Antioxidant Activities of Muntingia calabura, Syzygium cumini, Ocimum basilicum, and Eleutherine bulbosa using DPPH Method

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ABSTRACT

Antioxidants are substances that can provide endogenous protection and exogenous oxidative stress by capturing free radicals. Many plants are efficacious as antioxidants, namely plants that contain polyphenols, especially flavonoids, so many are formulated as natural antioxidants. Plants such as Muntingia calabura, Syzygium cumini, Ocimum basilicum and Eleutherine bulbosa contain polyphenol compounds, especially flavonoids which are efficacious as natural antioxidants. This research aimed to study antioxidant activity derived from some potential plants using the DPPH method by calculating the IC50 value of each plant extract. This research method starts from the determination process to prove the validity of the plants used, the extraction process using the maceration method with 70% ethanol solvent, then the antioxidant activity of extracts from each plant was carried out using the DPPH method. This research starts from the determination process to ensure the correctness of the plants used, then the extraction process is carried out using the maceration method with 70% ethanol solvent. After that the antioxidant activity was determined from the four plants using the DPPH method to see the strongest IC50 value among the four plants. IC50 is the concentration of the sample to inhibit 50% of free radicals. The results of IC50 values from methanol extract of Muntingia calabura leaves, Syzygium cumini leaves, Ocimum basilicum leaves and Eleutherine bulbosa bulbs, were 18.72; 63.84; 141.59 and 173.15 ppm. Ethanol extract of Muntingia Calabura has a smaller IC50 value of 18.72 ppm which has a very strong and most powerful antioxidant from the ethanol extract of Syzygium cumini, Ocimum basilicum and Eleutherine bulbosa.

Keywords: Antioxidant, Muntingia calabura, Syzygium cumini, Ocimum basilicum, Eleutherine bulbosa, DPPH Method

1. Introduction

Antioxidants defined as molecules having ability to inhibit the oxidation of other molecules mostly caused by free radicals, therefore can reduces the damages due to oxygen [1,2,3]. Antioxidants can be catagorized as either synthetic or natural and both are included in formulations. Synthetic antioxidants (e.g. butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and propyl gallate) has been largely used for many purposes since the ease of production leading to lower prices. However, high potential health risks following a high consumption of synthetic antioxidants has been reported by some studies. Despite the high demand for synthetic antioxidants by market, concerns on natural antioxidants have been increased in the last few years and it is expected to continue and growing. There are some reasons to explain this trend. Mostly, the consumer prefer organic and natural products, since they use less additives thus it is hoped to have less side effects than synthetic ingredients[4].

Natural antioxidants has been used in the cosmetic industries including a great number of substances and extracts obtained from a variety of plants, grains and fruits, either by reducing the skin oxidative stress or protecting the skin from oxidative degradation [4].

Plants that are efficacious as antioxidants are plants that contain carotenoids and polyphenols, especially flavonoids which can be formulated as natural antioxidants in oral dosage forms such as vitamins and topicals for skin care products.

This research aims to determine the antioxidant activity of Muntingia calabura leaves, Syzygium cumini leaves, Ocimum basilicum leaves and Eleutherine bulbosa bulbs since they contain polyphenol compounds, especially flavonoids which known to have efficacious as natural antioxidants. Muntingia calabura L. contains phenolic acids and flavonoids [5]. Syzygium cumini L. contains anthocyanin, gluicoside, isokuercin, kaempferol, myristin and high total phenolic compounds [6]. Ocimum basilicum L. contains apigenin and rosmarinic acid which can be used as anti-radical free [7]. Eleutherine bulbosa (Mill.) Urb contains three groups of compounds, namely naphtalen, naphtokuinon and anthraquinone [8].

Determination of antioxidant activity in this study

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using the DPPH method. The were conducted by DPPH method is used to obtain IC50 values from a plant extract. IC50s the concentration of the sample to inhibit 50% of free radicals. A compound is said to have very strong antioxidant activity if the IC50 value is less than 50 ppm, strong if the IC50 value is 50-100 ppm, moderate if it is 100-150 ppm and weak if the IC50 value is 150-200 ppm [9].

