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Analysis of the Antioxidant Potential of Ethanol Extracts from Pacing Plant (*Costus speciosus*) with DPPH Method

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ABSTRACT

Antioxidants are reducing compounds utilized to protect the body from oxidative degradation. One of the plants, Costus speciosus, commonly known as pacing, is believed to have potential as a natural source of antioxidants. The aim of this research is to determine the antioxidant content and secondary metabolite compounds present in the ethanol extracts of pacing stems, leaves, and flowers. Antioxidant measurements were conducted using the 1,1-diphenyl-2-picrylhydrazine (DPPH) method. The antioxidant content was analyzed using probit analysis and calculating the IC_{50} value. The content of secondary metabolite compounds was analyzed using thin-layer chromatography (TLC) tests. The research results indicate that the ethanol extracts of pacing stems, leaves, and flowers contain antioxidants. The highest antioxidant content was found in the leaves with an IC_{50} value of 17.8 ppm, followed by the stems with an IC_{50} value of 24.22 ppm, and the flowers with an IC_{50} value of 24.85 ppm. The secondary metabolite content in pacing plants showed the presence of flavonoids and alkaloids in the leaves, flavonoids and phenols in the stems, while only flavonoids were detected in the flowers.

***Keyword**: Antioxidant, Costus speciosus, DPPH, Flower, Leave, Stem*

ABSTRAK

Antioksidan adalah senyawa pereduksi yang digunakan untuk melindungi tubuh dari degradasi oksidatif. Salah satu tanaman yaitu Costus speciosus atau yang biasa dikenal dengan nama pacing diyakini

memiliki potensi sebagai sumber antioksidan alami. Penelitian ini bertujuan untuk mengetahui kandungan antioksidan dan senyawa metabolit sekunder yang terdapat pada ekstrak etanol batang, daun, dan bunga pacing. Pengukuran antioksidan dilakukan dengan menggunakan metode 1,1-difenil-2-pikrilhidrazil (DPPH). Kandungan antioksidan dianalisis dengan menggunakan analisis probit dan menghitung nilai IC50. Kandungan senyawa metabolit sekunder dianalisis menggunakan uji kromatografi lapis tipis (KLT). Hasil penelitian menunjukkan bahwa ekstrak etanol batang, daun, dan bunga pacing mengandung antioksidan. Kandungan antioksidan tertinggi terdapat pada bagian daun dengan nilai IC50 sebesar 17,8 ppm, diikuti oleh bagian batang dengan nilai IC50 sebesar 24,22 ppm, dan bagian bunga dengan nilai IC50 sebesar 24,85 ppm. Kandungan metabolit sekunder pada tanaman mondar-mandir menunjukkan adanya flavonoid dan alkaloid pada daun, flavonoid dan fenol pada batang, sedangkan pada bunga hanya terdeteksi adanya flavonoid.

Kata Kunci: Antioksidan, Batang, Bunga, *Costus speciosus,* Daun, DPPH,

INTRODUCTION

Oxidation is a biochemical process that occurs naturally in the body, where the chemical reaction between oxygen and molecules within cells produces free radicals. These free radicals can cause cellular damage that contributes to the development of various degenerative diseases such as cancer, heart disease, and aging (Halliwell & Gutteridge, 2015). Free radicals within the body can snatch electrons from other molecules with low stability in order to achieve their own stability (Dewi, 2019). Highly reactive free radicals have the potential to initiate chain reactions rapidly, leading to the production of abnormal compounds. The high reactivity of free radicals in electron grabbing can result in cell damage that may lead to various degenerative diseases. To counteract the negative effects of free radicals, the body requires antioxidants, compounds that can neutralize free radicals and protect body cells from oxidative damage.

The widespread use of antioxidants has been implemented to protect the body from oxidative degradation. Within the body, there is an antioxidant system consisting of enzymes such as superoxide dismutase, catalase, and glutathione. However, sometimes the quantity of these enzymes is insufficient to neutralize the excessive influx of free radicals into the body. Therefore, it is important to consume foods that contain antioxidant compounds, such as flavonoids, vitamin A, vitamin C, and vitamin E (Kurang & Andang, 2018). One natural source of antioxidants that has garnered attention is the pacing plant (Costus speciosus), which has a history of traditional medicinal use in various countries.

