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Antioxidant Activity Test of Sappan (*Caesalpinia sappan* L.) and Chinese Teak (*Senna alexandrina*) Extract Combination Using DPPH (*1,1 Diphenyl 2 Picrylhydrazyl*) Free Radicals Scavenging Method

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Abstract
Sappan (Caesalpinia sappan L.*) is applied as food and traditional medicine. Chinese teak (Senna alexandrina) is a plant from the tropics that can be developed as an antioxidant. This study was conducted to determine the antioxidant activity of the combination of Sappan and Chinese Teak by the DPPH (1,1 Diphenyl 2 Picrylhydrazyl) Free Radical Scavenging method. The extraction method used was maceration with Ethanol 96% as solvent. Determination of antioxidant levels was done quantitatively using the UV-Vis Spectrophotometer instrument at a wavelength of 514 nm. From the results of the study, the regression value obtained for the comparison of Quercetin is y = 3.1303x + 0.2292 with R^2 = 0.9974 and IC_{50} value of 15.899 µg/mL. For the antioxidant activity of Sappan, the regression value y=0.5769x + 18.543 with R^2=0.9969 and IC_{50} value of 54.53 µg/mL, Chinese Teak obtained regression value y=0.1421x + 17.506 with R^2=0.9989 and IC_{50} value of 228.67 µg/mL, and for the combination obtained regression value y=0.4304x + 36.622 with R^2=0.9954 and IC_{50} value of 40.38 µg/mL. The results of this study indicate that the combination of Sappan and Chinese Teak extracts has a very strong antioxidant effect (<50 µg/mL).*

I. Introduction

Chinese teak plants are often used by the community as herb medicine as anti-cholesterol, treat constipation, and anti-inflammatory. Chinese teak leaves are widely used as tea for easy consumption. And Chinese teak has a group of steroid compounds, alkaloids, tannins, monoterpenes, flavonoids, coumarins, glycosides, saponins, diterpenes, anthraquinones, and phenols. (Ngibad, 2023). Sappan is utilized in various fields, such as food and traditional medicine and many studies have been conducted to determine its chemical content. Ethanol extract of Sappan wood contains caesappin A and caesappin B which are part of protosappanin compounds. The ethanol extract of Sappan wood also contains terpenoids and phenols. The methanol extract of Sappan contains protosappanin A, sappanon B and brazilin (Kurniawan & Tukiran, 2021). Flavonoids are phenolic compounds most commonly found in plants, including roots, outer bark, leaves, and fruit. Phenolic compounds are widely distributed in nature and have many structures, easily found in all plants, leaves, flowers, and fruits. One of them is an antioxidant for the prevention and treatment of degenerative diseases, cancer, premature aging, and immune system disorders (Ahmad *et al*., 2015). Free radicals are relatively unstable molecules with atoms that have one or more unpaired electrons in their outer orbit. Free radicals are highly reactive chemical molecules and are said to be the cause of premature aging, cancer, narrowing of blood vessels (atherosclerosis), lung, liver, kidney, cataracts, rheumatism, and diabetes are often associated with free radicals (Handayani *et al*., 2020). Antioxidants can protect cells from damage caused by unstable molecules known as free radicals. Antioxidants are substances that can delay, slow down and prevent the oxidation process or neutralize free radicals (Herwin *et al*., 2022).

II. Research Method

II.1 Tools

Glassware (Pyrex), aluminum foil (Klin pak), stirring rod (Pyrex), blender (Panasonic), spray bottle, vial bottle, porcelain cup, label paper, filter paper, drying cabinet, micropipette (Dragonlab), drip pipette (Onemed), rotavapor (IKA HB10 basic), spatula, UV-Vis spectrophotometer (Thermo Scientific), analytical balance (KERN ABJ-NM/ABS-N), ultrasonic (Elmasonic), and waterbath (Memmert).

II.2 Materials

In this study the materials used were distilled water (Brataco), DPPH (Sigma), ethanol 96% (Marck), ethanol p.a (Marck), quercetin (Sigma), Sappan (*Caesalpinia sappan* L.), and Chinese Teak (*Senna alexandrina*).

II.3 Tools and Materials Preparation

Tools and materials are prepared according to the needs of the research to be carried out.