2. Materials and Methods

2.1 Materials

_Muntingia calabura_ leaves, _Syzygium cumini_ leaves, _Ocimum basilicum_ leaves and _Eleutherine bulbosa_ bulbs from Manoko plantation, Lembang, Bandung. Determination of plants is evaluated at the Laboratory of Taxonomy, Department of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University. The chemicals used in this study include ethanol 96% p.a. (Merck), DPPH (2,2-diphenyl-1-pycryl-hydrazyl) (Sigma), ascorbic acid (Merck). Absorbance of sample is analyzed using UV-Vis spectrophotometer (TECAN M200Pro).

2.2 Methods

2.2.1 Material collection and plant determination.

Plant material used was obtained from the Manoko plantation, Lembang, West Java. Plants were determined at the Taxonomy Laboratory, Biology Department, Faculty of Mathematics and Natural Sciences, Padjadjaran University.

2.2.2 Extraction

The extraction of _Muntingia calabura_ leaf, _Syzygium cumini_ leaf, _Ocimum basilicum_ leaf and _Eleutherine bulbosa_ bulbs plant were conducted using maceration with 70% ethanol solvent for 3 times 24 hours. Liquid extract concentrated with a rotary evaporator then steamed above a water bath until constant weight of the extract. The yield of the extract can be calculated by the formula:

\[
\text{Rendement} \times 100 = 1 + \frac{\text{Weight of Extract}}{\text{Weight of Simplicia}}
\]

2.2.3. In vitro Antioxidant Activity Test of Extract

a. Sample Preparation

Ethanol extracts were prepared with a stock solution of 1000 ppm, 1000 ppm stock solution is diluted to concentration 0, 5, 10, 15, 20 and 25 ppm for _Muntingia calabura_ L., 0, 20, 40, 60, 80 and 100 ppm for _Syzygium cumini_ L., 0, 50, 100, 150, 200 and 250 ppm for _Ocimum basilicum_ L. and _Eleutherine bulbosa_ (Mill.).

b. Preparation of Comparative Solutions

Ascorbic acid was prepared with a solution of 100 ppm. Diluted a stock solution standard to concentration 0, 1, 2, 3, 4, 5 ppm [10].

c. Preparation of DPPH solution (2,2-diphenyl-1-pycryl-hydrazyl)

DPPH was weighed and dissolved in ethanol p.a at a concentration of 160 ppm for immediate use and maintained in low temperatures and protected from light exposure[10].

d. Maximum Wavelength Determination

DPPH 0.2 mL solution was dissolved with 0.8 mL ethanol with a concentration of 250 ppm, measured at 500-530 nm wavelength to obtain an absorbance of ± 0.2-0.8[10].

e. Determination of IC50 with DPPH Method

The ethanol extract of _Muntingia calabura_ leaves, _Syzygium cumini_ leaves, _Ocimum basilicum_ leaves and _Eleutherine bulbosa_ bulbs were added 1 mL. DPPH for each concentration, then sonication and incubated for 30 minutes at room temperature. The absorbance was measured at 517 nm. The inhibition percentage was calculated using the equation [11]:

\[
\text{Inhibition} \times 100 = \frac{\text{Ab} - \text{As}}{\text{Ab}}
\]

3. Results

3.1. Determination

The results of plant determination show _Muntingia calabura_ leaves belong to the Family: Muntingiaceae, Genus: _Muntingia_, Species: _Muntingia calabura_ L, Indonesian name : Kersen. _Syzygium cumini_ leaves included in the Family: Myrtaceae, Genus: _Syzygium_, Species: _Syzygium cumini_(L.) Skeels., Indonesian name : Jamblang. _Ocimum basilicum_ leaves included in the Family: Lamiaceae, Genus: _Ocimum_, Species: _Ocimum basilicum_ L., Indonesian name : Kemangi. _Eleutherine bulbosa_ bulbs included in the Family: Tridaceae, Genus: _Eleutherine_, Species: _Eleutherine bulbosa_ (Mill.) Urb., Indonesian name : Bawangdayak.

