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Antioxidant Activity Test of (Acalypha indica L) Extract

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Abstract

The antioxidant activity test of Acalypha indica L has been carried out. This research sought to ascertain the IC50 value of the Acalypha indica L extract. The radical concentration of 2,2-diphenyl-1-picrylhydrazyl (DPPH) after adding Acalypha indica L extract was determined using a UV-Vis spectrophotometer. The concentration variations used in Acalypha indica L extract were 15.48 ppm, 30.96 ppm, 46.44 ppm, and 61.92 ppm. The findings indicated that the IC50 value of Acalypha indica L extract was 41,259 ppm, while the IC_{50} value of vitamin C as a comparison was 50.626 ppm. According to the given IC50 value data, it can be seen that the antioxidant of Acalypha indica L extract is robust compared to vitamin C.

Keywords: *Acalypha indica L* leaves, antioxidant, DPPH, UV-Vis spectrophotometer

Introduction

Using plants as drugs is related to the content of secondary metabolites synthesized by plants as an adaptation to the environment or as part of growth and development. The levels and types of secondary metabolites vary significantly from one type of plant to another. So, it is often also used as one for plant identification. *Acalypha indica L* is a medicinal plant that has long been used as an ingredient in traditional medicine. Phytochemical screening of water extracts of leaves stems, and roots *of Acalypha indica L* contain saponins, flavonoids, steroids, phenols, alkaloids, tannins, and glycosides (Nkumah et al., 2016). Using plants as traditional medicine is related to the content of secondary metabolites or their bioactivity. From the research results, *Acalypha indica L* has bioactivity as an antioxidant (Teklani & Perera, 2016).

The earring plant is used for joint rheumatism and lowers uric acid. The community uses the earring plant to cure enzymatic diseases, uterine bleeding, and dermatitis (Wijayakusuma, 2001). Antioxidants are substances that, in negligible amounts, can substantially inhibit or avoided the oxidation of substrates. Antioxidants are categorized into synthetic and natural type. Synthetic antioxidants that are allowed and commonly used for food, namely BHA, BHT, error profile and tocopherol, while natural antioxidants derived from plants are phenolic compounds that can be found as flavonoid groups, cinnamic acid derivatives, coumarins, tocopherols, and polyfunctional organic acids. In recent years, much attention has been paid to developing natural antioxidants for preventive medicine. The mechanism of action of phenolic antioxidants is a potent radical scavenger. Phenolic chemicals, as biologically active constituents, can donate hydrogen to free radicals and disrupt the chain of lipid oxidation processes at the first initiation phase (Isnindar et al., 2011).

Antioxidants can delay, slow, or inhibit oxidation reactions (Ade, 2018). Various antioxidant compounds can inhibit oxidative chain reactions to repair oxidative damage to body cells (Reksi & Sapri, 2018).

Free radicals are generated as a result of stress and can lead to cancer and cardiovascular disease. Antioxidant substances, including vitamin C, vitamin E, polyphenols, phenolic compounds, and flavonoids, can mitigate free radicals (Kavitha et al., 2009).

Free radicals are atoms or molecules whose valence electrons are unpaired in their outermost orbitals and are highly reactive and unstable. These atoms are hydrogen atoms, transition metals and oxygen molecules. To achieve stability, free radicals need electrons around them to react to obtain electron pairs (Masrifah et al., 2017). If it is in the body, this reaction will occur continuously, and if it is not stopped immediately, it will damage cells and tissues in the human body (Nasution & Rahma, 2014). Cellular damage can lead to aging and a range of diseases, including cancer, diabetes, rheumatoid arthritis, cardiovascular disorders, chronic inflammation, and stroke (Shanmugam et al., 2018).

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An easy-to-do antioxidant activity check technique uses stable free radicals, 2.2-diphenyl-1-picrylhydrazyl (DPPH). DPPH offers data on the reactivity of chemicals when assessed against a stable radical. DPPH provides strong absorption at a wavelength of 517 nm with a dark violet colour. The compound functioning as a free radical eliminator will reduce DPPH, as indicated by the color change of DPPH from purple to yellow, due to the unpaired electron of the DPPH radical pairing with hydrogen from the free radical eliminator, leading to the creation of reduced DPPH-H (Molyneux, 2004). This study aims to determine the antioxidant activity of earring plant extract as a natural antioxidant and its antioxidant power category in inhibiting free radicals.

Methods

Tool

The equipment used is UV-Vis spectrophotometry, blender, 25 mL measuring flask, cuvette, digital balance, drop pipette, knife, baking dish, 500 mL measuring cup, 500 mL beaker, stirring rod, funnel, filter paper, rotary evaporator, and glassware every day in the laboratory.

Material

The ingredients used are earring leaves (*Acalypha indica L*), 96 % absolute ethanol, vitamin C, and 2.2-diphenyl-1-picrylhydrazil (DPPH).

