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Phytochemical and Antioxidant Activity of Extracts Red *Cambodia* Sap (*Plumeria Rubra* L.)

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ABSTRACT: Red Cambodian (*Plumeria rubra* L.) is a medicinal plant cultivated by the community with antimicrobial, anti-cancer, and antioxidant bioactivity. This research aims to obtain information regarding secondary metabolite content by phytochemical testing on red Cambodian sap (*Plumeria rubra* L). Phytochemical screening is carried out by looking at the colour-testing reaction using a reagent of methanol and n-hexane. The antioxidant test was carried out using DPPH (1,1-diphenyl-2-picrylhydrazyl) at a wavelength of 517 nm. The results showed that the methanol faction contained alkaloids and saponins. Meanwhile, the n-hexane fraction contained flavonoid compounds, tannins, terpenoids and saponins. The antioxidant test was based on the IC_{50} calculation; the n-hexane fraction had an IC_{50} value of 19.8282 mg/L.

KEYWORDS: Phytochemical, antioxidant, secondary metabolite, *Plumeria rubra* L.

I. INTRODUCTION

Free radicals are chemical compounds with unpaired outer electrons [1], reactive and unstable [2]. The source of free radicals can come from the rest of the body's metabolic products and from outside the body, such as food, UV rays, pollutants, and cigarette smoke. The number of free radicals continues to increase in the body can lead to cell oxidative stress because there is an imbalance between the number of free radicals and antioxidants produced by the body, which can trigger the emergence of degenerative diseases, such as diabetes mellitus, parkinsonism, cardiovascular, respiratory, cancer, cataracts, and rheumatoid arthritis [3]. Therefore, antioxidants are needed from outside the body to slow down the oxidation process of free radicals by donating hydrogen atoms or protons so that they become more stable radical compounds [4,5].

Antioxidant needs can be obtained from synthetic materials, such as Butyl Hydroxy Anisol (BHA) and Butyl Hydroxy Toluene (BHT) [6]. However, synthetic antioxidants can be a promoter compound of carcinogenesis [7]. This may occur if the dosage of synthetic antioxidants exceeds the prescribed limit of 0.01% - 0.1%. Natural antioxidant sources in many plants contain secondary metabolite compounds, such as flavonoids, phenolics, tannins, and anthocyanins [8]. Cambodia is one of the medicinal plants that can be used as an antioxidant.

Red Cambodia (*Plumeria rubra* L.) is an Apocynaceae plant. It contains flavonoid and phenolic compounds distributed in plant parts, such as bark, stems, leaves, roots, flowers, and sap [9]. Red Cambodia sap contains alkaloids, tannins, flavonoids, and triterpenoids that have not been utilized as antioxidants. In connection with this, it is necessary to know the content of secondary metabolites in red Cambodia sap extract using methanol fraction and n-hexane fraction through phytochemical tests. Antioxidant activity can be measured by measuring the absorbance value of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical in red Cambodia sap using a UV-VIS spectrophotometer.

II. METHODS

A. Materials and Equipment

The materials used were *Plumeria rubra* L. plants, Whatman filter paper, technical methanol, methanol p.a, technical n-hexane, ethyl acetate (Sigma), 5% hydrochloric acid (Merck), ascorbic acid (Merck), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Merck) 0.4%, concentrated sulfuric acid (Merck), $FeCl_3$ 1% and $CHCl_3$. The tools used were a condenser, Erlenmeyer (pyrex), measuring cup (Pyrex), beaker (Pyrex), and analytical balance (Explorer Ohaus),

Methanol Extraction of Red Cambodian Leaves

50 ml of P. rubra L plant methanol liquid-liquid extraction macerate and 50 ml filter solution were added until two layers formed. The remaining phase of *Plumeria rubra* L. plant methanol macerate plus 50 ml of filter solution. Moreover, allowed to stand until separation occurs again. Repeat this until the filter solution is clear.

Phytochemical Screening

Phytochemical tests were carried out to determine the content of secondary metabolite compounds qualitatively in methanol extracts of red Cambodia plants (*Plumeria rubra* L.) consisting of alkaloids, flavonoids, saponins, tannins, steroids, and terpenoids.

Alkaloid Test

5.0 ml of methanol extract of red Cambodia (*Plumeria rubra* L.) was put into a test tube. Add five drops of Dragendroff. If formed, brick red on dragendroff reagent indicates alkaloids [10].

Flavonoid Test

Put 5.0 ml of methanol extract of the red Cambodia plant (*Plumeria rubra* L.) into a test tube. Add 5.0 drops of 10% NaOH and shake until homogeneous and filtered. Yellow colour is formed, a positive sign containing flavonoid compounds.

