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Activity Test of Methanol Extract of Young Stem Bark of *Semambu rattan* Plant (*Calamus Scipionum* Lour) with DPPH Method

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Abstract

The Semambu rattan (Calamus scipionum Lour) plant contains phenolic compounds, such as flavonoids and polyphenols that have the ability as antioxidants. Therefore, the community often consumes C scipionum Lour, and the stiff stems are made of crafts. However, the skin, the content of which is not scientifically known, is currently considered a waste in the community. This study was conducted to determine the results of phytochemical tests and the activity of methanol extract of young bark of the Semambu rattan plant (C. scipionum Lour). The bark of young Semambu rattan stems is extracted by maceration, and then the extract obtained is carried out by phytochemical screening. Antioxidant acrypability was measured using the DPPH method at a wavelength of 517nm. The results of phytochemical testing of young bark extract of C. scipionum Lour contain flavonoids, saponins, tannins, alkaloids and terpenoids. The research results on antioxidant young stem bark C. scipionum Lour with positive control in the form of ascorbic acid obtained results IC_{50} = 65.10 ppm, which is classified as a potent antioxidant. The conclusion of this study is that the extract of the young bark of Semambu rattan (C. scipionum Lour) can be used as an alternative antioxidant.

Keywords: *Semambu rattan, Antioxidant, IC_{50}*

Introduction

Rattan is one of the Non-Timber Forest Products (HHBK) crops. Rattan has an essential role in Indonesia's economic growth. This is because rattan is known as a multifunctional plant. After all, it has many benefits. The young stems can be consumed as vegetables by the community, while the old stems are widely used in making handicrafts such as household furniture (Putra, 2021).

Besides being consumable parts of rattan plants (Calamus Sp), such as fruits and stems, people also believe that the plant can be used as traditional medicine. One of them is as a remedy for asthma, canker sores, and febrifuge (Ketapang et al., 2021). Therefore, several researchers conducted tests on several types of rattan plants, including those conducted by Ulfayani, who showed that young stems of *manan rattan* (*C.* manan) contain secondary metabolites that can ward off free radicals such as phenolic derivatives, namely flavonoids and tannins (Mayasari, 2022a). As for the fruit part, based on research conducted by Sandra, the fruit of the rattan plant (*Calmaus* sp) contains phenolic compounds that have activity as antioxidants with a value of IC_{50} = 6.09ug / ml with a firm category (S. T. Fendri, 2021).

Various scientific evidence shows that rattan plants are active as antioxidants. However, of the many types of rattan plants, there are still rattan that have not been studied by researchers, one of which is the *Semambu rattan* (*Calamus scipionum* Lour), which is widely found in the South Sumatra area, Indonesia (Jasni, 2007). *Semambu rattan* plants (*C.scipionum Lour*) amid South

Sumatran society are usually part of the young stems traded to be processed into side dishes or regional specialities. Community consumptive activities on young stems of *Semambu rattan* will undoubtedly leave skin waste from the young stems. Therefore, in this study, the content of secondary metabolite compounds will be tested in the bark of young stems of *Semambu rattan* (C scipionum Lour). The test is believed that the *Semambu rattan* plant (C scipionum Lour), which comes from the same family as *Manan rattan* (C manan), will have the same secondary metabolic content (Salusu et al., 2018). In addition, based on morphology, the skin of plants usually contains phenolics, which are thought to also act as antioxidants (Abror Rahman, 2020).

Based on the description above, antioxidant testing will be carried out on the bark of young stems of *Semambu rattan* plants (*C scipionum* Lour). The test aims to determine the content of secondary metabolite compounds and the ability to ward off free radicals in the bark of young *Semambu rattan*. Given that in this day and age, there has been pollution in the environment causes exposure to free radicals to increase (Cahyaningsih et al., 2019). Therefore, alternative free radical antidotes from natural materials are needed that are more natural and can reduce waste in the environment (Simanjuntak & Zulham, 2020).

Therefore, antioxidant testing will be carried out from the bark of young stems of *Semambu rattan* plants (*C scipionum* Lour). The method chosen is the DPPH method because this method is sensitive even with a small amount and is also sensitive to the antioxidant activity of natural compounds (Putu, 2021).

Method

Tools and Materials

The tools and materials used in this study are maceration bottles, UV-Vis spectrophotometers (*Shimadzu*), rotary evaporators (*Buchi*), beaker glass (*Pyrex*), petri dishes, measuring pipettes, test tubes (*pyrex*), measuring flasks, drip pipettes, measuring pipettes, ABJ 320-4NM type scales (Cranes), filter paper, spatels, glass funnels, aluminum foil, plastic wrap. The materials used in this study were young bark of *Semambu rattan* (*Calamus scipionum* Lour), methanol, aquades, DPPH, ascorbic acid, Mg and HCl (p.a) powders, $FeCl_3$, acetic acid (p.a), H_2SO_4 (p.a) and Dragendroff reagent.

