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A COMPARISON OF ANTIOXIDANT ACTIVITY IN TEA MISTLETOE (*Scurrula atropurpurea* (Bl.) Dans.) LEAF EXTRACTS USING DPPH ASSAY

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ABSTRACT

The general public often regards mistletoe as a parasitic plant that can harm other plants. However, some communities aware of its benefits have utilized mistletoe as an alternative treatment for various diseases. Tea mistletoe (*Scurrula atropurpurea* (Bl.) Dans.) is one type of mistletoe that the community has used to treat various ailments. Based on phytochemical analysis, this plant contains active compounds such as flavonoids, alkaloids, steroids, tannins, saponins, phenolics, and glycosides, which act as antioxidants. Therefore, this study was conducted to provide new information about the potential of tea mistletoe (*Scurrula atropurpurea* (Bl.) Dans.) leaf extract as a natural antioxidant with low toxicity effects through the DPPH method and to demonstrate the effectiveness of this natural antioxidant in inhibiting free radicals. This research is an experimental laboratory study with a quantitative approach. A series of sample concentrations was prepared, with 0,15 mL of each concentration placed into a 12-well plate and homogenized with 2,85 mL of 0,1 mM DPPH solution. The mixture was incubated for 30 minutes before its absorbance was measured using a UV-Visible Spectrophotometer. The results of this study showed that tea mistletoe (*Scurrula atropurpurea* (Bl.) Dans.) leaf extract exhibited very strong antioxidant activity, with an IC_{50} value of 3,32 ppm, comparable to quercetin.

Keywords: *tea mistletoe, DPPH, antioxidant, extract.*

INTRODUCTION

The Indonesian general public has long used plants as an alternative form of medicine. One of the plants with potential as a raw material for medicine is mistletoe (Sjakoer & Mubarakati, 2021). Traditional medicinal plants are processed in various ways, including boiling, grinding, pounding, soaking, applying, burning, squeezing, chewing, or consuming them directly (Fakaubun et al., 2022). The general public often regards mistletoe as a parasitic plant that can harm other plants. However, some people familiar with its benefits have used mistletoe as an alternative treatment for various diseases (Prabandari et al., 2024).

Tea mistletoe (*Scurrula atropurpurea* (Bl.) Dans.) is one type of mistletoe that the community has used to treat various ailments. Phytochemical analysis has shown that this plant contains active compounds such as flavonoids, alkaloids, steroids, tannins, saponins, phenolics, and glycosides, all of which act as antioxidants (Mustafa et al., 2021). These compounds make tea mistletoe effective as an antihypertensive (vasodilator). This is supported by research conducted by Athiroh dan A'yun (2020) which showed that tea mistletoe reduces blood pressure in experimental animals and is safe for consumption. Tea mistletoe (*Scurrula atropurpurea* (Bl.) Dans.) was also found to reduce the contractility of isolated rat tail artery vessels due to the role of endothelial cells (Athiroh et al., 2019).

There are several methods for testing antioxidant activity to neutralize free radicals, such as DPPH, ABTS, ORAC, CUPRAC, and FRAP. In this study, the DPPH method was chosen due to its simplicity, effectiveness, speed, and reproducibility (Poli et al., 2022). This research aims to provide new insights into the potential of tea mistletoe (*Scurrula atropurpurea* (Bl.) Dans.) leaf extract as a natural antioxidant with low toxicity, using the DPPH method. Additionally, it seeks to present the effectiveness of this natural antioxidant in inhibiting free radicals.

METHODS

This research was an experimental laboratory research with a quantitative approach conducted from November to December 2024 at the Integrated Laboratory of the Islamic University of Malang.

Tools and Materials

The tools used in this research included dark glass bottles, glass funnels, dropper pipettes, 12-well plates, volumetric flasks, beakers, watch glasses, erlenmeyer flasks, cuvettes, spatulas, refrigerators, rotary evaporators, UV-Visible spectrofotometers, analytical balances, blenders, vials, microppettes, vortex mixers, shakers, moisture anayzers, alumunium foil, plastic wrap, filter paper, sponges, bottle brushers, labels, and camera. The materals used were

tea mistletoe leaves, DPPH reagent powder (1,1-Diphenyl-2-picrylhydrazyl), quarcetin powder, ethanol pro analysis, 96% ethanol, and diswashing soap.

Sample Preaparation

Tea mistletoe (*Scurrula atropurpurea* (Bl.) Dans.) leaves were sorted, washed under running water, and dried using an oven. The dried leaves were blended and sieved into a fine powder. The moisture content of the sample must meet the quality standard requirement ≤10% (Utami et al., 2017). Once the moisture content complied with the standard, the sample was used for the extraction process.

