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Ethanol Extract Antioxidant Activity Test Of Leaves Of Tembelekan (Lantana Camara L.) Using 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) Reagent)

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Abstract

The plant Prickly lantana (Lantana Camara L.) contains alkaloids, flavonoids, saponins, tannins, and triterpenoids and has the potential to be used as a natural antioxidant source. The research objective is to determine the IC_{50} value for the antioxidant activity of prickly lantana leaf extract. Prickly lantana leaves were macerated in a 96% ethanol solvent for 3x24 hours. Phytochemical analysis of Prickly Lantana leaves revealed that the leaves contained alkaloids, flavonoids, saponins, and tannins. This antioxidant activity test was performed using the reagent 1,1-diphenyl-2-picrylhydrazyl (DPPH), whose absorption at 517 nm was measured using a UV-Vis spectrophotometer, and by varying the sample concentration after the addition of prickly lantana leaf extract. The concentrations of prickly lantana leaf extract used varied from 20 ppm to 40 ppm, 60 ppm, and 80 ppm. The positive control was vitamin C at the same concentration variation, and the negative control was DPPH solution dissolved in 96% ethanol. The results showed that the IC_{50} of prickly lantana leaf extract was 164.639 ppm, while vitamin C had an IC_{50} of 18.754 ppm.

Keywords: Prickly lantana, antioxidant, DPPH, maceration, IC_{50}

Introduction

The biodiversity of Indonesia is the greatest of any country, and it can be used to meet the needs of the entire community. Biodiversity may be used for a variety of purposes, including food and medicine. Traditional medicine has been practiced in the community for a long time and has been passed down from generation to generation (Venkatachalam et al., 2011). However, because humans are unaware of the majority of the benefits of various plant types, their use is inefficient.

Prickly lantana (*Lantana camara L.*) is a plant that is used to treat a variety of ailments. This plant is a wild species rich in secondary metabolites, specifically volatile compounds, flavonoids, phenols, saponins, alkaloids, steroids, tannins, terpenoids, and quinones, according to (Bhaka & Ganjewala, 2009) in (Lestari et al., 2018). Secondary metabolites found in prickly plant leaves are antioxidants, anticancer, blood anticoagulants, antibiotics, and antibacterials (Pakaya et al., 2015). The community does not properly utilize this prickly lantana plant, particularly in the Tondo area of Palu City, Central Sulawesi. In the Tondo area, prickly lantana is commonly regarded as a wild plant; however, some people are only aware of its use as a wound medicine.

Prickly lantana (*Lantana camara L.*) is an antioxidant, which is a compound capable of neutralizing free radicals by donating one electron (Rahmi, 2017). Antioxidants benefit the body's health by protecting cells from free radical damage. Compounds or molecules with one or more unpaired electrons in their outermost orbital are classified as free radicals. (Sami & Rahima, 2015).

Free radicals can weaken the immune system, raise 'bad' cholesterol levels in the body, damage cell DNA structures, and cause inflammation. Excessive and continuous free radical exposure can increase the risk of premature aging and diseases such as dementia, cancer, and heart disease. Frequent exposure to free radicals can also make the body more susceptible to illness and increase the likelihood of developing cataracts. As a result, the body needs antioxidants to combat the effects of free radical exposure. Flavonoids, polyphenols, beta carotene, lutein, lycopene, selenium, zinc, anthocyanins (colorants found in fruits and vegetables), vitamin A, vitamin C, and vitamin E are examples of antioxidant substances. (Rahmi, 2017).

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One of several methods for reducing free radicals is the use of DPPH (1,1-diphenyl-2 picrylhydrazyl). The DPPH antioxidant activity test method was selected because it is simple, quick, easy, and sensitive, requiring only a small sample of the compound material to evaluate its antioxidant activity (Molyneux, 2004). A standard or positive control should be used in this antioxidant activity test. Ascorbic acid is the commonly used standard (vitamin C). Vitamin C is used as a comparison because it is a potent natural antioxidant. Vitamin C is more polar than vitamin E and vitamin A. Vitamin C has more antioxidant activity than vitamin E and vitamin A because it has four hydroxyl groups as opposed to one in vitamin E and zero in vitamin A. (Lung & Destiani, 2017).

