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Methanolic Extracts of Rose Flowers (*Rosa chinensis* Jacq.): Phytochemical Evaluation and Total Antioxidant Capacity

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ABSTRACT

Oxidative stress is an imbalance between oxidants and antioxidants that can lead to impaired signaling and redox control. Free radicals in large quantities will stimulate the production of IL-6 and TNF- α , which act as proinflammatory cytokines and trigger the release of polymorphonuclear cells (PMN); this makes antioxidants are needed to inhibit the oxidation process and free radicals prevention. One of the natural antioxidants that is easy to find and become a symbol of beauty and love is the rose (*Rosa chinensis* Jacq.). The purpose of this study was to know secondary metabolic compounds and total antioxidant capacity on methanol extracts of rose flower. This research is an experimental research using methanol solvent with maceration method that includes phytochemical screen³¹ test consisting of 12 secondary metabolic compounds by Harborne and total antioxidant capacity test by DPPH method (1,1-Diphenyl-2-Picrylhydrazyl) by Blois. In the qualitative test, rose flower extracts contain cardioglycosides, alkaloids, flavonoids, anthocyanins and betacyans, coumarins, glycosides, phenolics, quinones, tannins, and terpenoids. The test results of the total antioxidant capacity showed that the IC₅₀ of rose flower was 69,42 μ g/mL. These results indicate that rose flower extract has potential as a strong antioxidant to prevent many diseases such as cancer.

Keywords: Oxidative Stress, Reactive Oxygen Species, *Rosa chinensis* Jacq., Total Antioxidant Capacity.

1. INTRODUCTION

Oxidative stress is a condition of imbalance between oxidant and antioxidant such as antioxidant deficiency, increased reactive oxygen species (ROS), reactive nitrogen species (RN²⁴) and reactive sulfur species (RSS) which contribute to the pathogenesis of several diseases such as cancer, diabetes mellitus, inflammation, and the aging process.¹

Antioxidant can protect cells and organs of the body from the harmful effects of oxidative stress through various body defense mechanisms through enzymatic and non-enzymatic reactions that work synergistically.² One of the ornamental plants which is an exogenous antioxidant is the rose plant. This plant symbolizes beauty, love, joy and glory in order that it is dubbed as "Queen of Flowers".^{2,3} Every part of the rose plant has been utilized as traditional medicine.⁴ Since ancient time, every part of the rose plant has been utilized as traditional medicine and has various benefits such as antioxidant,

antibacterial, antiseptic, antispasmodic.^{2,5} Although the rose plant has been used widely as a medicine and it is also known that its content can act as an antioxidant, but research on the rose plant species *Rosa chinensis* Jacq is still rare.

2. MATERIAL AND METHOD

This research is an experimental research that applies two test techniques, namely in vitro and bioassay. In vitro test that includes the phytochemical screening test by Harborne⁶, consists of 12 secondary metabolite compounds including cardioglycosides, alkaloids, flavonoids, anthocyanins and betacyanins, coumarins, glycosides, phenols, quinones, saponins, steroids, tannins, and terpenoids, and total antioxidant capacity test by DPPH method by Blois⁷.

Samples of roses (*R²⁶chinensis* Jacq.) were taken from Cikole Village, Lembang Sub-district, West Bandung Regency, West Java Province, Indonesia.

Samples were sent in the form of cut roses as many as 120 stalks. In this research, it was used the petals part so as it was obtained rose petals weigh 1,044 grams that was then dried for 5-7 days in a room with room temperature, good air flow, and were not exposed to direct sunlight. A total of 56 grams of dried petals were mashed and then macerated by using a maceration tube covered with aluminum foil and cotton in it. This simplicia-methanol mixture was stirred everyday twice a day both in the morning and evening without touching the cotton. After that, the extract was evaporated by using a rotatory evaporator in order that 17.93 grams of sample methanol extract was obtained in the form of pasta.

Phytochemical test using several methods or reagents. Keller Killiani method for cardioglycosides, mayer and wagner for alkaloids, NaOH for flavonoids, betacyanins, and anthocyanins, NH₃ for coumarins, Bortrager for glycosides, Folin Ciocelteau for phenolics, H₂SO₄ for quinones, Foam for saponins, Liebermann Burchard for steroids, Ferric Chloride for Tannins, and Salkowski for terpenoids.