Procedure

Materials Preparation

Semambu rattan plants (*Calamus scpionum* Lour) were taken from Muara Enim Regency, then Identification plants at the FMIPA Biology Laboratory of Sriwijaya University Palembang. *Semambu rattan* plants are sorted by peeling young stems of *Semambu rattan* (*C. scipionum* Lour) and then taking the bark. The bark of young stems of *Semambu rattan* is washed with water and then dried in the sun. The dried bark of the young stems of *Semambu rattan* is then chopped and mashed using a blender until fine powder is obtained from the young stems of *Semambu rattan*.

Sample Extraction

A total of 50 g of young bark powder of *Semambu rattan* (*C. scipionum* Lour) was extracted by putting it in a large glass jar with the addition of 100 ml of methanol, then allowed to stand for 24 hours. The extract obtained was evaporated using a rotary evaporator at 40°C until the solvent evaporated and obtained thick methanol extract from the bark of young *Semambu rattan* (Rantina et al., 2022).

Phytochemical Screening

Photochemical screening of the bark extract of young *Semambu rattan* (Calamus scipionum Lour) includes the following tests:

A. Alkaloids

Take 1 ml of methanol extract from the young bark of *Semambu rattan*, then add 1-2 drops of Dragendroff reagent. The positive reaction of alkaloids is characterised

by the formation of yellow or orange colour (Putri & Hidajati, 2015).

B. Flavonoids

Methanol extract from the young bark of *Semambu rattan* is taken 1 ml, followed by a small quantity of Mg of metal and a few drops of HCl. The appearance of yellow-orange to red colour indicates the presence of flavonoid compounds (Putri & Hidajati, 2015).

C. Terpenoids and Steroids

1 ml of methanol extract of young *Semambu rattan* bark is taken, then put into a test tube, and 1 ml of CH_3COOH and 1 ml of concentrated H_2SO_4. The formation of blue or purple indicates the presence of steroids and terpenoids (S. T. J. Fendri et al., 2022)

D. Tannins

Methanol extract from the young bark of *Semambu rattan* is taken 1 ml, then 1-2 drops of FeCl3 are added. Positive tannins if the colour changes to blackish-green (Mayasari, 2022a).

E. Saponins

Take 1 ml of methanol extract from young rattan bark, put it in a test tube, and then shake. If a permanent foam is formed (± 10 minutes), it shows the presence of saponins (Fitri Yani & Dirmansyah, 2021).

Solution Manufacturing

DPPH Solution Preparation

A total of 1.5 mg of DPPH is put in a 25 ml measuring flask, then add methanol to the limit mark (Damanis et al., 2020).

Manufacture of Vitamin C Solution

A total of 12.5 mg of vitamin C was put into a 50 ml measuring flask, then added methanol to the limit mark so that a concentration of 250 ppm was obtained (Wahdaningsih, 2022).

Manufacture of Extract Solution

A total of 12.5 mg of young bark extract of *Semambu rattan* (*C scipionum* Lour) was put into a 50 ml measuring flask, then added methanol to the limit mark so that a concentration of 250 ppm was obtained (S. T. Fendri, 2021).

Determination of Antioxidant Activity with DPPH

Determination of DPPH Maximum Wavelength

Pipette 2 ml DPPH 50 ppm, add 2 ml of methanol to the bottle and cover with aluminium foil. Then, leave for 30 minutes in a dark place. Measure the absorbance of a solution using a UV-Vis spectrophotometer with a maximum absorption wavelength of 517 nm (Salusu et al., 2018).

Determination of antioxidant activity of vitamin C

Pipettes of Vitamin C solution 250 ppm each pipette (2, 4, 6, 8, 10) mL Put in a 10 mL measuring flask, add methanol to the limit mark to the concentration (50; 100; 150; 200; 250) ppm.

Pipetted each solution as much as 2 ml, then added 2 ml DPPH 50 ppm into the vial. Let stand for 30 minutes in a dark place. Determine the absorption of the solution using a UV-Vis spectrophotometer at a maximum wavelength of 400-800 nm (Nur'amala, 2019).

Determine antioxidant activity by determining % inhibition and IC_{50}.

Determination of Bark Extract Activity of Young *Semambu rattan* (*C scipionum* Lour)

Pipette a solution of young bark extract of *Semambu rattan* 250 ppm each pipette (2, 4, 6, 8, 10) ml into a measuring flask of 10 mL. Add methanol to the limit mark to the concentration (50; 100; 150; 200; 250) ppm.

Pipetted each solution as much as 2 ml, then put into the vial 2 ml DPPH 50 ppm. Let stand for 30 minutes in a dark place. Determine the absorption of the solution using a UV-Vis spectrophotometer at a maximum wavelength of 400-800 nm. Determine antioxidant activity by

determining % inhibition and IC_{50} (Damanis et al., 2020).