This article aims to discover how much antioxidant activity *(IC_{50})* prickly lantana (*Lantana camara L.*) leaf extract has.

Methods

Equipment and materials

An analytical balance, rotary vacuum evaporator, UV-Vis spectrophotometer, blender, 70 mesh sieve, Erlenmeyer, funnel, shaker, stirring rod, dropper pipette, test tube, and measuring flask are among the equipment used.

Prickly Lantana leaves, 96 percent ethanol, 37 percent HCl, Dragendorff reagent, Magnesium powder, $FeCl_3$, aquades, CH_3COOH, H_2SO_4, DPPH, chloroform, vitamin C, and filter paper were used in this study.

Procedures for work

Preparation of the sample

Prickly Lantana leaves are collected and placed in a container. Young Prickly Lantana leaves are used in the picking process. The sample is then thoroughly washed with running water until it is clean. The Prickly Lantana leaves were then dried/aired for 7 days without direct sunlight exposure. After drying, the Prickly Lantana leaves are blended until smooth. After that, the mixture was sieved through a 70-mesh sieve. The resulting fine sample is now ready for use (Rachmani et al., 2018).

Extraction of samples

The dried and mashed Prickly Lantana leaves are weighed up to 20 grams. Furthermore, Prickly Lantana leaf powder was placed in the provided Erlenmeyer, followed by 200 mL of 96 percent ethanol solvent. The sample was then allowed to stand for 3×24 hours, with occasional stirring. Following that, the sample was shaken for 90 minutes before being filtered through a funnel. The extraction results are then concentrated using a rotary vacuum evaporator (Salamah & Widyasari, 2015).

Phytochemical test

a. Alkaloid test

In a test tube, a 0.5 mL thick extract of Prickly Lantana leaves was added, followed by 5 drops of 2 N HCl and 1 drop of Dragendorff's reagent. When a test is positive, a brown or orange precipitate forms (Tanzaq et al., 2013).

b. Flavonoid test

In a test tube, 0.5 mL of a thick extract of Prickly Lantana leaves was added, followed by 5 mL of 96 percent ethanol. The mixture was then treated with 10 drops of concentrated HCl and 0.1 grams of magnesium powder. A positive test results in an orange, pink, or red solution (Fajriah & Megawati, 2015).

c. Saponin test

In a test tube, a 0.5 mL thick extract of Prickly Lantana leaves was placed, followed by 2 mL of distilled water, and shaken. After that, the mixture was heated and 2 N HCl was added. A positive test resulted in the presence of foam that lasted less than 10 minutes after the mixture was vigorously shaken (Tanzaq et al., 2013).

d. Tannin test

In a test tube, 0.5 mL of a thick extract of Prickly Lantana leaves was added, followed by 5 mL of 96 percent ethanol. Then, 2-3 drops of 1 percent $FeCl_3$ were added. A positive test results in a solution that is red, blue, or blackish green (Minarno, 2015).

e. Triterpenoid test

In a test tube, a 0.5 mL thick extract of Prickly Lantana leaves was placed, followed by 2-3 drops of chloroform and 5 drops of anhydrous CH_3COOH. Then 1 mL of H_2SO_4 was added. A positive test is indicated by the presence of a red or purple color (Mangela et al., 2016).

Solution preparation and measurement

1 mg of DPPH was placed in a 10 mL volumetric flask. The volume is then filled with absolute ethanol to the mark and shaken until homogeneous.

Pipette 0.5 mL of DPPH solution into a volumetric flask of 10 mL. The volume was then filled with absolute ethanol. The solution is then homogenized.

10 mg of Prickly Lantana leaf extract was placed in a 10 mL volumetric flask. Absolute ethanol was then used to make up the difference in volume.

To make the vitamin C comparison solution, 10 mg of vitamin C was placed in a 10 mL volumetric flask. After that, the mixture was dissolved by adding up to the mark of absolute ethanol.