The pacing plant, also known as the spiral ginger, has been traditionally used in medicine for various health conditions including diabetes,

inflammation, infections, and skin diseases (Jagtap & Rosenberg, 2012). The pacing plant contains bioactive compounds such as flavonoids, alkaloids, phenolics, and terpenoids that have antioxidant potential (Prasad et al., 2016). This highlights the potential of the pacing plant as a natural source of antioxidants.

Several previous studies have explored the antioxidant potential of the pacing plant. For example, a study by Prakasha et al. (2013) found that methanol extract from the stems of the pacing plant exhibited significant antioxidant activity through DPPH measurement. Additionally, another study by Mukherjee et al. (2014) showed that methanol extract from the leaves of the pacing plant had antioxidant activity and could protect red blood cells from oxidative damage. Goyal et al. (2019) demonstrated significant antioxidant activity of methanol extract from the leaves of the pacing plant based on DPPH testing. Another study by Kumari et al. (2016) revealed that ethanol extract from the flowers of the pacing plant had high flavonoid content and exhibited strong antioxidant activity through FRAP and DPPH testing.

However, there is a need for more detailed research in the past decade to better understand the overall antioxidant potential of the pacing plant, specifically in its stems, leaves, and flowers, as well as to identify the active compounds present. Therefore, this study aims to analyze the antioxidant potential of ethanol extracts from different parts of the pacing plant, namely the stems, leaves, and flowers. This research is expected to provide a deeper understanding of the antioxidant potential of the pacing plant and lay a scientific foundation for its use in the treatment and prevention of oxidative-related diseases. The information obtained from this study will contribute to the development of safer and more effective natural medicines to improve human health

METHOD

This research was conducted in September 2020. The pacing plant used in the study was sourced from Buring Kencana Village, Blambangan Pagar District, North Lampung Regency (Figure 1). The study took place at the Integrated Laboratory and Technology Innovation Center of Lampung University. The pacing plants used were approximately 100 days old and were collected systematically. The research involved several steps, including sample preparation and simplification, extraction, and identification of compound groups in the samples using thin-layer chromatography (TLC).

Figure 1. Costus speciosus
(Source: Personal documentation)

Procedures

Sample Preparation and Simplification

Fresh pacing plant samples were collected from the Kotabumi area, North Lampung, then cleaned from impurities using flowing water and dried in an oven. The dried samples appeared slightly rolled or wrinkled with a brown color, then were cut into small pieces and blended into powder.

Extraction

A total of 600 g of pacing plant simplisia was divided into three parts: flower, stem, and leaf (each 200 g). Each sample was then macerated with 250 mL of 96% ethanol solvent at room temperature for 3x24 hours. The longer the maceration time, the more compounds were extracted due to prolonged contact between the material and the solvent, optimizing the solvent's ability to absorb flavonoids in the material. After that, each sample was placed in an Erlenmeyer flask, tightly covered with aluminum foil, and occasionally stirred. The extraction results were then filtered to obtain the extract. The extract from the obtained samples was evaporated using a rotary evaporator to obtain a concentrated extract from the pacing flowers. The same steps were performed for the stem and leaf of the pacing plant. The extract yield was determined using the formula (Edison et al., 2020):

$$\% \text{ Yield} = \frac{\text{weight of extract } (g)}{\text{weight of sample } (g)} \text{ x } 100\%$$

DPPH Test

The DPPH test was conducted by preparing the DPPH solution. The first step was to prepare a 100 ppm DPPH solution by mixing 10 mg of DPPH powder with 100 mL of 96% ethanol in a beaker. A 10 ppm solution was then prepared by taking 10 mL from the 100 ppm DPPH solution and diluted to a volume of 100 mL. Next, the DPPH solution was incubated for 30 minutes at 37°C, and its wavelength was measured in the range of 450-650 nm to observe the highest spectrum absorption of colored compounds. A blank solution was prepared by taking 1 mL from the 10 ppm DPPH solution in a test tube and diluted to a volume of 3 mL with 96% ethanol. The solution was then homogenized and incubated for 30 minutes before being measured with a UV-Vis spectrophotometer at a wavelength of 517 nm. The testing on the pacing leaf extract was prepared at a concentration of 100 ppm by mixing 10 mg of the leaf extract with 100 mL of 96% ethanol in an Erlenmeyer flask. Various concentrations of 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm were prepared by taking 2.5 mL of the 100 ppm extract for each concentration and diluted to a volume of 100 mL in separate Erlenmeyer flasks for each concentration. The antioxidant test was performed by mixing 1 mL of the 10 ppm DPPH solution with the extract solution with concentrations of 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm, each 2.5 mL in a test tube. Then, the solution was homogenized and incubated for 30 minutes. After that, the solution was tested using a UV-Vis spectrophotometer at a wavelength of 517 nm, where the highest spectrum absorption would occur. The same steps were performed for the flower and stem samples. Vitamin C was used as a control in the DPPH test.