Sample preparation

The sample preparation carried out in this study includes several stages: the earring plant is picked and washed with running water until clean, the earring plant is taken from the leaves, and the leaves of the earring plant are mashed using a blender. The purpose of blending is To minimize the sample size, hence facilitating rapid contact between the solids (samples) and the solution during the extraction process, due to the increased surface area of the sample, ensuring optimal diffusion of the sample and solvent (Shah & Modi, 2015). Then, the acceptable samples are extracted using ethanol.

Extraction with ethanol

Earrings leaf extract was weighed as much as 50 grams using a digital balance, then macerated using absolute ethanol as much as 500 mL for 2 × 24 hours while stirring occasionally; after 2 × 24 hours, the extract was filtered, and after filtering, the residues obtained were re-macerated in the same way. After that, the extract results were filtered, and then the filtrate obtained was concentrated using a rotary evaporator at a temperature of 40 °C.

Antioxidant activity test

Earrings leaf extract was weighed as much as 25 mg using an analytical balance. Next, it is put into a measuring flask of 25 mL, then dissolved with ethanol, and the volume is sufficient to the limit mark so that 1000 ppm is obtained. Furthermore, each solution was taken in 0.5 mL, 1 mL, 1.5 mL, and 2 mL, Subsequently, each was combined with 2.5 mL of DPPH. The volume of ethanol was enough up to the boundary mark, resulting in an extract solution with concentrations (15.48, 30.96, 46.44, and 61.92 ppm) was obtained. The concentration of the test solution was adjusted to assess the degree of color immersion resulting from antioxidant chemicals that may diminish the intensity of the purple hue of DPPH (Molyneux, 2004).

Vitamin C, as a comparison, was weighed as much as 25 mg, put into a 25 mL measuring flask, and then dissolved with ethanol, and the volume was sufficient to the limit mark so that 1000 ppm was obtained. Furthermore, each solution was taken as much as 0.5 mL, 1 mL, 1.5 mL and 2 mL, after which each solution was added 2.5 mL of DPPH, then the volume was sufficient with ethanol until the boundary mark. So that an extract solution with concentrations (15.48, 30.96, 46.44, and 61.92 ppm) was obtained. Vitamin C is used as a comparison solution because vitamin C or ascorbic acid is one of the natural antioxidant substances. In addition to being easy to obtain and a common standard often used in the comparison of antioxidants, vitamin C is a natural antioxidant substance BPOM recommends to be consumed by the general public. Therefore, this study used vitamin C as a positive control or comparison (Purwati, 2012).

The determination of antioxidant activity at each concentration was taken as much as 0.5 mL of sample solution with a micropipette and put into a vial, adding 3.5 mL of DPPH 50 mm solution. The mixture is homogenized, and absorption is measured with a UV-Vis spectrophotometer at a wavelength of 517 nm. The antioxidant activity of the sample is determined by the magnitude of the radical resistance of DPPH through the calculation of the percentage of inhibition of DPPH uptake using the formula:

$$Inhobitor\ (\%) = \frac{blanko\ absorbance - sample\ absorbance}{blanko\ absorbance} x\ 100$$

Description:
Blanko absorbance: An absorbant DPPH 50 mM
Sample absorbance:An absorbance of test sample (Nurhaeni et al., 2019).

The results of the calculations obtained are included in the regression equation with the concentration of extract (ppm) on the x-axis and the value of % of inhibition (antioxidants) on the y-axis to determine the IC_{50} with the equation:

$$Y = ax + b$$

$$50 = ax + b$$

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

(Hestiningtyas et al., 2019)

Antioxidant activity in earring leaf extract was tested using DPPH as a free radical provider. The antioxidant test method using DPPH is a

quantitative test method to find the activity power of earring leaves as an antioxidant. DPPH was chosen because it is a simple, easy, fast, and sensitive method and requires only a tiny sample for antioxidant evaluation of natural compounds (Salamah & Widyasari, 2015).

Results and Discussion

Extraction with ethanol

The extraction method used is maceration (cold extraction) to prevent the damage of chemical compounds that are not resistant to heating, especially flavonoids. The principle of this extraction is that the liquid enters the cell cavity containing the active substance and will dissolve into the solvent because there is a difference in concentration between the solution of the active substance inside the cell and outside the cell. The active substance will diffuse out of the cell (Estikawati & Lindawati, 2019). Extraction is a selective process of taking substances in a mixture using appropriate solvents (Verdiana et al., 2018). Earrings leaf extract was weighed as much as 50 grams using a digital balance, then put into a 500 mL chemical glass and then macerated using 500 mL ethanol solvent for 2 × 24 hours. The purpose of immersing a sample with a solvent volume of 10 times the sample weight is an effective way in the extraction process so that the amount of solvent used is 500 mL for 50 grams of simplicia (Salamah & Widyasari, 2015). The purpose of using ethanol as a solvent is because ethanol penetrates cell membranes more easily, dissolves secondary metabolite compounds from plant materials and is easily vaporized. The selection of ethanol solvents is based on *like dissolve,* meaning that polar solvents will dissolve polar compounds, and non-polar solvents will dissolve non-polar compounds (Sastrohamidjojo, 2016).

