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Free-Radical Scavenging Activity and Total Phenolic Compounds of Red and Green Poinsettia Leaves (*Euphorbia pulcherrima* Willd.) from Lombok Island

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Abstract

To determine the free-radical scavenging activity and total phenolic content of the ethanol extract of Poinsettia leaves (*Euphorbia pulcherrima* Willd.) from Lombok Island, we used the 1,1-diphenyl-2-picrylhydrazyl method and expressed the total phenolic content as gallic acid equivalent (GAE) per gram of ethanol extract. The results show the strong antioxidant activity (79.77 ppm) of the ethanol extract of red Poinsettia leaves and the moderate antioxidant activity of green Poinsettia leaves (118.350 ppm). The ethanol extracts of red and green Poinsettia leaves possessed an average of 63.276 ± 0.228 and 22.715 ± 0.090 mg GAE/g extract total phenolic contents, respectively. In addition, the ethanol extracts of red and green Poinsettia leaves exhibited moderate and strong free-radical inhibitory activities, respectively. These findings indicate that color affects the antioxidant ability of leaves and their phenolic content.

Keywords: Euphorbia pulcherrima Willd., free radical scavenging, Gallic Acid Equivalent (GAE), phenolic compound, 2,2-diphenyl-1-picrylhydrazyl (DPPH)

Introduction

Free-radical scavenging represents the antioxidant activity under the principle that compounds can inhibit oxidation reactions by binding to free-radical molecules and therefore prevent cell damage to the body [1], especially in cell parts, such as DNA, brain cells, and skin tissue [2]. The human body can naturally produce enzymatic antioxidant compounds [3], such as superoxidase dismutase, catalase, and nonenzymatic antioxidants (vitamin A, coenzyme Q-10, and glutathione) [4]. However, the body cannot endure excessive exposure to oxidants. Therefore, the body needs a supply of antioxidants from external sources [5]. The consumption of numerous antioxidants can help in maintaining adequate amounts of antioxidants in the body. Antioxidant compounds that react with free radicals in vitro can prevent oxidative damage [6]. Natural antioxidant compounds include flavonoids, tocopherols, β-carotene, vitamin C, and vitamin E [4]. According to Ardhie (2011), the primary defense mechanism of chain-breaking or scavenger antioxidants involves the neutralization of free radicals by donating one electron. Antioxidant molecules that have lost one electron become new free radicals but are relatively stable because other antioxidants may neutralize them [7]. Flavonoid antioxidant compounds are commonly found in *Euphorbia* plants, such as in the isolate of *E. heterophylla* leaves [8]. *Euphorbia* is known as the largest genus in Euphorbiaceae family with approximately 2000 species [9]. Medicinal plants such as *E. hirta* have been traditionally used for the treatment of respiratory ailments (cough coryza, bronchitis, and asthma), worm infestations in children, digestive problems, and tumors [10]. Several species from the genus *Euphorbia*, such as *E. hirta* L. (asthma plant) [11], *Euphorbia heterophylla* L. (fire plant) [12], *Euphorbia prunifolia* Jacq. (Patikan Emas) [13], and *E. pulcherrima* found in Mexico exhibit antioxidant activities [14]. To our knowledge, the free-radical inhibitors and phenolic compounds of this genus have never been reported quantitatively.

Furthermore, according to previous studies, the ethanol extract of *Euphorbia pulcherrima* Willd. contains flavonoids, tannins, and terpenoids. The quantitative test using 1,1-diphenyl-2-picrylhydrazyl (DPPH) showed that this plant has potential as a free-radical inhibitor, as indicated by yellow spots observed during thin-layer chromatography after spraying of the DPPH reagent [15]. The total phenolic content of extracts can be determined using Folin-Ciocalteu assay [16]. The Folin–Ciocalteu method, an electron transfer-based assay, provides a reducing capacity that is expressed as phenolic content with gallic acid (GA) as the standard. DPPH-radical

scavenging assay was carried out in accordance with the method reported by Öztürk et al. with some modifications [17]. The DPPH assay is a free-radical scavenging method based on the radical-scavenging activity of antioxidants with acidic hydrogens (AH) toward the purple-colored DPPH in MeOH solvent. The free-radical DPPH is reduced to the corresponding stable diamagnetic molecule hydrazine (yellow colored) when it reacts with hydrogen donors (AH) [15,18]. Naturally, antioxidants can be polar or nonpolar and can act as radical scavengers via electron or hydrogen donating mechanisms.