Thin-Layer Chromatography (TLC)

TLC was used to identify compounds in the ethanol extract of the pacing stem, flower, and leaf, using silica gel 60 F_{254} plates measuring 4 x 3 cm^2. Sample spots were placed 0.5 cm from the bottom edge of the plate and eluted with a mobile phase suitable for

separating the compound group (Fajriaty et al., 2018).

a. For phenolic compounds, the mobile phase consisted of a mixture of ethyl acetate: methanol (3:2). After elution, the TLC plate was observed under UV 254 nm light, and phenolic compounds were detected by spraying with $FeCl_3$, resulting in a dark blue color.
b. For flavonoid compounds, the mobile phase consisted of a mixture of ethanol: ethyl acetate (3:1). After elution, the TLC plate was observed under UV 254 nm light, and flavonoid compounds were detected by spraying with $AlCl_3$, resulting in a yellow color (Dahlia & Ahmad, 2014).
c. For alkaloid compounds, the mobile phase consisted of a mixture of methanol: chloroform (1:1). After elution, the TLC plate was observed under UV 254 nm light, and alkaloid compounds were detected by spraying with dragendorff reagent, resulting in a brown or orange color (Arnida et al., 2018).

Data analysis

The percentage of inhibition obtained was analyzed using probit analysis to create a graph of the log concentration of the sample (x-axis) against the percentage of antioxidant activity (y-axis) to find the linear regression equation that will provide the IC_{50} value. The percentage of inhibition is calculated using the following formula (Aminah et al., 2016):

$$\% \text{ inhibition} = \frac{(\text{Initial Absorbance} - \text{Absorbance after reaction})}{\text{Absorbance after reaction}} x\ 100\%$$

The IC_{50} value is calculated using the linear regression equation from the graph of log concentration of the sample (x-axis) against the percentage of inhibition (y-axis) with the equation y = a + bx. Then, the IC_{50} value can be calculated using the following formula:

$$IC_{50} = \frac{(50-b)}{a}$$

Note: y = 50 (percentage of inhibition at 50%); x = IC_{50} (concentration of the extract that can inhibit the oxidation process by 50%); a = slope; b = intercept

Table 1. Antioxidant Categories Based on IC_{50} Values (Tristantini et al., 2016)

IC_{50} Value	**Antioxidant**
50 ppm≤	Very Strong
50-100 ppm	Strong
100-150 ppm	Moderate
150-200 ppm	Weak
> 200 ppm	Very Weak

RESULTS AND DISCUSSION

Extraction Results

Extraction was performed using the maceration method with 200 grams of the crude drug in 250 mL of ethanol solvent. The extraction yields are presented in the following Table 2:

Table 2. Ethanol extraction yields of powdered stems, leaves, and flowers of Costus speciosus.

No	Organ Name	Weight of		Ethanol Extract Yield (%)
		Simplicia (grams)	Ethanol Extract (gram)	
1	Leaves	200	11,2	5,6
2	Flowers	200	9,84	4,92
3	Stems	200	10,5	5,25

Table 2, it can be observed that the ethanol extract of pacing leaves has the highest yield, indicating that pacing leaves contain the highest amount of bioactive compounds compared to the other samples

Antioxidant Results

The antioxidant activity of ethanol extracts from the leaves, flowers, and

stems of pacing plants was tested using the DPPH free radical scavenging method. The research showed that the ethanol extracts from the leaves, flowers, and stems exhibited positive antioxidant activity. This was evident from the color change of DPPH to pale yellow, indicating a reduction reaction by the free radical scavenging compounds. Before measuring with a spectrophotometer, the test solutions were incubated for 20-30 minutes in the dark, as DPPH is sensitive to light. Antioxidant activity was observed by the decrease in DPPH absorption after the addition of the samples (Nurfadillah et al., 2016). The results of the antioxidant tests are listed in the following Table 3.