Pipette 0.2 mL mother liquor, 0.4 mL, 0.6 mL, and 0.8 mL reference solution, in that order. Then pour into a 10 mL volumetric flask. After adding 1 mL of DPPH solution, the volume was made up by adding absolute ethanol to the limit mark. Set aside for 30 minutes after that.

The blank solution, test solution, and comparison solution were placed in the cuvette. The solution was measured at 517 nm using a UV-Vis spectrophotometer.

The formula y = ax + b is used to calculate the IC50 value, where y equals 50 and x equals IC50 (Amin et al., 2014).

Results and Discussion

Extraction of prickly lantana leaves.

The extraction of 20 grams of Prickly Lantana leaf sample with 200 mL absolute ethanol produces 14.78 grams of thick extract abbreviated as EDT, with a 73.9% yield value.

Results of phytochemical tests

Table 1 shows the results of phytochemical tests on Prickly Lantana leaf extract for alkaloids, flavonoids, saponins, tannins, and triterpenoids.

Table 1. Results of phytochemical tests on Prickly Lantana leaf extract

No	Test	Reactor	Result
1.	Alkaloid	Reagen dragendroff	+
2.	Flavonoid	Mg and metals Concentrated HCl	+
3.	Saponin	Water and HCl 2 N	+
4.	Tanin	$FeCl_3$ Solution 1%	+
5.	Triterpenoid	CH_3COOH anhidrat and H_2SO_4	–

Results of absorption measurements

Table 2 shows the results of the absorbance measurement of the sample and comparison (vitamin C) to which DPPH solution was added at various concentrations.

Table 2. Results of the absorbance measurement of the sample and (vitamin C)

No	Concentration (ppm)	Absorbance	
		Leaves	Vitamin C
1.	0 (blanko)	1.394	1.394
2.	20	1.061	0.694
3.	40	1.016	0.678
4.	60	0.976	0.665
5.	80	0.904	0.638

Antioxidant activity of prickly lantana leaves

Prickly lantana leaf extract was tested for antioxidant activity using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) reagent as a free radical source. The DPPH antioxidant activity test method was chosen to measure antioxidant activity because it is simple and easy to use, has high sensitivity, can analyze data quickly, and requires only a small number of samples to test antioxidant activity (Ridho et al., 2014). The activity of DPPH was measured using a UV - Vis spectrophotometer with a wavelength of 517 nm.

In this study, color intensity was measured at concentrations of 20 ppm, 40 ppm, 60 ppm, and 80 ppm to determine the level of color reduction caused by the presence of antioxidant compounds that can reduce the intensity of DPPH's purple color (Dris & Jain, 2004).

The reaction that occurs between DPPH and antioxidant compounds can be seen in **Figure 1**.

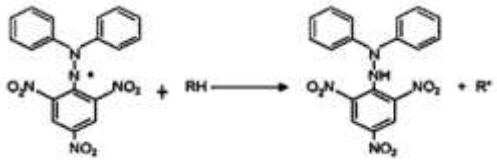

Figure 1. The reaction of DPPH free radicals with antioxidant compounds (Prakash, 2001)

The absorbance measurements of the prickly lantana leaf ethanol extract are illustrated in **Figure 2.**

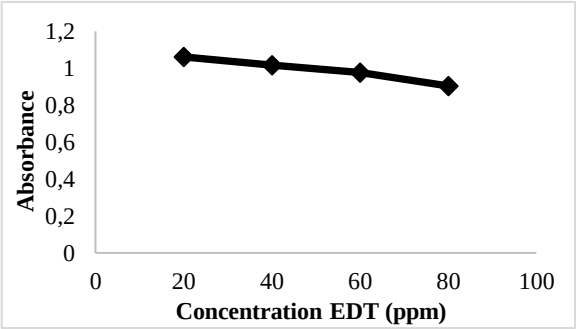

Figure 2. The relationship between the DPPH absorbance value and the concentration of prickly lantana leaf extract.

Figure 2 shows that the decrease in absorbance value in Prickly Lantana leaf extract occurs due to the reduction of DPPH radicals by antioxidants, where the higher the concentration of the sample, the more particles of antioxidant compounds contained, so the greater the antioxidant activity and causes the absorbance of DPPH to decrease (Talapessy et al., 2013).