Materials and Methods

Plant material. Red and green Poinsettia leaves were randomly collected from several different trees in East Lombok Regency, West Nusa Tenggara, Indonesia Sample determination was conducted at the Biology Department Laboratory, Faculty of Mathematics and Natural Sciences, University of Mataram.

Preparation of ethanol extract of poinsettia leaves. A total of 100 g of each simplicial powder from green and red Poinsettia leaves were macerated with 1,000 mL ethanol 96%, placed in a sanitized glass jar, and allowed to stand for 24 h in a place shielded from sunlight while occasionally being stirried [19]. Then, the mixture was evaporated with a rotary evaporator to obtain ethanol extract from the Poinsettia leaves.

Determination of total phenolic content. The phenolic quantification test is based on the Folin–Ciocalteu method [16, 17]. A total of 10 mg of each extract was dissolved in 10 mL methanol:water mixture at a ratio of 1:1. As much as 300 µL of the solution obtained was then added to 1.5 mL Folin–Ciocalteu reagent (1:10), shaken, left for 3 min, mixed with 1.2 mL 7.5% Na_2CO_3, and finally allowed to stand within the operating time range and at maximum wavelength (λ_{max}).

Determination of free-radical inhibitory activity. The free-radical inhibitory activity was determined using the DPPH method [20, 21]. First, 2 mL of each 20, 40, 60, 80, and 100 ppm Poinsettia extract were placed a test tube and then added with 0.1 mM DPPH solution. Next, the mixture was homogenized using a vortex and incubated in a dark room for 30 min. The absorption was measured at a wavelength (λ) of 516 nm. The absorbance was then plotted on the regression equation of the standard's (vitamin C) concentration series. The linear regression equation was used to evaluate the half maximal inhibitory concentration (IC_{50}) of each sample.

Results and Discussion

Determination of total phenolic content of the ethanol extract of Poinsettia leaves. The Folin–Ciocalteau test was used to determine the total phenolic content of the ethanol extract from Poinsettia leaves. In this method, phenolic ions from the sample reduce phosphomolybdic acid and phosphotungstic acid in the Folin–Ciocalteau reagent to form a complex blue molybdenum compound during the phenol oxidation process under an alkaline atmosphere. The greater the concentration of phenolic compounds, the more phenolic ions are formed. As a result, more phosphomolybdate and phosphotungstate are reduced by phenolic ions, intensifying the blue color observed. Hence, the measurable absorbance increases. In this study, the Folin–Ciocalteu test was conducted through the formation of a standard GA curve between the absorbance and concentration. The calibration curve was generated from the linear standard (GA) analysis (Figure 1) ($y = 0.0041x + 0.1994$). This standard curve was used to calculate the total phenolic content expressed in mg GA equivalent (GAE) per gram of extract. A regression equation with a good linearity has a coefficient of determination (R^2) approaching 1 (one) [22]. The better the linearity value, the better the resulting correlation.

The total phenolic content of the ethanol extract of red Poinsettia leaves was determined at a concentration of 1,000 ppm with three replications (Table 1). Measurement was performed at the operating time of 30 min and the λ of 745 nm based on the λ_{max} of the GA absorbance curve (Figure 2).