Sample Extraction

The sample was extracted using the maceration method. A total of 100 grams of powdered sample was weighed and placed in a dark glass bottle, then 700 mL of 96% etanol was added. The mixture was homogenized repeatedly and allowed to stand for 3 days and 2 nights. The first maceration filtrate was then filtered. Subsequently, 300 mL of 96% ethanol was added to the residue in the dark glass bottle from the first maceration, homogenized, and filtered again into an erlenmeyer flask. The filtrate from the first and second macerations was combined and evaporated using a rotary evaporator to produce a concentrated extract.

Antioxidant Testing Using the DPPH Method

1. **Determination of Maximum Wavelength of 0,1 mM DPPH Solution**

   A 0,1 mM DPPH solution was prepared by weighing 4 mg of DPPH powder and dissolving it in ethanol pro analysis in a 100 mL volumetric flask up to the marked line. Then, 3 mL of the DPPH solution was pipetted into a cuvette and measured using a UV-Visible spectrophotometer over a wavelength range of 400–600 nm. Once the maximum wavelength was identified, the absorption measurement was conducted by mixing 2,85 mL of the DPPH solution with 0,15 mL of ethanol pro analysis, and the absorption was measured at the identified wavelength (Theafelicia & Wulan, 2023).
2. **Preparation of Standard Solution as Comparator**

   A standard comparator solution was prepared by weighing 10 mg of quercetin and dissolving it in ethanol pro analysis in a 10 mL volumetric flask up to the marked line. The stock solution was homogenized using a vortex mixer and divided into a series of concentrations (5 ppm, 10 ppm, 15 ppm, 20 ppm, 25 ppm). Each concentration was pipetted 0,15 mL into a 12-well plate and mixed with 2,85 mL of 0,1 mM DPPH solution. The mixture was incubated for 30 minutes, and its absorption was measured using a UV-Visible spectrophotometer at a wavelength of 517 nm (Theafelicia & Wulan, 2023).

3. Preparation of Test Solution

Tea Mistletoe Leaf Extract

The tea mistletoe (*Scurrula atropurpurea* (Bl.) Dans.) leaf extract sample was prepared by weighing 10 mg of the extract and dissolving it in ethanol pro analysis in a 10 mL volumetric flask up to the marked line. The sample solution was homogenized using a vortex mixer and divided into a series of concentrations (100 ppm, 125 ppm, 150 ppm, 175 ppm, 200 ppm). Each concentration was pipetted 0,15 mL into a 12-well plate and mixed with 2,85 mL of 0,1 mM DPPH solution. The mixture was incubated for 30 minutes, and its absorption was measured using a UV-Visible spectrophotometer at a wavelength of 517 nm (Theafelicia & Wulan, 2023).

Free Radical Scavenging Activity of DPPH

Antioxidant activity was determined through the inhibition value using a UV-Visible spectrophotometer. The absorbance data obtained were used to calculate the percentage of DPPH free radical inhibition. The antioxidant capacity for scavenging free radicals was expressed using the following formula.

$$\text{DPPH Free Radical Inhibition (\%)} = \frac{A_0 - A_1}{A_0} \times 100\%$$

Keterangan: A_0 = Control Absorbance
A_1 = Sample/Standard Absorbance

The absorbance of the control was the absorbance data of DPPH mixed with ethanol pro analysis, while the absorbance of the sample/standard was the absorbance data of the standard/sample solution mixed with DPPH (Khalil et al., 2020).

Data Analysis

Antioxidant activity was analyzed using Microsoft Excel with the data represented through graphs and expressed as IC_{50} values obtained from the linear regression equation y = ax + b (Werdyani et al., 2019). The higher the IC_{50} value, the lower the antioxidant activity of the sample, whereas the lower the IC_{50} value, the higher the antioxidant activity of the sample.

RESULTS AND DISCUSSION

RESULTS

The antioxidant activity test of tea mistletoe leaf extract, using quarcetin as a comparator revealed the presence of antioxidant activity.

Table 1. Antioxidant activity measurements of tea mistletoe *(Scurrula atropurpurea* (Bl.) Dans.) leaf extract and quarcetin

Sample	*Konsentrasi (ppm)*	*Rata-Rata Absorbansi*	*% Inhibisi*	*IC_{50} (ppm)*
Benalu Teh	100	0,781	30,173	3,320007589
	125	0,660	40,966	
	150	0,548	50,984	
	175	0,480	57,036	
	200	0,458	59,034	
Quarcetin	5	0,552	50,656	0,972065064
	10	0,493	55,874	
	15	0,239	78,623	
	20	0,125	88,790	
	25	0,103	90,757	

Figure 1. Linear regression curve of antioxidant activity of tea mistletoe *(Scurrula atropurpurea* (Bl.) Dans.) leaf extract