The earring leaf extract is filtered with a separate funnel to separate the filtrate and residue. The residue obtained is added 500 mL of 96 % ethanol. The mixture is macerated for 2 × 24 hours, so the diffusion process takes place optimally. Then, it is filtered through a separate funnel to obtain a filtrate. The filtrate obtained is concentrated with *a rotary evaporator*. The purpose of the concentration is to separate and evaporate between the solvent and the extract so that a thick extract of the green leaves of the earrings is obtained, a concentrated extract of the leaves of the earrings that is ready for further analysis.

Antioxidant activity test

The antioxidant activity of earring leaf extract was tested by reacting samples and DPPH solution and measuring at 517 nm, the maximum wavelength of DPPH. This maximum wavelength provides the maximum absorption of the test solution and the most incredible sensitivity. This method was chosen because it is easy, fast, simple, has a high level of sensitivity, and can analyze samples quickly (Widiastuti, 2016).

This study uses the DPPH method to determine the value of antioxidant activity. The antioxidant activity test method with DPPH (2,2-diphenyl-1-picrylhydrazyl) was chosen because it is simple, fast, and sensitive. It only requires a small sample to evaluate the antioxidant activity of natural material compounds, so it is widely used to test the ability of compounds that act as electron or hydrogen donors (Pienyani et al., 2018).

Antioxidant activity testing was carried out by determining the percentage of inhibition of earring leaf extract at each concentration, which was 15.48 ppm, 30.96 ppm, 46.44 ppm and 61.92 ppm. The absorbance value of the earring leaf extract decreases with increasing concentration. This occurs due to the reduction of DPPH radicals by antioxidants, where the higher the concentration of earring leaf extract, the greater its antioxidant activity, and causes its absorption to decrease (Molyneux, 2004). The results of the DPPH absorbance measurement in the earring leaf extract can be seen in **Figure 1** and the DPPH absorbance is 0.153.

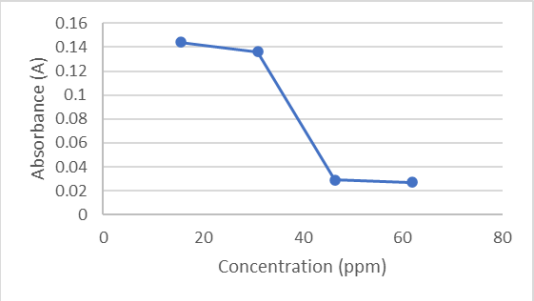

Figure 1. Concentration relationship curve (ppm) of earring leaf extract with absorbance value (A)

The IC_{50} value in the earring leaf extract was determined using the linear regression equation of the sample concentration relationship curve to the percentage of inhibition presented in **Figure 2.**

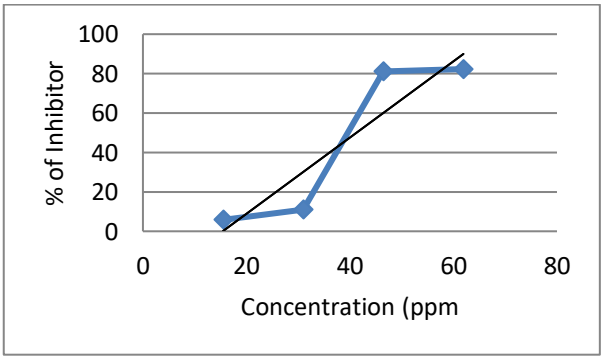

Figure 2. Concentration relationship curve (ppm) of earring leaf extract with inhibition percentage

The curve in **Figure 2.** The value for linear regression Y=1.9341x - 29.8 for the earring leaf extract was obtained. The data shows that the greater the concentration of earring leaf extract, the

greater the percentage of DPPH radical inhibitors. The highest percentage value of inhibition for earring leaf extract was 82.3 %. The IC_{50} value for earring leaf extract was 41.259 ppm.

The absorbance value of earring leaf extract decreases with increasing concentration. This occurs due to the reduction of DPPH radicals by antioxidants, where the higher the concentration of earring leaf extract, the more antioxidant compound particles will be contained, so the more significant the antioxidant activity and cause the absorption to decrease (Molyneux, 2004).