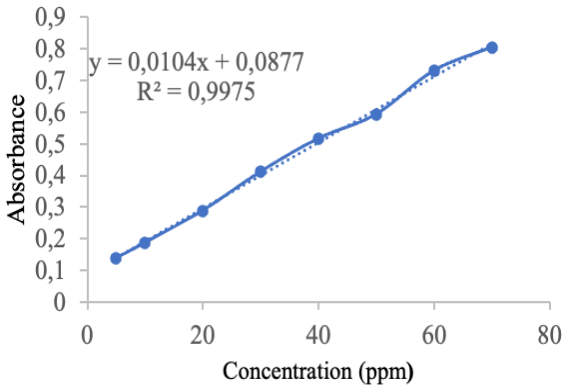

Figure 1. Calibration Curve of GA

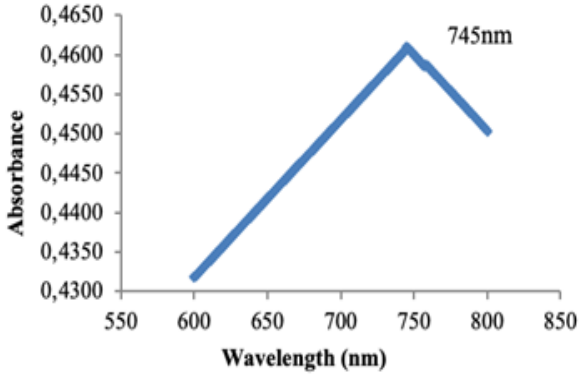

Figure 2. GA Absorbance Curve

The sample absorbance obtained was then applied to the determined linear regression equation. The calculation showed that the average phenolic content was 63.276 ± 0.228 mg equivalent to the gram GA of the ethanol extract of red Poinsettia leaves. The coefficient of variation (CV), which describes the relative precision of an analytical method, had a value of 0.004%. The smaller the CV, the higher the precision of the method used. In general, the precision standard is achieved if a method provides a CV ≤ 2%. Hence, in this study, the Folin–Ciocalteau method presented good precision for determining the total phenolic content in the ethanol extract of Poinsettia leaves.

Table 2 displays the average total phenolic content obtained using the ethanol extract of green Poinsettia leaves (22.715 ± 0.090 mg GAE per gram of extract). The resulting % CV was 0.004%, which is less than 2% and thus indicates the good precision of data [23].

Free-radical inhibitory activity of the ethanol extract of Poinsettia leaves. Free radicals are critical causative factors in the development of chronic diseases. Plant polyphenols benefit human diets given their antioxidant activity and capacity to alleviate oxidative-stress-induced tissue damage associated with several chronic diseases[24]. Medicinal plants, including the Castanese plant, which are potential sources of natural antioxidants, have been extensively studied. Furthermore, the DPPH radical-scavenging method is the most widely used testing method for antioxidant activity because it has good sensitivity and is simple, easy, and economical [25]. Tests using the DPPH method are based on the theory that antioxidants can donate hydrogen to free radicals. Thus, the antioxidant activity of the tested samples is directly proportional to the loss of DPPH radicals. In this research, the DPPH radical appeared purple and exhibited the maximum absorbance at the operating time of 30 min at a λ of 516 nm (Figure 3). According to Molyneux (2004), the sample color changes from purple to yellowish because the DPPH radicals transform into a more stable form due to the presence of hydrogen donors from the antioxidant [21].

Phenolic compounds show potential as antioxidants in the presence of hydroxyl groups. During the reaction with radical compounds, the hydroxyl groups contribute hydrogen atoms through an electron transfer mechanism by inhibiting the oxidation process [26]. Moreover, the capability of phenolic compounds to act as antioxidants is shown through their role as reduction agents, free-radical scavengers, metal chelating agents, dampers that form singlet oxygen, and electron donors [27]. These data are depicted in the graphs in Figure 4.