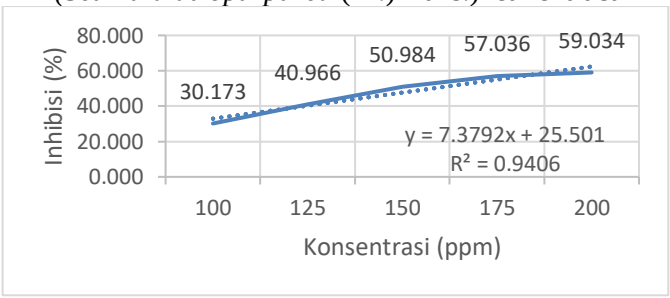
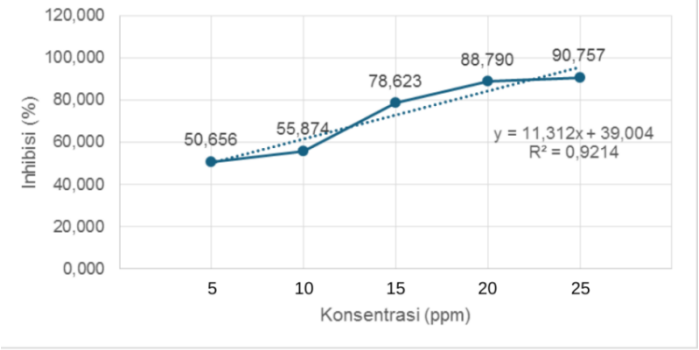


Figure 2. Linear regression curve of antioxidant activity of quarcetin



DISCUSSION

The determination of antioxidant activity began by preparing leaf extracts of tea mistletoe *(Scurrula atropurpurea* (Bl.) Dans.) using ethanol as the solvent through the maceration method. Ethanol was chosen because it is capable of extracting both polar and non-polar soluble components, thereby allowing the extraction of all chemical compounds present in tea mistletoe (Dianda & Suharti, 2023). The obtained tea mistletoe leaf extract was then

tested using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method with quarcetin as a standard reference. The DPPH method was used because it is simple, fast, sensitive, and widely used to evaluate the ability of chemical compounds in samples to scavenge free radicals (Putri & Mahfur, 2023).

The antioxidant activity test of the extract and the standard solution was conducted by reacting a series of sample and quarcetin concentrations with DPPH reagent, followed by measuring their absorbance using a UV-Visible spectrophotometer at a wavelength of 517 nm. A color change in the sample from deep purple to yellow indicated the presence of antioxidants in tea mistletoe *(Scurrula atropurpurea* (Bl.) Dans.) leaf extract.

The absorbance and inhibition percentage results for the extract and the standard solution are presented in Table 1. The data show that as the sample concentration increased, the absorbance decreased while the % inhibition increased. This occurs because higher sample concentrations contain more chemical compounds that inhibit DPPH free radicals. Inhibtion percent is a parameter that reflects the effectiveness of an antioxidant in inhbiting free radicals. The parameter used to measure the antioxidant capability of a compound is the IC_{50} value. The IC_{50} value represents the concentration of antioxidant compounds required to scavenge 50% of DPPH free radicals (Pratiwi et al., 2023). Tabel 1. indicated that the IC_{50} value for the tea mistletoe *(Scurrula atropurpurea* (Bl.) Dans.) leaf extract has a free radical scavenging activity of less than 50 ppm, similar to quarcetin. Both samples exhibited very strong antioxidant activity, although the inhibtion capacity of quarcetin was higher than that of the tea mistletoe leaf extract. A sample is categorized as having very strong antioxidant activity if its IC_{50} value is less than 50 ppm, strong activity ranges between 50-100 ppm, moderate activity between 100-150 ppm, weak activity between 150-200 ppm, and very weak or no antioxidant activity if the IC_{50} value exceeds 200 ppm (Sulistyani, 2024). The inhibitory concentration was calsulated using a linear regression equation to determine the relationship between sample concentration (x) and % inhibiton (y). The IC_{50} value was obtained by substituting 50 as the y-value into the linear regression equation derived from the log concentration versus % inhibition graph (Figures 1 and 2). From the curve, a linear regression equation was obtained with a good corelation for the tea mistletoe *(Scurrula atropurpurea* (Bl.) Dans.) leaf extract (R^2 = 0,9406). This R^2 value indicates a linear relationship between concentration and inhibition percentage. The closer the R^2 value is to 1, the clearer the relationship between increased extract concentration and antioxidant activity.

CONCLUSION

Based on this study, it can be concluded that the extract of tea mistletoe leaves *(Scurrula atropurpurea* (Bl.) Dans.) exhibits very strong antioxidant activity with and IC_{50} value of 3,32 ppm, which is comparable to quarcetin.

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