Rose petals extract (*Rosa chinensis* Jacq.) was pondered 10 mg then dissolved with 10 mL of methanol solvent in 10 mL volumetric flask so that it was obtained a concentration of 1 mg/mL. After that, the extract solution was diluted with methanol solvent so as it was obtained concentrations of 30 µg/mL, 50 µg/mL, 70 µg/mL, 90 µg/mL, and 120 µg/mL and used a comparison standard of Vitamin C with concentrations of 2 µg/mL, 4 µg/mL, 6 µg/mL, 8 µg/mL and 10 µg/mL. Then, 0.5 mL of the extract solution from each flask was mixed with 3.5 mL of DPPH solution. This process was conducted twice and this mixture was allowed to become homogeneous for 30 minutes in a dark place. Absorption was measured by using a Genesys 30 visible spectrophotometer with a maximum wavelength. The absorbance results obtained from both tubes with the same concentration were calculated on average and the inhibition of each concentration. The value of percent inhibition was calculated by using the formula:

$$\%Inhibition = \frac{Control\ Abs - Test\ Abs}{Control\ Abs} \times 100\% \quad [1]$$

The results of calculation of the value of % inhibition were entered into the linear equation curve $y = ax + b$ to determine the antioxidants needed to inhibit DPPH free radicals by 50% (IC₅₀)

Statistical analysis of this research used GraphPad Prism v.9.0.2 La Jolla, California, USA statistical program application. Data was presented in the form of graphs and tables.

3. RESULTS

3.1 Extraction and Phytochemical Test Results

The total weight of the rose petals simplicia was 56 grams. The extraction was conducted in order that the weight of the sample methanol extract was 17.93 grams. Then calculated by using the formula so that the yield of 32.01% was obtained.

$$\begin{aligned} \%yield &= \frac{\text{weight of extract}}{\text{weight of simplicia}} \times 100\% \quad [1] \\ &= \frac{17,93 \text{ gram}}{56 \text{ gram}} \times 100\% = 32,01\% \quad [2] \end{aligned}$$

Rose petals extract contains phytochemical content in the form of cardioglycosides, alkaloids, flavonoids, anthocyanins and betacyanins, coumarins, glycosides, phenolics, quinones, tannins, and terpenoids. While saponins and steroids were not found (Table 1)

Table 1. Phytochemical Content

Phytochemical	Extract	Method
Cardioglycoside	+	Keller Killiani
Alkaloids	+	Mayer/Wagner
Flavonoids	+	NaOH
Anthocyanins	+	NaOH
Betacyanins	+	NaOH
Coumarins	+	NH ₃
Glycosides	+	Bortrager
Phenolics	+	Folin Ciocelteau
Quinones	+	H ₂ SO ₄
Saponins	-	Foam
Steroids	-	Liebermann Burchard
Taninms	+	Ferric Chloride
Terpenoids	+	Keller Killiani

3.2 Total Antioxidant Capacity Test

It was obtained maximum wavelength and control absorbance by using genesys 30 vis spectrophotometer, for the rose petals extract test were 515 nm and 0.662.

For each extract concentration, the absorbance value was searched by using genesys 30 vis spectrophotometer with a wavelength of 515 nm and the percent inhibition was searched (Table 2). The concentration of rose petals extract in the X variable and the percent inhibition in the Y variable then it was made a linear equation curve so that it was obtained IC₅₀ result (Figure 1).

Table 2. Concentration of Rose Petals Extract, % Inhibition, and IC₅₀

Concentration (µg/mL)	Percent Inhibition (%)	IC ₅₀ (µg/mL)
30	18,42	69,42
50	32,17	
90	72,35	
120	86,85	

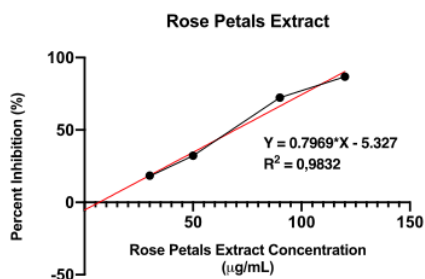


Figure 1 DPPH Test Curve of Rose Petals Extract

On the curve, it was obtained $Y = 0.7969X - 5.327$ and $R^2 = 0.9832$. After calculating, IC_{50} result of rose petals extract was $69.42 \mu\text{g/mL}$.

Vitamin C Comparison Standard Test

Each concentration of vitamin C was measured for absorbance by using genesys 30 vis spectrophotometer and the percent inhibition was searched (Table 3). Then a standard curve of vitamin C was made with the concentration value of rose petals extract being the X axis and the percent inhibition on the Y axis. Then IC_{50} value was searched with linear equation curve. On the curve, it was obtained $Y = 6.934X + 12.52$ and $R^2 = 0.9988$ (Figure 18). After calculating, IC_{50} standard result of vitamin C was $5.4 \mu\text{g/mL}$.