In Figure 4, it can be observed from the probit analysis that the antioxidant content of ethanol extract from the flower, stem, and leaf of the pacing plant does not differ significantly from the antioxidant content found in vitamin C

Table 3. IC_{50} Values of DPPH Ethanol Extracts from Leaves, Stems, and Flowers of Pacing Plants (*Costus speciosus*)

Sample name	Consentration	% Inhibition	Log Consentration	Probit	IC_{50} Value (ppm)	IC_{50} Probit Value (ppm)
Ethanol extract of pacing leaves	2	27,67	0,3010	4,39	5,20	17,8
	4	34,57	0,6021	4,59		
	6	59,55	0,7782	5,23		
	8	81,87	0,9031	5,88		
	10	87,29	1	6,13		
Ethanol extract of pacing flowers	2	8,92	0,3010	4,12	7,95	24,85
	4	16,25	0,6021	4,23		
	6	18,37	0,7782	4,87		
	8	40,40	0,9031	5,05		
	10	52,05	1	5,41		
Vitamin C	2	25,29	0,3010	4,33	4,94	16,63
	4	32,08	0,6021	4,53		
	6	57,30	0,7782	5,18		
	8	79,78	0,9031	5,81		
	10	91,30	1	6,28		
Ethanol extract of pacing stems	2	19,54	0,3010	3,66	7,40	24,22
	4	22,61	0,6021	4,05		
	6	45,14	0,7782	4,12		
	8	52,48	0,9031	4,77		
	10	66,09	1	5,08		

* Absorbance of control 0,4430

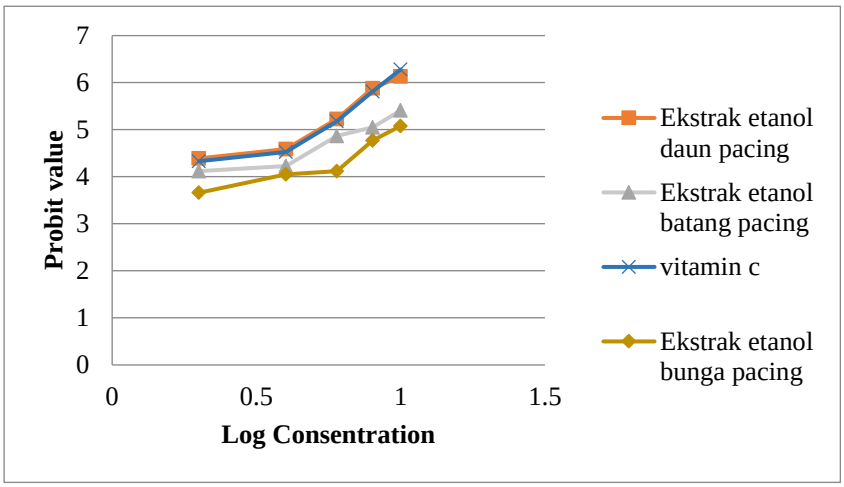

Figure 4. Correlation between the concentration of ethanol extract of flower, stem, leaf of *Costus speciosus* (pacing plant), and vitamin C with antioxidant activity

KLT Results

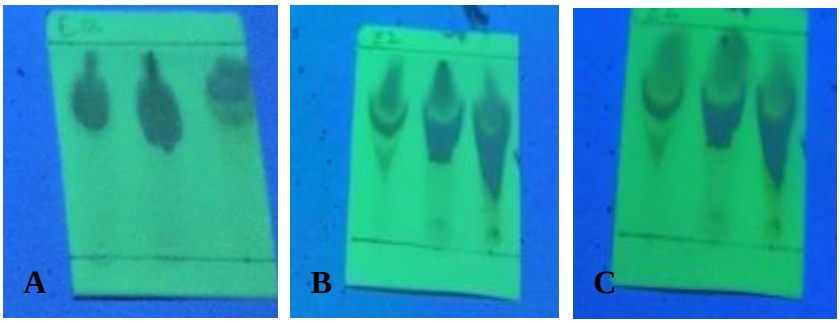

Figure 5 Shows the chromatography identification of phenols (A), alkaloids (B), and flavonoids (C) from the ethanol extract of the leaf, stem, and flower of the pacing plant.