Saponin Test

Put 5 mL of filtrate from red Cambodia plant extract (*Plumeria rubra* L.) dissolved in methanol into a test tube, then add 10 mL of hot water. The solution is cooled and shaken for 10 seconds; if foam is formed that stays for ± 10 seconds with a height of 1-10 cm and does not disappear if one drop of HCl 2N is added, it means there is Saponin. [10].

Tannin Test

The test was conducted by adding 2 mL of methanol extract from a red Cambodia plant (*Plumeria rubra* L.) into a test tube and adding 2-3 drops of $FeCl_3$ 1% solution. Tannins appear when a bluish-black or blackish-green colour is formed during the reaction [11].

Steroid Test

To 5 mL of methanol extract from the red Cambodia plant (*Plumeria rubra* L.), add three drops of concentrated sulfuric acid (H_2 SO_4). The orange colour in the extract solution indicates the presence of steroid compounds.

Terpenoid Test.

Enter 5 mL of red Cambodia plant extract filtrate (*Plumeria rubra* L.), which has been dissolved in m to ethanol in a test tube; add 2 mL each of $CHCl_3$ and 2- 3 drops of H_2 SO_4, then the test tube is shaken and allowed to stand for a few minutes, a change follows the reaction that occurs in colour if reddish brown or greenish brown means positive terpenoids.

Antioxidant Activity Test

Preparation of 0.5 mM DPPH Solution

DPPH 0.5 mM solution was prepared by weighing 9.8 mg of DPPH and dissolved in 10 mL of methanol. The blank solution was made from 1 mL of 0.5 mM DPPH solution dissolved in 5.0 mL of methanol.

Preparation of Blank Solution

Pipette 1 ml of 0.5 mM DPPH solution and put it into a 5 ml volumetric flask. Add methanol p.a in the volumetric flask to a volume of 5 ml. thus obtaining a DPPH solution (blank).

Preparation of Ascorbic Acid Standard Solution

4 mg ascorbic acid powder was dissolved with 5 mL methanol p.a. Stock solution was taken as much as 200, 300, 400, 500 and 600 µL. Add 1 mL of DPPH solution and methanol p.a until the concentration of ascorbic acid standard solution of 10, 20, 30, 40 and 50 µg/mL is obtained.

Plant Extract Test of *Plumeria rubra* L.

The test solution was pipetted as much as 200, 300, 400, 500 and 600 µL, then put into a 5 mL flask to obtain solutions with various concentrations of 10 µg/mL, 20 µg/mL, 30 µg/mL, 40 µg/mL, and 50 µg/mL respectively. Then, 1 mL of 0.4 mm DPPH solution was added to each flask, and the volume was adjusted with methanol until the marked line.

Antioxidant test

Antioxidant tests were carried out by first incubating DPPH solution (blank), test solution + DPPH and ascorbic acid + DPPH solution at 37° C for 30 minutes until the respective absorbance of each solution was obtained. The percentage of inhibition is calculated using the blank absorption minus the sample absorption divided by the blank absorption and multiplied by 100%. The percentage inhibition equation can be seen below:

$$\%\ \text{Inhibition} = \frac{\text{blank absorbance} - \text{sample absorbance}}{\text{blank absorbance}}\ 100\%$$

III. RESULTS AND DISCUSSION

A. Methanol Extraction of Red Cambodia Sap (*Plumeria rubra* L.)

Extraction of *Plumeria rubra* L. sap by liquid-liquid extraction method using methanol. Methanol solvent has polar hydroxyl (OH) and nonpolar alkyl (-CH_3) groups. The first extraction separates polar, semi-polar, and nonpolar compounds. In extraction, the solvent liquid penetrates the cell wall and enters the cell cavity containing the active substance. The concentrated solution is pushed out due to the difference in concentration between the active substance inside and outside the cell. Secondary metabolite compounds in the cytoplasm will dissolve in methanol solvent so that the *Plumeria rubra* L. sample is maximally extracted.

The methanol extraction obtained was partitioned using an n-hexane solvent in a separatory funnel to attract nonpolar compounds during the extraction. The fractions were concentrated using a rotary evaporator and evaporated with a hot plate at 40°C. The partition resulted in an n-hexane fraction, and the remaining methanol extract was cooked into beakers for phytochemical and antioxidant activity testing.

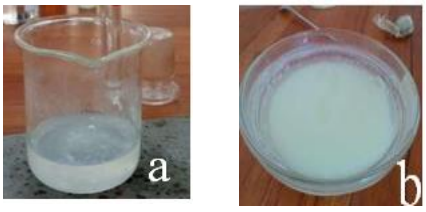

Figure 1. methanol fraction(a) and methanol fraction(b)

B. Phytochemical Screening

Phytochemical screening was conducted to determine secondary metabolite compounds in red Cambodia sap (*Plumeria rubra* L.). The results of phytochemical screening can be seen in Table 1.