Observation of the color intensity of prickly lantana leaf extract with DPPH can be seen in Figure 3, whereas seen from the left to right samples (20, 40, 60, and 80 ppm) the color of the sample is getting faded. This happens because of the reduction reaction in DPPH by antioxidant compounds.

Figure 3. Level of color reduction in Prickly lantana leaf extract

Based on the results of measuring the absorbance value of the sample, the percent inhibition value of the Prickly Lantana leaf extract was calculated from the percentage of free radical inhibitors shown in **Figure 4.**

Figure 4 shows that the percentage of inhibition of Prickly Lantana leaf extract increased with increasing sample concentration. The calculated percentage of inhibition matched the color change of DPPH when added to the Prickly Lantana leaf extract solution, with the lightest color indicating a higher percentage of inhibition.

Vitamin C antioxidant activity test

In the same way, the antioxidant activity of vitamin C was tested on Prickly Lantana leaf extract and DPPH as a negative control. The antioxidant activity of Prickly Lantana leaf extract was tested using the same vitamin C concentrations, namely 20, 40, 60, and 80 ppm. Based on the research findings, the absorbance value for vitamin C is shown in **Figure. 5**.

Figure 5 shows that vitamin C concentration is inversely proportional to DPPH absorbance value, with higher vitamin C concentration resulting in a lower absorbance value. Because vitamin C contains more antioxidant compounds and is more potent, it serves as a positive control of antioxidant compounds in this study.

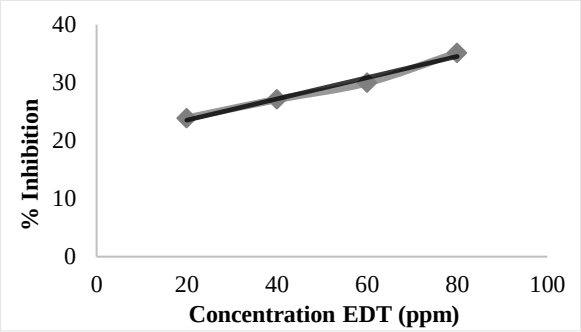

Figure 4. The percentage value of Prickly Lantana leaf extract inhibition

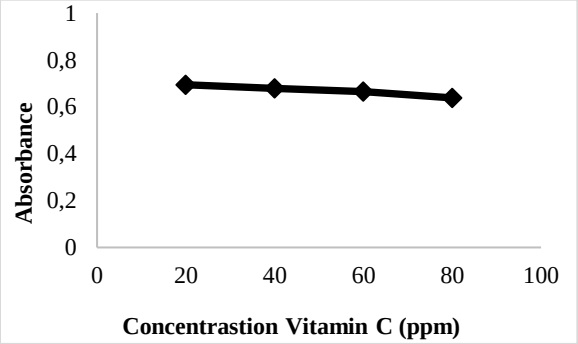

Figure 5. The relationship between DPPH absorbance and vitamin C concentration

Observations on the intensity of vitamin C with DPPH can be seen in **Figure 6**, whereas seen from the left to right samples (20, 40, 60, and 80 ppm) the color of the sample is getting lighter.

Figure 6. Level of color reduction vitamin C

Following the determination of the absorbance value of vitamin C, the percent inhibition value is computed using the previously determined absorbance value; **Figure 7** depicts the percent value of vitamin C inhibition against DPPH free radicals.

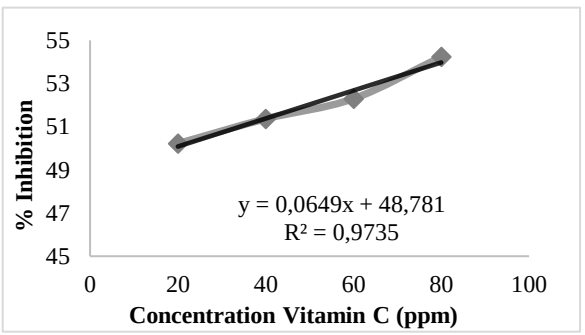

Figure 7. Vitamin C inhibition percentage.