Table 1. Total Phenolic Content in Red Poinsettia Leaves

Concentration **(ppm)**	Total Phenolic Content **(mg GAE/g extract)**	**x ± SD**	CV
1,000	62.9712 63.3365 63.5192	**63.276 ± 0.228**	0.004%

Table 2. Total Phenolic Content in Green Poinsettia Leaves

Concentration **(ppm)**	Total Phenolic Content **(mg GAE/g extract)**	**x ± SD**	CV
1,000	22.6058 22.7115 22.8269	**22.715 ± 0.090**	0.004%

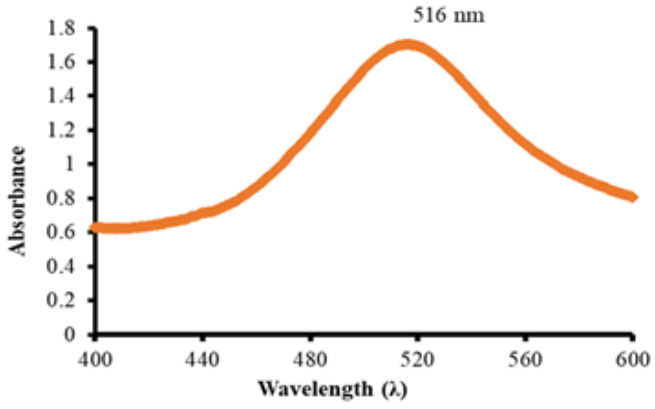

Figure 3. DPPH Absorbance Curve

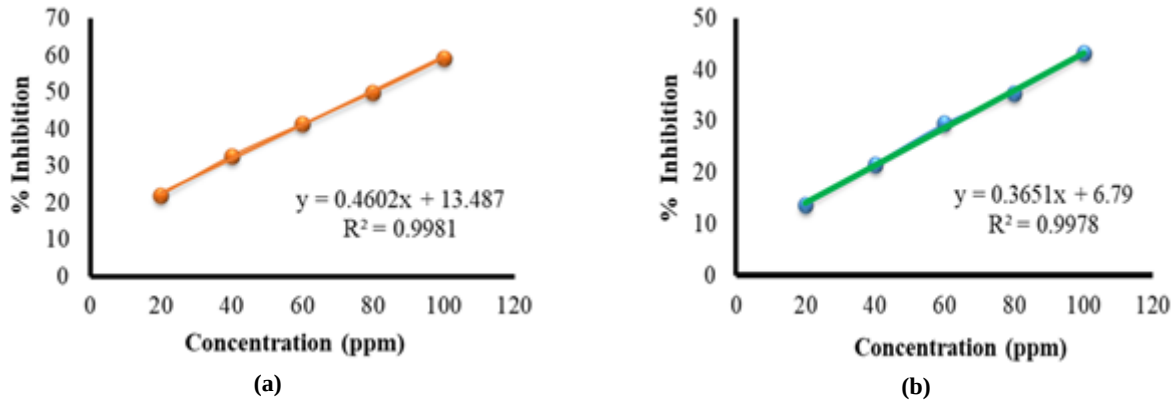

Figure 4. Inhibition Curve of (a) Red and (b) Green Poinsettia Leaves

The antioxidant activity in terms of the IC_{50} was obtained from the percent inhibition of each sample concentration. IC_{50} indicates the concentration required by a sample to inhibit radical activity by 50% [21]. The smaller the IC_{50}, the greater the compound's capability as an antioxidant. Different IC_{50} were obtained based on the linear regression equations y = ax + b of the inhibition curves in Figure 4, where x represents the value of IC_{50}, and y is equal to 50. In this study, vitamin C was used to compare the free-radical inhibitory activities of the ethanol extracts of Poinsettia leaves. Vitamin C or ascorbic acid was selected because of its robust antioxidant activity, with an IC_{50} of approximately 4.006 ppm compared with vitamins E and A, which have an equal average IC_{50} of 14.79 ppm [28].