Table 1. Phytochemical Content of Red Cambodia Sap Extract (*Plumeria rubra* L.)

Name Sample	SECONDARY METABOLITES					
	Alkaloids	**Flavonoids**	**Tannins**	**Steroids**	**Terpenoids**	**Saponins**
Methanol fraction	+ (Orange Yellow)	- (white cloudy	+ (Blackish Green	- (Yellowish White)	- (White)	+ Slight Foam
Faction n-Hexan	- (Yellow)	+ (Yellow)	- (Yellow)	– (Green)	+ (Greenish)	+ Foaming

Based on the results of the phytochemical screening test of methanol extract of red Cambodia (*Plumeria rubra* L.) alkaloids, tannins, saponins, flavonoids, and terpenoids. Testing alkaloids, tannins, and saponins with methanol fraction obtained positive results for brick red, bluish-black, and foam formation. Meanwhile, the n-hexan fraction positively contains flavonoids, terpenoids and saponins [12].

C. Antioxidant Activity Test

Antioxidant activity analysis of the methanol extract of Red Cambodian Flower was carried out using by DPPH method. This method uses UV-Vis spectrophotometry with the principle of light absorption at specific wavelengths. In this study, the optimum wavelength is 517 nm. The results of the antioxidant activity test of Red Cambodian Flower extract are shown in Table 2.

Table 2. Antioxidant Activity Test Results of Cambodian Red Flower Extract (*Plumeria rubra* L.)

Sample concentration of DPPH (ppm)	Absorbance measurement to			% Inhibition		
	I	II	III	I	II	III
10	0.378	0.425	0.348	47.552	40.979	51.613
20	0.356	0.408	0.326	50.489	43.338	54.558
30	0.336	0.384	0.304	53.427	46.499	57.504
40	0.313	0.366	0.283	56.364	49.229	60.504
50	0.296	0.346	0.267	59.104	52.240	63.114

From Table 2, the concentration of DPPH absorbance value decreases with increasing sample concentration, and the percentage of antioxidant inhibition increases. This can occur due to the reduction of DPPH radicals by the percentage of antioxidant inhibition [13] so that the absorbance decreases.

IC_{50} values were determined using a linear regression equation, as shown in Table 3.

Table 3. IC_{50} values of fractions, n-hexane, and vitamin C.

Sample	IC_{50}Value (mg/L)
N-Hexan fraction	19.82818
Methanol fraction	43.85714
Vitamin C	4.965278

Table 3 shows that the n-hexane and methanol fractions are categorized as vigorous antioxidant activity with IC_{50} values between 50-100 µg/L. while vitamin C has an IC_{50} value ≤ 50 ppm and is a powerful antioxidant.

The presence of antioxidants in the fraction is thought to be due to the presence of phenolic compounds such as polyphenols, which are polar bioactive components and have groups that donate electrons to free radicals so that they are stable.

The antioxidant activity of DPPH samples was measured at a wavelength of 517 nm, which is the maximum wavelength. Table 4 shows the results of antioxidant activity measurement of n-hexane fraction and methanol fraction of red Cambodia flower (*Plumeria rubra* L.) and Vitamin C.

Table 4. Antioxidant Activity Test Results of n-hexane Fraction and Methanol Fraction Red Cambodian Flower and VitaminC

Sample	Concentration (mg/L)	%inhibition (mg/L)	IC_{50}	Description
N-Hexan	10	47.5524	19.8282	Very strong
	20	50.4895		
	30	53.4266		
	40	56.3636		
	50	59.1036		
Methanol	10	51.6129	43.8571	strong
	20	54.5582		
	30	57.5035		
	40	60.5042		
	50	52.2409		
Vitamin C	10	51.6129	4.96528	Very strong
	20	54.5582		
	30	57.5035		
	40	60.5042		
	50	63.1136		

Antioxidant activity was measured at a wavelength of 517 nm, the maximum wavelength of DPPH, with a DPPH concentration of 50 mM. The n-hexan fraction is active and strongly inhibits radicals where the value of n-hexan is greater than the IC value$_{50}$ = 19.8282 mg/L. Similarly, the methanol fraction actively inhibits free radicals with a value greater than the IC_{50} value = 43.8571.

CONCLUSIONS

Secondary metabolite compounds contained in the plant sap of *Plumeria rubra* L. are flavonoids, tannins, terpenoids and saponins that have the potential to inhibit antioxidant activity. From the results of the antioxidant activity test using the (DPPH) method from *Plumeria rubra* L. extract, the n-hexane fraction has a powerful ability to inhibit antioxidant activity with an IC_{50} value of 19.8282 mg/L, compared to the methanol fraction, which has antioxidant activity with an IC_{50} value of 43.8571 mg/L and vitamin C has an IC_{50} value = 4.965278.

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