II.4 Sampling

The obtained samples were wet-sorted to remove soil and other impurities that were still attached to the obtained samples. Samples that have been sorted are then dried. After drying the sample is weighed and recorded the dry weight and then pollinated after which the weight of the powder sample obtained is reweighed.

II.5 Extract Making

A total of 200 grams of samples were macerated using 96% ethanol solvent for 3x24 hours and then re-macerated twice. The maceration process is assisted by occasional stirring so that the extraction process takes place optimally. The filtrate obtained from the maceration results was combined, then evaporated with a rotary evaporator.

II.4 Antioxidant Activity Test using DPPH

II.4.1 DPPH Solution Making

DPPH solution with 30 ppm concentration was prepared by weighing 3 mg of DPPH dissolved with 100 mL of methanol pro analysis in a measured flask.

II.4.2 Sample Solution Making

1000 ppm stock solution was made by weighing 10 mg of extract and dissolved with methanol p.a while stirring and homogenized and then the volume was adjusted to 10 mL. Furthermore, dilutions were carried out, pipetted 0.1 mL, 0.2 mL, 0.3 mL, 0.4 mL, and 0.5 mL, then the volume was adjusted to 5 mL to obtain concentrations of 20, 40, 60, 80, and 100 ppm.

II.4.3 Standard Solution Making

1000 ppm stock solution was made by weighing as much as 10 mg of Quercetine, then dissolved with methanol p.a while stirring and homogenized and then the volume was sufficient to 10 mL. Furthermore, dilution was carried out, pipetted 0.01 mL then adjusted the volume to 5 mL (2 ppm), pipetted 0.02 mL then adjusted the volume to 5 mL (4 ppm), pipetted 0.03 mL then adjusted the volume to 5 mL (6 ppm) and pipetted 0.04 mL then adjusted the volume to 5 mL (8 ppm) and pipetted 0.05 mL then adjusted the volume to 5 mL (10 ppm).

II.4.4 Blank Antioxidant Activity Test

Tests were carried out by pipetting 4 mL of DPPH. Vortexed and incubated at 37°C in a dark room. The absorbance was measured at a wavelength of 400-800 nm.

II.4.5 Sample Antioxidant Activity Test

The test was performed by pipetting 0.5 mL of sample solution from various concentrations (20, 40, 60, 80 and 100 µg/mL). Then each of them was added with 3.5 mL of DPPH. Then vortexed and incubated in a dark room for 30 minutes. The absorbance was measured at a wavelength of 516 nm.

II.4.6 Standard Antioxidant Activity Test

The test was conducted by pipetting 0.5 mL of quinine solution from various concentrations (2, 4, 6, 8, 10 µg/mL). Then each was added with 3.5 mL of DPPH. Then vortexed and incubated in a dark room for 30 minutes. The absorbance was measured at a wavelength of 516 nm.

III. Results and Discussion

Chinese Teak Leaf (*Senna alexandrina*) comes from the Fabaceae family and is found in subtropical and tropical regions like Pakistan, Mexico, Saudi Arabia, Africa, and India. Some species of this plant are also native to high-temperature areas. Senna grows wild and was first seen in the historic and holy city of Makkah in the center of the ancient Hijaz region (Ikram *et al.*, 2023). *Caesalpinia sappan* L. is another member of the Leguminosae family and is commonly known as Brazilwood or Sappan. Throughout Southeast Asia, sappan has long been used as a traditional food and beverage ingredient. This study intends to assess the antioxidant potential of a combination of Sappan and Chinese Teak ethanol extracts by determining their IC_{50} values through the DPPH free radical scavenging method. This research utilized Sappan and Chinese Teak. They were cleaned and crushed to reduce particle size, increasing the effectiveness of the solvent in extracting chemical compounds. Due to its simplicity, ease, and lack of heat, maceration was chosen as the extraction method. The acquired extract was evaporated using a rotavapor at 60°C and then with a water bath to produce a concentrated extract. DPPH inhibition method was used to determine antioxidant activity and a UV-

Vis spectrophotometer was used to determine amount of DPPH remaining after addition of extract. The DPPH method is preferred because it is simple, user-friendly, rapid, requires a small number of samples, and is appropriate for all samples containing antioxidant compounds (Dahlia *et al*, 2013). Flavonoid compounds will capture DPPH free radicals. Flavonoids react with DPPH free radicals to create more stable radicals with low reactivity. They donate hydrogen radicals (H^+) from their aromatic rings to reduce toxic free radicals. As a result, the flavonoid radicals become resonance-stabilized and non-toxic (Karim *et al*., 2015). A 1000 ppm stock solution was made using ethanol p.a. Several concentration series were created from the stock solution. When testing antioxidant activity using the DPPH method, the IC_{50} value is used to express the results quantitatively. The IC_{50} value indicates the concentration of extracts and standards that provides 50% antiradical activity compared to the control. This is determined through a linear regression line equation based on the levels against the percentage of radical capture (Andriani & Murtisiwi, 2020).