The results of thin-layer chromatography in Figure 5 indicate the presence of secondary metabolite compounds in the tested samples. TLC was performed repeatedly using various types of eluents with different polarities to find a solvent that provides good separation and spot detection (Alen et al., 2017). The content of secondary metabolite compounds in the spots obtained from TLC can be determined by calculating the Rf values as shown in the following Table 4.

Table 4. Rf values of ethanol extract from pacing leaves, stems, and flowers

No	Ethanol extract	Compound groups	Rf
1	Leaves	Fenol	0,63
		Alkaloid	0,85
		Flavonoid	0,86
2	Stems	Alkaloid	0,86
		Flavonoid	0,84
3	Flowers	Fenol	0,66
		Flavonoid	0,88

Discussion

Maceration of simplisia was performed for 3 x 24 hours, then the filtrate was filtered and concentrated using a rotary evaporator. Ethanol solvent was used for extraction to absorb polar compounds. The effectiveness of using plant extract biostimulants is influenced by the type of solvent used in the extraction process. The solvent type affects the types and levels of secondary metabolites carried in the extraction process due to the polarity relationship between the solvent and the extracted material (Cikita et al., 2016). During the extraction process of polar total phenolic compounds, polar solvents such as acetone, water, ethyl acetate, and alcohol (methanol, ethanol, and propanol) need to be carefully considered (Khoddami et al., 2013). The amount of bioactive content in the extract can be traced by calculating the sample yield value. The yield is calculated as the ratio of the dry weight of the product to the weight of the raw material. The yield value reflects the amount of bioactive content in the sample (Whika et al., 2017).

In Table 3, it can be seen that vitamin C has the highest IC_{50} value as it is a single compound that is effective as an antioxidant. Furthermore, ethanol leaf extract has better antioxidant activity compared to ethanol stem and flower extracts of pacing plants. Ethanol leaf extract of pacing plant has antioxidant activity with an IC_{50} value of 17.8 ppm. Ethanol stem extract of pacing plant has an IC_{50} value of 24.22 ppm, while ethanol flower extract has an IC_{50} value of 24.85 ppm. The IC_{50} value for vitamin C (ascorbic acid) is 16.63 ppm. The smaller the IC_{50} value, the higher the antioxidant activity (Handayani et al., 2014). Based on the antioxidant activity category, ethanol leaf, stem, and flower extracts of pacing plants have very strong activity as their IC_{50} values are below 50 ppm. Ethanol leaf extract shows better antioxidant activity compared to ethanol stem and flower extracts of pacing plants (Rahmiyani & Zustika, 2016).

The results of TLC were examined using visible light. The best separation was obtained with this TLC method as the mixed components were separated based on the distribution difference between the stationary phase (silica gel F254 plate) and the mobile phase (eluent) (Yuda et al., 2017). Identification of bioactive compounds in TLC was performed using spray reagents (Suhaenah & Nuryanti, 2017). The results of the flavonoid test on leaves, flowers, and stems of pacing plants in table 4 show positive results with Rf values of 0.86, 0.84, and 0.88, respectively. The study shows that the leaf, flower, and stem extracts of pacing plants contain flavonoids with Rf values of 0.85-0.87, which are indicated by a red color and yellow fluorescence after hydrolysis. Flavonoids have antioxidant activity due to the presence of phenolic hydroxyl groups in their structure (Rohmah et al., 2019). The alkaloid test on leaves and stems of pacing plants shows positive results with Rf values of 0.85 and 0.86, indicating the presence of alkaloid compounds. The phenol test on leaves and flowers shows positive results with Rf values of 0.63 and 0.66. Phenolic compounds have many OH groups, making them polar and soluble in polar solvents like ethanol (Suhaenah & Nuryanti, 2017).

CONCLUSION

The research results indicate that the ethanol extracts of pacing stems, leaves, and flowers contain antioxidants. The highest antioxidant content was found in the leaves with an

IC_{50} value of 17.8 ppm, followed by the stems with an IC_{50} value of 24.22 ppm, and the flowers with an IC_{50} value of 24.85 ppm. The secondary metabolite content in pacing plants showed the presence of flavonoids and alkaloids in the leaves, flavonoids and phenols in the stems, while only flavonoids were detected in the flowers.

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