Free radical scavenging activity of prickly lantana leaf extract versus vitamin C

This study's findings aimed to compare the free radical scavenging activity of Prickly Lantana leaf extract with vitamin C as a positive control, as

evidenced by the percentage of inhibition. The data showed that the percent inhibition of vitamin C was higher in the Prickly Lantana leaf extract sample. Figure 6 depicts a comparison of the percentage of inhibition of prickly lantana leaves and vitamin C.

Figure 4 and **7** shows that prickly lantana leaf extract has a lower percentage of inhibition than vitamin C. Since the phytochemical assays of Prickly Lantana leaf extract continue to produce negative results for the tested antioxidant compounds, the difference in percentage inhibition between the sample extract and vitamin C appears to be quite significant. This, of course, inhibits the Prickly Lantana leaf extract. Furthermore, vitamin C is a pure isolated compound that has very strong antioxidant (Fitriani et al., 2019), while the sample extract is a mixed compound, which can affect the inhibitory power of the Prickly Lantana leaf extract due to non-antioxidant compounds. Another factor, according to Wikanta et al. (in Fauziah et al., 2021), is the low levels of antioxidants contained in the leaf extract, which could be due to impurities. Impurities in the extract can reduce the levels of active compounds, requiring their removal. Chlorophyll, minerals, and others are examples of impurities.

Calculation of the IC_{50} value

The inhibitor concentration *(IC_{50})* value, which is the concentration that results in a 50% loss of DPPH activity, is a commonly used parameter to interpret DPPH test results. The lower the IC_{50} value, the higher the antioxidant activity. By graphing the relationship between the percent inhibition value and the concentration value obtained from the DPPH free radical inhibitor test, the IC_{50} value can be calculated, with the X-axis representing the percent inhibition value and the Y-axis representing the concentration value. Figures 3 and 4 depict a graph of the relationship between the percentage of inhibition and the concentrations of Prickly Lantana leaf extract and vitamin C, respectively.

Based on **Figures 4** and **7**, the linear regression equation for Prickly Lantana leaf extract is y = 0.183x + 19.871 and y = 0.065x + 48.781 for vitamin C (positive comparison/control). After replacing y with 50, this equation yields the value of x as IC_{50}. In addition to the linear regression equation, the image above provides the value of R^2 (correlation coefficient). R^2 values are the linear relationship between inhibition and concentration percentages. The calculated data is very strong if the calculated graph has an R^2 value close to or equal to one, indicating that the two variables are closely related. The R^2 values for prickly Lantana leaf extract and vitamin C were 0.981 and 0.974, respectively, indicating that the obtained data are excellent because their R^2 values are close to 1.

Rizkayanti et al., (2017) classify compounds as very strong if the IC_{50} value is <50 ppm, strong if the IC_{50} value is 50-100 ppm, moderate if the IC_{50} value is 100-150 ppm, weak if the IC_{50} value is 150-200 ppm, and inactive or very weak if the IC_{50} value is >200 ppm.

Prickly Lantana leaf extract has an IC_{50} value of 164.639 ppm, and vitamin C has an IC_{50} value of 18.754 ppm. It implies that the antioxidant activity of the bioactive compounds found in Prickly Lantana leaf extract is low, whereas vitamin C has extremely high antioxidant activity. The high antioxidant activity difference between Prickly Lantana leaves and vitamin C is due to the presence of secondary metabolites that act as antioxidants in the Prickly Lantana leaf extract indicated in the phytochemical test, which still yields negative results, namely the triterpenoid test, which is one of the factors causing the low antioxidant activity.

According to the IC_{50} value, vitamin C is a stronger antioxidant when compared to the Prickly Lantana leaf extract used as a sample in this study, where the IC_{50} value of vitamin C is much lower, 18.754 ppm, which means the lower value. The greater the antioxidant activity, the lower the IC_{50}. As a result, prickly lantana leaves may be useful as natural antioxidants.

Conclusions

The phytochemical test on Prickly Lantana leaf extract revealed that it contained alkaloids, flavonoids, saponins, and tannins. Based on the IC_{50} value, the antioxidant activity of prickly lantana leaf samples is 164.639 ppm, or in the range of 150-200, indicating that it is a weak antioxidant.

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