Table 3. Concentration of Rose Petals Extract, % Inhibition, and IC_{50} Vitamin C Standard

Concentration ($\mu\text{g/mL}$)	Percent Inhibition (%)	IC_{50} ($\mu\text{g/mL}$)
2	26,85	5,4
4	39,11	
6	54,97	
8	67,87	
10	81,81	

Comparison Standard of Vitamin C

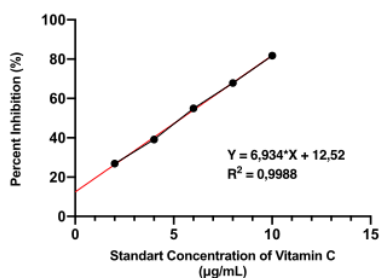


Figure 2 Comparison Standard Curve of Vitamin C

4. DISCUSSION

4.1 Phytochemical Test

In this research, researchers conducted a phytochemical test of the methanol extract of rose petals to see secondary metabolites contained in rose flower. The results of qualitative phytochemical test on rose petals extract showed positive results in several tests, namely, cardioglycosides, alkaloids, flavonoids, anthocyanins and betacyanins, coumarins, glycosides, phenolic, quinones, tannins, and terpenoids. This positive reaction is characterized by a visible color change.

The results of this research are in line with research conducted by Luo Y et al⁸, which has identified 80 compounds including fifty flavonols, eleven phenolic acids, two amino acids, forty tannins, one monosaccharide, and one benzyl alcohol derivative. Research on the analysis of phytochemical activity of rose leaves (*Rosa chinensis* Jacq.) by Afifah DN et al⁹, showed positive phytochemical screening results for the presence of flavonoids, tannins, and saponins. In research conducted by Cai YZ et al², that there is anthocyanin content in rose extract (*Rosa chinensis* Jacq.). The results of research conducted by Qing LS et al¹⁰, found that there are 12 types of flavonoid glycoside compounds from rose flower extract (*Rosa chinensis* Jacq.).

4.2 Total Antioxidant Capacity

This research measures the total antioxidant capacity of rose petals extract and ascorbic acid by using *1,1-Diphenyl-2-Picrylhydrazyl* (DPPH) method. This method is used to measure the overall antioxidant capacity and has been successfully used to research the antioxidant capacity of wheat, vegetable, oil, and flour in various solvents such as ethanol, methanol, benzene, and aqueous acetone. The advantages of DPPH method compared to other methods are it is easy to implement, fast, does not require a lot of cost, and can react with almost all types of antioxidants because of the stability of DPPH even with weak antioxidant.¹¹

The final results of this research showed that the amount of antioxidants needed to reduce the initial DPPH concentration by 50% and the time needed to reach a stable state to reach the IC_{50} concentration. In this research, the R^2 value of 0.9988 was obtained from the standard linear equation curve of ascorbic acid. From the high value of R^2 obtained, then the results of the standard linear equation of ascorbic acid have a high level of confidence. After calculating the linear equation, the IC_{50} value was $5.4 \mu\text{g/mL}$. While, on DPPH test curve of rose petals extract, the R^2 value of 0.9832 was obtained and continued with the calculation of the linear equation so that it was obtained an IC_{50} value of $69.42 \mu\text{g/mL}$.

According to the research of Phongpaichit et al¹², if the IC₅₀ value of a test material is > 250 µg/mL then it is said to be inactive, if it is 100-250 µg/mL then it is weak or less active, if it is 50-100 µg/mL then it has a strong enough ability, if it is 10-50 µg/mL then it is strong, and if <10 µg/mL it has a very strong antioxidant ability. In this research, it was found that the antioxidant capacity of rose petals extract (IC₅₀ of 69.42 µg/mL) was included in the category of having strong antioxidant abilities. The results of the calculation of the antioxidant capacity of ascorbic acid (IC₅₀ of 5.4 µg/mL) are included in the category that has a very strong antioxidant ability. According to the research of Cai YZ et al², that rose petals extract has an IC₅₀ of 21.3 µg/mL which shows that there is a high antioxidant ability in *R. chinensis* rose petals extract so it is often utilized in traditional medicine in China. In the research of Afifah et al⁹, it was found that the methanol extract of rose leaves has a very strong antioxidant ability in warding off free radicals by 50% with an IC₅₀ value of 8.69 µg/mL. In the research of Luo Y et al⁸, it was said that *R. chinensis* roses besides of being useful as antioxidant, namely as anticancer, antiviral, antibacterial, and can boost body's immunity.

5. CONCLUSION

After obtaining the results and discussion of the search that has been conducted with the title Methanolic Extracts of Rose Flowers (*Rosa chinensis* Jacq.): Phytochemical Evaluation and Total Antioxidant Capacity, it can be concluded:

1. In rose petals extract, it was obtained content of secondary metabolites, namely cardioglycosides, alkaloids, flavonoids, anthocyanins, betacyanins, coumarins, glycosides, phenolics, quinones, tannins, and terpenoids.
2. The total antioxidant capacity in rose petals extract with an IC₅₀ value of 69.42 µg/mL so that it can be categorized as a strong antioxidant.

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AUTHORS' CONTRIBUTIONS

17 The authors contributed equally to all aspects of the article.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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