Determination of antioxidant activity

The magnitude of DPPH radical resistance determines the antioxidant of the sample and is then calculated through the calculation of % DPPH uptake inhibition, using the following formula (Abror Rahman, 2020).

$$\%\text{Inhibisi} = \frac{abs\ kontrol - abs\ sampel}{Absorban\ kontrol} x\ 100\%$$

Ket :
Abs control : Absorption of DPPH radical solution
Abs sample: Absorption of sample solution plus DPPH solution, reduced absorption until without DPPH

IC_{50} Value Determination

The IC50 value is calculated using the linear regression equation of the solution concentration relationship with the % inhibition value. The following formula can determine the determination of the IC50 value (Maesaroh et al., 2018):

Y = a + bx

Research Results and Discussion

Phytochemical Extraction and Test

The extract was obtained from the bark of young stems of *Semambu rattan* (C scipionum Lour) macerated with methanol. Maceration is carried out three times, and then the solvent is evaporated with an evaporator to obtain a thick extract of Semambu rattan bark. The maceration method in this study was chosen because the implementation is simple and reduces the possibility of reducing the active substance in the sample due to the sample's resistance to heat (termolabi) (Cahyaningsih et al., 2019). The concentrated extract of the bark of young rattan stems can be seen in **Figure 1.**

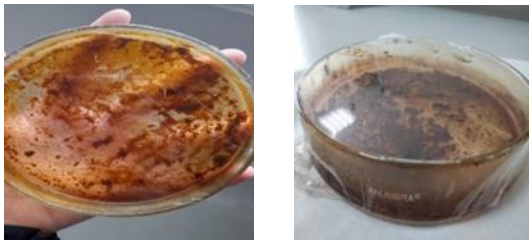

Figure 1. Semambu rattan Bark Ekstract

The thick extract of young rattan bark from methanol solvent has a thick texture like jelly with a brownish-green colour, yielding 4,080g (12.25%). The purpose of the drying shrinkage examination is to show volatile parts and water lost during heating and to provide a maximum limit on the amount of compounds lost in the drying process in the extract (Handoyo, 2020).

Phytochemical Test

Phytochemical testing aims to identify what compounds are found in plants (Solichah et al., 2021)Phytochemical tests were qualitatively carried out on samples of methanol extract from the young bark of Semambu rattan. The test results are in **Table 1.**

Tablel 1. Phytochemical Test Results

Secondary Metabolic Compound	Positive Color	Test Results
Flavonoids	Bright yellow	(+)
Tannins	Greenish black	(+)
Saponins	There is foam	(+)

Terpenoids	Reddish brown	(+)
Alkaloids(dragendroff)	Reddish brown precipitate	(+)

Based on phytochemical tests, the methanol extract of young rattan bark contains flavonoids, tannins, saponins, terpenoids and alkaloids, where the results show the same compounds as *rattan manau* plants from tests conducted by Mayasari (Mayasari, 2022b). This is because it is based on the same family (Solichah et al., 2021). In addition, according to Fatimah (2022), secondary metabolites contained in a plant can be influenced by several factors, including temperature, growing location, climate, rainfall, age, nutrition, allelopathy, nutrition and light; therefore, *rattan manau* does not show positive results containing alkaloids (Fatimah, 2022)Positive test results in each phytochemical test show that polar methanol solvents attract secondary metabolites contained in the extract of young rattan bark.

Antioxidant Test

After the phytochemical screening, the methanol extract's antioxidant activity from the rattan plants' young bark was determined using the DPPH method. The DPPH method was chosen because it is simple and sensitive even with a small sample, so that it can be widely used for testing (Maesaroh et al., 2018)The maximum wavelength is determined in the wavelength region of 400-800 nm to determine the maximum absorption area indicated by the absorbance value of the DPPH solution measured using UV-Vis Spectrophotometry. The wavelength reading results obtained a stable absorbance value at a wavelength of 517 nm with an absorbance of 0.999.

After determining the maximum wavelength of DPPH, antioxidant testing is continued. Test antioxidant activity on samples using spectrophotometric principles where compounds with antioxidant activity will absorb free radicals by donating hydrogen atoms. The hydrogen atom of the antioxidant compound will bind to DPPH and form DPPH-H (*1,1-diphenyl-2-pycryhydrazine*) (Cahyaningsih et al., 2019)DPPH-H is a more stable form of reduced DPPH. Its stable formation is characterised by a change in the solution's colour from deep purple to pale yellow or a decrease in the absorbance value. Discoloration occurs during the solution's incubation period with DPPH.

