Indarto, 2015). With the addition of Liebermann-Buchard reagent, the molecules of acetic anhydride and sulfuric acid will bind to the steroid compound molecules to produce reactions that appear to change color (Sangi et al., 2012).

2. Antioxidant Activity Test with DPPH

Based on the measurement results in table 2 and table 3, the mean value of IC_{50} Ascorbic acid obtained is 5.22 ppm, which indicates that it is classified as a very strong antioxidant, while the average IC_{50} value of buni leaf ethanol extract is 61.8 ppm which is classified as a strong antioxidant (Lung & Destiani, 2017).

Tabel 2: The results of the antioxidant test

| Sampel | Concentration (ppm) | Absorbance | % inhibition |
|----------|------------------------|------------|--------------|
| Ascorbic | Control | 0,5703 | |
| acid | 1 | 0,5299 | 7,083576856 |
| | 2 | 0,4897 | 14,1320865 |
| | 3 | 0,4185 | 26,62185856 |
| | 4 | 0,3714 | 34,88018703 |
| | 5 | 0,3109 | 45,4880187 |
| | 6 | 0,2193 | 61,54295733 |
| Extract | Control | 0,6429 | |
| | 5 | 0,6254 | 2,716855913 |
| | 10 | 0,5770 | 10,24005807 |
| | 20 | 0,5105 | 20,58381293 |
| | 40 | 0,4344 | 32,43117126 |
| | 50 | 0,388 | 39,64846788 |
| | 80 | 0,2284 | 64,46310987 |

| Tabel 3: IC50 | Ascorbic | Acid and | Extract |
|---------------|----------|----------|---------|
|---------------|----------|----------|---------|

| Sample | Linear Regression Equations | IC ₅₀ (ppm) |
|---------------|--------------------------------|------------------------|
| Ascorbic acid | y = 10,704x - 5,8375 | 5,22 |
| Extract | y = 0,7842x + 1,5533 | 61,8 |

The ethanol extract of Buni leaves has antioxidant activity due to the presence of certain secondary metabolites that have antioxidant properties. Each compound has its own mechanism in counteracting free radicals. Alkaloids have a mechanism as antioxidants by donating hydrogen antoms to free radicals. This mechanism shows that alkaloids work as primary antioxidants (Kurniati, 2013). The mechanism of alkaloids as an antioxidant can be seen in Figure 1.

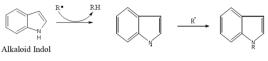


Figure 1. The mechanism of alkaloids as antioxidants (Yuhernita & Juniarti, 2011)

Saponins can be effective as antioxidants because they have the ability to reduce superoxide through the formation of hydroperoxide intermediates so as to prevent biomolecular free radical damage (Handayani, Ahmad, & Sudir, 2014). Tannin also have a mechanism as an antioxidant by donating hydrogen atoms or electrons to radicals (Amarowicz, 2007). The mechanism of tannins as antioxidants can be seen in Figure 2.

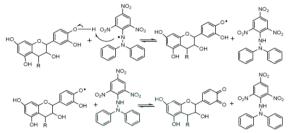


Figure 2. Mechanism of tannins as antioxidants

In addition, the mechanism of flavonoids as antioxidants is by transferring an electron to free radical compounds and also forming complexes with metals (Yuhernita & Juniarti, 2011). The mechanism of these flavonoids can be seen in Figures 3 and 4.

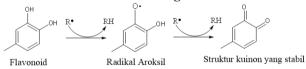


Figure 3. The process of reducing free radicals by flavonoids by transferring electrons (Yuhernita & Juniarti, 2011)

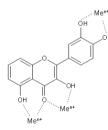


Figure 4.The process of reducing free radicals by flavonoids with the formation of metal complexes (Yuhernita & Juniarti, 2011)

Secondary metabolites of steroids and triterpenoids also have the ability to act as antioxidants. These two compounds are classified as lipophilic antioxidants (Hardiningtyas, Purwaningsih, & Handharvani, 2014). Based on their mechanism as antioxidants, these two compounds are classified as primary antioxidants which have a mechanism of action by reducing the formation of new free radicals through breaking chain reactions and turning them into more stable products (Maulida, Fadraersada, & Rijai, 2016). Especially for triterpenoid compounds, the antioxidant mechanism can be carried out in 2 ways, namely by capturing reactive species and by chelating metals (Fe²⁺ and Cu²⁺). Triterpenoids can inhibit lipid peroxidation. at the initiation stage by inhibiting peroxyl radicals and in the final stage by inhibiting secondary products (Hardiningtyas et al., 2014).

In phenolic compounds, the ability of antioxidant activity occurs by donating hydrogen which causes a neutralization reaction of free radicals that initiates the oxidation process or stops the chain radical reactions that occur (Yuhernita & Juniarti, 2011). The mechanism of phenolic as an antioxidant can be seen in Figure 5.

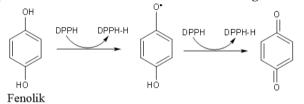


Figure 5. Mechanism of phenolics as antioxidants (Marliana, 2012)

Conclusion

Based on the research that has been done, it can be concluded that the ethanol extract of buni leaves contains phenolic compounds, flavonoids, tannins, saponins, alkaloids, terpenoids and steroids and has relatively strong antioxidant activity with an IC_{50} of 61.8 ppm.

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