The test for the antioxidant activity on the ethanol extract of red Poinsettia leaves was carried out through the preparation of five concentration series, namely, 20, 40, 60, 80, and 100 ppm, with three replications. As shown in Figure 4, the antioxidant activity of the red leaves exhibited a considerable difference. The higher the concentration, the higher the percentage of inhibition. The % inhibition was used to create a linear regression equation, $y = 0.4602x + 13.487$ with an r value of 0.9981. As displayed Figure 4a, the concentration of the ethanol extract of red Poinsettia leaves was directly proportional to its % inhibition. The greater the concentration, the more electron donors that reduced DPPH, which caused fading of the compound's color. The IC_{50} (x) was calculated by using 50 as the value of y in the linear regression equation obtained:

$$\begin{aligned} y &= 0.4602x + 13.487 \\ 50 &= 0.4602x + 13.487 \\ 50 - 13.487 &= 0.4602x \\ x &= \frac{(50-13.487)}{0.4602} \\ x &= 79.77\ \mu g/mL \end{aligned} \quad (1)$$

An IC_{50} of 79.77 ppm was obtained, indicating the strong free-radical inhibitory activity of red Poinsettia leaves.

Moreover, the ethanol extract of green Poinsettia leaves exhibited a similar antioxidant activity. As presented in Figure 4b, the concentration of the ethanol extract of green Poinsettia leaves was directly proportional to its % inhibition. The curve regression equation for green Poinsettia leaves (Figure 4b) gave a regression equation of $y = 0.3651x + 6.79$ with an r value of 0.9978.

$$\begin{aligned} y &= 0.3651x + 6.79 \\ 50 &= 0.3651x + 6.79 \\ 50 - 6.79 &= 0.3651x \\ x &= \frac{(50-6.79)}{0.3651} \\ x &= 118.35\ \mu g/mL \end{aligned} \quad (2)$$

Therefore, the IC_{50} of green Poinsettia leaves was 118.350 ppm, which reflects a moderate free-radical inhibitory activity. The different IC_{50} were extrapolated data from the percent inhibition curve. We focused more on the comparison of the percent inhibition values between red and green Poinsettia leaves at the same concentration.

The IC_{50} obtained indicates the strong free-radical inhibitory activity of the ethanol extract of red Poinsettia leaves. By contrast, the ethanol extract of green Poinsettia leaves exhibited a moderate free-radical inhibitory activity. Variations in the compounds' contents can caused the difference in IC_{50} between the ethanol extracts of the red and green Poinsettia leaves. The preliminary test revealed that the ethanol extracts of green and red Poinsettia leaves contained flavonoids, tannins, and terpenoids. However, the amounts of flavonoids and tannins were notably higher in the ethanol extract of red Poinsettia leaves than that of green Poinsettia leaves.

The presence of flavonoids considerably increases the antioxidant activity of extracts because radicals oxidize flavonoids to produce a more stable, less reactive form. Specifically, stabilization of reactive oxygen species occurs when flavonoids react with a radical's reactive component. Radicals are rendered inactive due to the

strong reactivity of the hydroxyl group of flavonoids [29]. These secondary metabolites possess aglycones and glycosides. The phenolic hydroxy groups attached to glycoside in flavonoids are active antioxidants and radical scavengers binding sugar groups. Hence, aglycones generally exhibit higher antioxidant and radical scavenging activities than glycosides in flavonoids.

Conclusions

The red and green Poinsettia leaf ethanol extracts showed strong and moderate free-radical inhibition activities, respectively. The results indicate that the color of the leaves affected their ability as antioxidants and their phenolic content. The IC_{50} of the ethanol extract of red Poinsettia leaves was 79.77 ppm, indicating a strong free-radical inhibitory activity. Meanwhile, the green Poinsettia leaves has an IC_{50} of 118.350 ppm, suggesting a moderate free-radical inhibitory activity. Furthermore, the total phenolic contents of the ethanol extracts of the red and green Poinsettia leaves were 63.276 ± 0.228 and 22.715 ± 0.090 mg GAE/g, respectively.

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