The positive control in this study was ascorbic acid (Vitamin C). Vitamin C is used to compare the strong antioxidant potential in the methanol extract of the young stem bark of *Semambu rattan* plants. The higher the concentration used, the higher the antioxidant activity. Antioxidant activity is expressed by an Inhibitor Concentration of 50% (IC_{50}), namely the ability of sample concentration to reduce as much as 50% of free radicals. The results of the standard antioxidant activity test of vitamin C can be seen in Table 2 and Figure 2.

From the equation, The curve between the concentration of vitamin C and % inhibition obtained IC50 of vitamin C is 12.47ppm, which means that with a concentration of 12.47%, vitamin C can ward off free radicals by 50%. The smaller the IC_{50} value, the higher the antioxidant activity, so the antioxidant activity of vitamin C is high. Where a substance has robust antioxidant activity

if the IC_{50} value is less than 50ppm. If IC_{50} is 50-100 ppm, antioxidant activity is robust to moderate (Wahdaningsih, 2022).

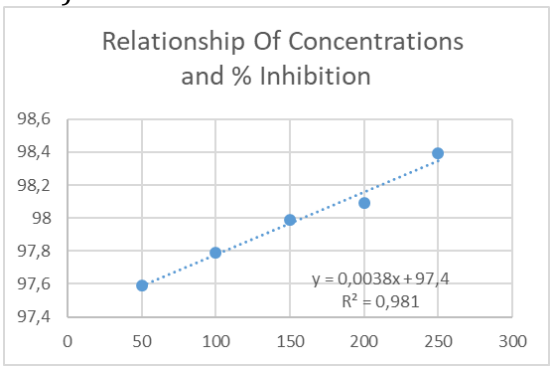

Figure 2. Relationship of Contingration & % Inhibition of Vitamin C

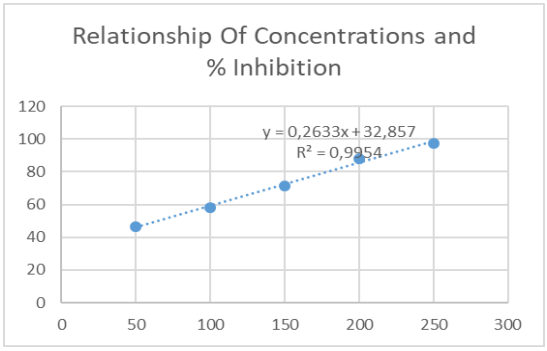

Figure 3. Relationship of Concentration & % Inhibition of Bark Extract

The results of the antioxidant activity test of methanol extract of young rattan bark, when viewed from the curve of extra concentration of young rattan grey bark and the % inhibition value, showed that the higher the concentration, the greater the inhibition of the extract against free radicals. So, from this equation, the IC50 value of methanol extract of young rattan bark of Semambu rattan amounts to IC50 = 65.10ppm, a concentration included in the vital antioxidant group. Antioxidant activity in the methanol extract of young rattan bark can be caused by the content of flavonoids that can provide effectiveness as antioxidants by inhibiting oxidation reactions caused by free radicals. The content of phenolic compounds such as tannins and flavonoids. The test results of the methanol extract of the young bark of *Semambu rattan* can be seen in Figure 3 and Table 2.

Table 2. Test Results of Antioxidant Activity of Skin Extract and Vitamin C

Sample	Concentration (ppm)	Abs Sample	Abs control	% Inhibisi	IC_{50}
	50	0,533		46,54	
	100	0,417		58,25	65,10
Metanol	150	0,284		71,57	ppm
Extract	200	0,122		87,78	(strong)
	250	0,024		97,59	
	50	0,024	0,999	97,59	
	100	0,022		97,77	12,47
Vitamin C	150	0,020		97,99	ppm
	200	0,019		98,09	(Very
	250	0,016		98,39	Strong)

If we look at **Table 2**, the activity of methanol extract of young rattan bark has far different results compared to vitamin C as a control, namely with a value of IC50 = 12.47 ppm, classified as a powerful antioxidant. This is also because vitamin C is a pure antioxidant compound with a solid activity that prevents free radicals. However, the antioxidant activity of the young bark extract of *Semambu rattan* also cannot be said to be weak because it has a strong ability as an antioxidant if we look at the ability of its servants. Therefore, methanol extract of young rattan bark can be used as a natural antioxidant alternative. In addition, it is hoped that further research will be able to isolate and further research on the skin of *Semambu rattan* rods.

Conclusion

The maceration results of methanol extract of young rattan bark were obtained by a thick extract of 4,080g (12.25%). The phytochemical test results of methanol extract were identified as containing flavonoids, tannins, saponins, terpenoids and alkaloids that act as antioxidants, with test results IC_{50}=65.10 ppm. Although the IC_{50} value of viscous extract is not the same as vitamin C. Namely IC50 = 12.47ppm (very strong), methanol extract is still classified as a potent antioxidant, so it can still be used as an alternative antioxidant.

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