3.2. Extraction

_Muntingia calabura_ leaves extraction (250 g) was macerated with 70% ethanol solvent resulting leaf extract of 78,836 g (rendement = 31,53 %). _Syzygium cumini_ leaves, _Ocimum basilicum_ leaves and _Eleutherine bulbosa_ bulbs extraction (100 g) was macerated with 70% ethanol solvent resulting 28,49; 19,94; 13,24 g (rendement% = 28,49; 19,94; 13,24 %) (Table 2 - 5 and Figure 1 – 4)

3.3 Antioxidant Activity

In this study, antioxidant activity was tested for all four plants, using the DPPH method. The principle of the DPPH method is based on measurements of capture of antioxidant capacity. DPPH is a free
radical is stable in purple, can be reduced by the presence of antioxidant molecules resulting in changes in color from purple to yellow. The results of testing the antioxidant activity indicate IC50 values can be seen in Table 6.

**Table 1.** Phytochemical Screening Results of Ethanol Extracts of *M. calabura* leaves, *S. cumini* leaves, *O. basilicum* leaves and *E. bulbosa* bulbs.

| Parameter                  | Mc | Sc | Ob | Eb |
|----------------------------|----|----|----|----|
| Alkaloid                   | +  | +  | +  | +  |
| Flavonoid                  | +  | +  | +  | +  |
| Polifenol                  | +  | +  | +  | +  |
| Tanin                      | +  | +  | -  | -  |
| Saponin                    | +  | +  | -  | -  |
| Steroid & Terpenoid        | +  | +  | -  | -  |
| Monoterpenoid & Sesquiterpenoid | + | + | + | + |
| Kuinon                     | +  | +  | -  | +  |

**Note:**

(+): Identified  
(-): Not Identified

**Table 2.** IC50 of *Muntingia calabura* L.

| Concentration (ppm) | Absorbance | % Inhibition |
|---------------------|------------|--------------|
| 0                   | 0,8199     | 0            |
| 5                   | 0,69495    | 15,23966     |
| 10                  | 0,58015    | 29,24137     |
| 15                  | 0,4642     | 43,38334     |
| 20                  | 0,3598     | 56,1166      |
| 25                  | 0,3164     | 61,40993     |

**4. Discussion**

Phytochemical screening was carried out on *Muntingia calabura* leaves extract, *Syzygium cumini* leaves, *Ocimum basilicum* leaves and *Eleutherine bulbosa* bulbs. Phytochemical screening aims to determine and determine the secondary metabolites contained therein[12]. The results of phytochemical screening showed that all four plants contained alkaloid compounds, flavonoids, polyphenols, monoterpenoids and sesquiterpenoids. Compounds such as flavonoids and polyphenols are compounds that have...
antioxidant activity. Based on the compound content in it, the four plants have positive antioxidant activity [13].

The antioxidant activity test was carried out using a UV-Viss spectrophotometer. This test was conducted to determine the absorbance of the remaining DPPH after adding the sample. If the DPPH solution is dissolved with a compound that has antioxidant activity, there will be a decrease in the value of DPPH absorbance which is characterized by a change in color from purple to yellow after incubation for 30 minutes.

This study uses the DPPH method to obtain IC_{50} values from a plant extract. IC_{50} is the concentration of the sample to inhibit 50% of free radicals. A compound is said to have very strong antioxidant activity if the IC_{50} value is less than 50 ppm, strong if the IC_{50} value is 50-100 ppm, moderate if it is 100-150 ppm and weak if the IC_{50} value is 150-200 ppm (Zuhra et al., 2008). Based on the results of the study of IC_{50} Muntingia calabura leaf ethanol extract of 18.72 ppm including very strong, the IC_{50} Syzygium cumini leaf value of 63.84 ppm was strong, IC_{50} Ocimum basilicum leaf value was 141.59 ppm including moderate and IC_{50} Eleutherine bulbosa leaf value is 173.15 ppm including weak. Based on IC_{50} values produced from the four plants, it can be seen that the ethanol extract of Muntingia calabura has the strongest antioxidant activity because it has a very high content of phenolic compounds, especially flavonoids.

5. Conclusion

The result showed that ethanol extract of Muntingia calabura produced an IC_{50} value of 18.72 ppm so it can be concluded that ethanol extract of Muntingia
calabura leaf has the strongest and most powerful antioxidant activity between Syzygium cumini, Ocimum basilicum and Eleuthereine bulbosa.

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