From the results obtained in Table 1,2,3,4 respectively are the IC_{50} value of Quercetin is 15.899 µg/mL which includes a very strong antioxidant because it is in the range of IC value 50 <50 µg/mL, IC_{50} value for Sappan ethanol extract is 54,83 µg/mL which is included in Strong antioxidant activity (50-100 ppm), Chinese Teak ethanol extract is 227,46 µg/mL that included in Very Weak antioxidant activity (>200 ppm), and the extract combination is 40,38 µg/mL which included in Very Strong antioxidant activity (<50 ppm) (Pratiwi & Wardaniati, 2019). Figure 1 shows the graph that connects the percent inhibition with the concentration of Quercetin with the linear regression equation of Quercetin is y = 3.1303x + 0.2292 with R value2 = 0.9974, Figure 2 showed the relationship between percent inhibition with Sappan extract concentration, the linear regression results of the sample is y = 0,5653x+19,006 with R2 value = 0.9953, Figure 3 showed the relationship between percent inhibition with Chinese Teak extract concentration, the linear regression results of the sample is y=0,1416x+17,792 with R2 value = 0,9989, and the correlation graph between extract combination is y=0,4304x+32,622 with R2 value = 0,9954. From the linear regression, it is then entered into the equation y = bx + a, where y is the % inhibition of 50 and x is the IC_{50} value. This shows that the extract combination of Sappan and Chinese Teak is proven to have stronger antioxidant activity than individual sample.

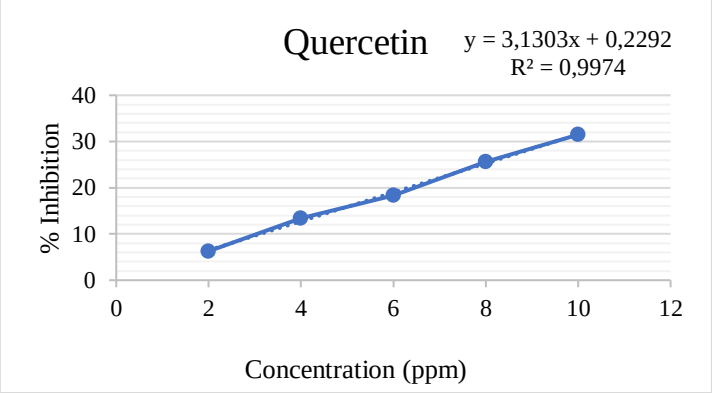

Figure 1. Correlation graph between concentration of Quercetin and % Inhibition

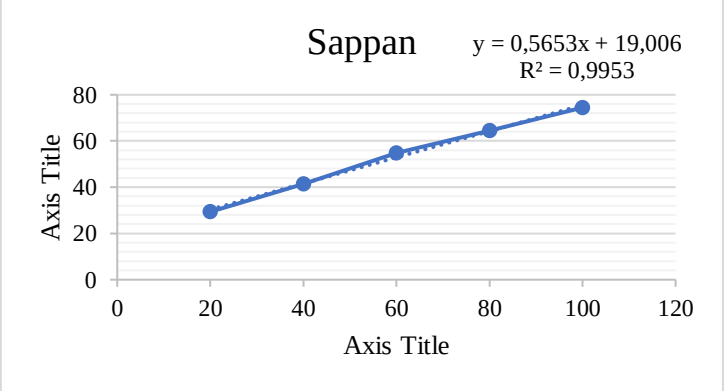

Figure 2. Correlation graph between concentration of Sappan Ethanol Extract and % Inhibition