In this test, vitamin C (ascorbic acid) compares antioxidant activity with a concentration of 15.48 each, 30.96, 46.44, and 61.92 ppm. The greater the concentration of vitamin C, the greater the percentage of DPPH free radical inhibition that occurs. This is because the more significant the concentration of vitamin C, the more particles can oxidise particles from existing DPPH free radicals (Mariani et al., 2018). The antioxidant activity test against vitamin C can be shown in **Figure 3.**

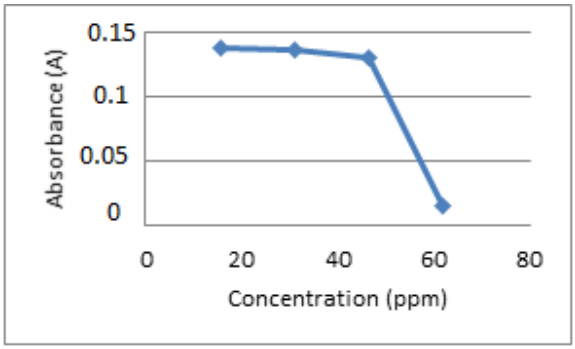

Figure 3. Curve of the relationship between vitamin C concentration (ppm) and absorbance value (A)

The absorbance value in the vitamin C antioxidant test obtained the percentage of DPPH free radical inhibition, as seen in **Figure 3.** showing the relationship between vitamin C concentration and DPPH free radical inhibition percentage. The greater the concentration of vitamin C, the greater the percentage of DPPH free radical inhibition. This is because the more significant the concentration of vitamin C, the more particles can oxidize particles from the existing DPPH free radicals (Molynux, 2004). The IC_{50} value of vitamin C was determined by using the linear regression equation of the relationship curve of sample concentration to percent inhibition presented in **Figure 4.**

The percentage of inhibition increased drastically from the earring leaf extract from the concentration of 30 to 45 and vitamin C from the concentration of 45 to 60. This is suspected to be due to the synergy between the antioxidant compounds in the extract, which ensures that the antioxidant activity remains stable and increases significantly. This is also due to the influence of solvents on the sample, which results in the sample not completely dissolving in DPPH (Dewi et al., 2007).

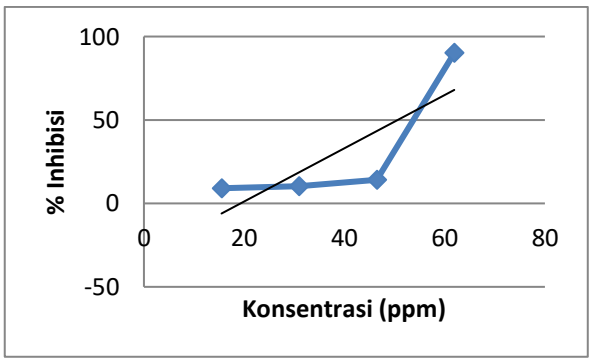

Figure 4. Ascorbic acid concentration (ppm) concentration relationship curve with inhibition percentage (%)

This arises from errors resulting from inadequate preparation of the solution sequence employed. The spectrophotometer is not calibrated accurately, and there are contaminants in the test tube utilized as the initial container for the solution (Day, 1999). Multiple factors can contribute to subpar data, including inadequate preparation of the concentration series of solutions, improper calibration of UV-Vis spectrophotometer devices, and contaminants in the cuvette utilized at the onset of the solution (Day & Underwood, 2002).

The IC50 value of the earring leaf extract is 41.259 ppm when compared to the IC50 value of the vitamin C comparator. This indicates that the antioxidant properties of the bioactive compounds in the earring leaf extract are robust and have good antioxidant activity compared to ascorbic acid. A compound is said to have good antioxidant activity if the IC_{50} value is less than 200 ppm. If a compound has an IC_{50} value above 200 ppm to 1000 ppm, it can be stated that it still has potential as an antioxidant, but its activity is not good (Widianingsih, 2016).

The sample was declared as a powerful antioxidant if the IC_{50} value < 50 ppm, as a potent antioxidant if the IC_{50} value was 50 - 100 ppm, as a moderate antioxidant if the IC_{50} value was 100 - 150 ppm, as a weak antioxidant if the IC_{50} value was 151 - 200 ppm and was declared inactive if it had an IC_{50} value > 200 ppm (Artanti & Lisnasari, 2018). The stability of antioxidants can be influenced by various circumstances, including temperature, pH fluctuations, light exposure, oxygen presence, and metal ions. Furthermore, the process of analysis is not conducted immediately upon the preparation of the viscous extract, resulting in the degradation of the suspected phenolic compounds due to the sample's condition and storage duration. In addition, some factors affect active substances in plants, namely the nutrient content in plants (Sari & Ayati, 2018).

Conclusions

According to the research findings, earring leaf extract can be classified as a potent natural antioxidant, with an IC_{50} value of 41.259 ppm.

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