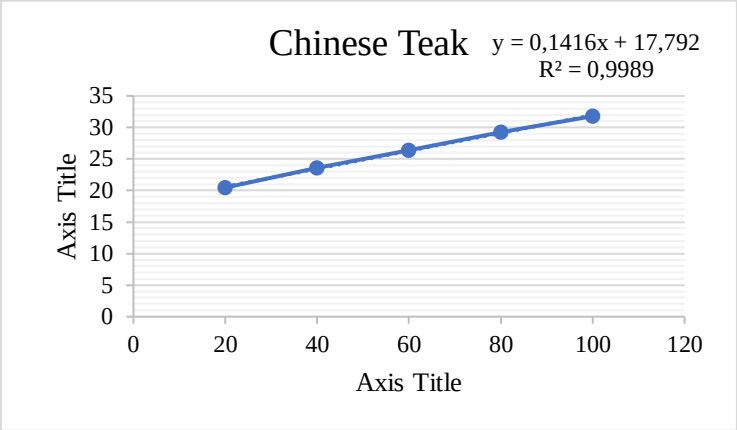

Figure 3. Correlation graph between concentration of Chinese Teak Ethanol Extract and % Inhibition

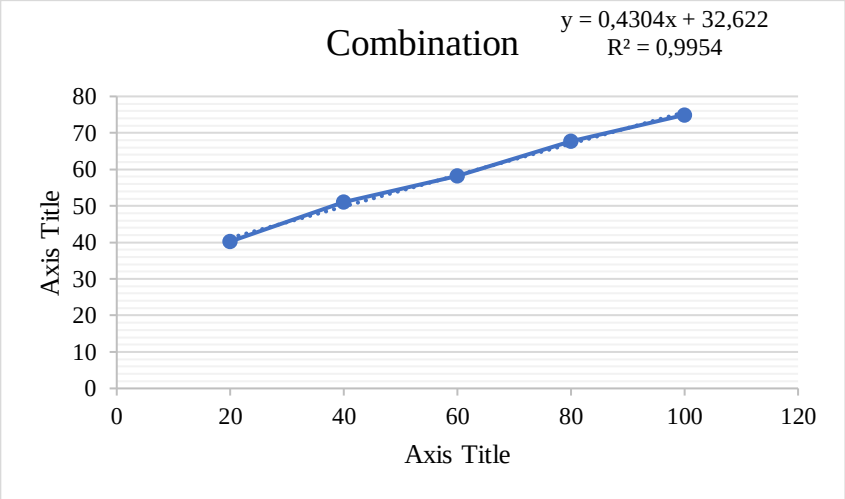

Figure 4. Correlation graph between extract combination of Sappan and Chinese Teak with % Inhibition

Table 1. IC_{50} value of quercetin as standard

Sample	Concentration (ppm)	Blank Absorbance	Sample Absorbance	% Inhibition	IC_{50} (µg/mL)
Quercetin	2	0.829	0.777	6.272	**15.899**
	4	0.829	0.718	13.389	
	6	0.829	0.677	18.335	
	8	0.829	0.617	25.572	
	10	0.829	0.568	31.487	

Table 2. IC_{50} value of sappan ethanol extract

Sample	Concentration (ppm)	Blank Absorbance	Sample Absorbance	% Inhibition	IC_{50} (µg/mL)
Sappan	20	0.865	0.611	29.36	**54.83**
	40	0.865	0.506	41.50	
	60	0.865	0.390	54.91	
	80	0.865	0.308	64.39	
	100	0.865	0.221	74.45	

Table 3. IC_{50} value of chinese teak ethanol extract

Sample	Concentration (ppm)	Blank Absorbance	Sample Absorbance	% Inhibition	IC_{50} (µg/mL)
Chinese Teak	20	0.865	0.688	20.46	**227.46**
	40	0.865	0.661	23.58	
	60	0.865	0.637	26.36	
	80	0.865	0.612	29.25	
	100	0.865	0.590	31.79	

Table 4. IC_{50} value of sappan and chinese teak extract combination

Sample	Concentration (ppm)	Blank Absorbance	Sample Absorbance	% Inhibition	IC_{50} (µg/mL)
Combine	20	0.862	0.515	40.26	**40.38**
	40	0.862	0.422	51.04	
	60	0.862	0.360	58.26	
	80	0.862	0.278	67.75	
	100	0.862	0.216	74.94	

IV. Conclusions

Based on the research that has been conducted, it can be concluded that the combination of Sappan and Chinese Teak ethanol extract has antioxidant activity with IC_{50} 40.38 µg/mL which is included in Very Strong antioxidant activity (IC_{50} <50 ppm).

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