



ANTIOXIDANT ACTIVITY OF RED ROSE LEAVES (*Rosa chinensis* Jacq.) EXTRACT

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ABSTRACT

Background: Background: Antioxidants are compounds that can neutralize free radical reactions. The red rose leaves (*Rosa chinensis* Jacq.), usually disposed of as waste, have been shown to have antioxidant activity.

Objective: This study aims to determine the antioxidant activity of red rose leaves extracts.

Methods: The red rose leaves were extracted by the maceration method in ethanol, methanol, ethyl acetate, and n-hexane. The antioxidant activity was measured by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and was expressed as IC₅₀ and an antioxidant activity index (AAI).

Results: The results showed that the red rose leaves ethanol, methanol, ethyl acetate, and n-hexane extracts had an antioxidant activity with an IC₅₀ value of 1.8, 8.7, 210.3, 48.7 µg/mL, respectively, and an AAI value of 17.3, 3.5, 0.1, 0.6, respectively. The main content of ethanol and methanol extracts are flavonoid, saponin, and tannin.

Conclusion: The ethanol and methanol extract showed the most potent antioxidant activity.

Keywords : Antioxidant, DPPH, *Rosa chinensis* Jacq.

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INTRODUCTION

The malfunctioning of body cells causes degenerative diseases. In addition, lifestyle and environmental conditions can worsen the quality of life. The World Health Organization (WHO) reported that degenerative diseases are among the top 10 causes of death globally[1]. The prevalence of degenerative diseases worldwide shows 70-80% in Canada, China, India, Nigeria, and the United Kingdom. The Ministry of Health (2018) reported an increase in degenerative diseases in Indonesia by 13.3% [2].

Free radicals may cause malfunction of the cells, which can disrupt cell integrity. Free radicals can react with cell components such as cell membranes, enzymes, and DNA [3]. Damage to these components can lead to degenerative diseases, such as cancer, atherosclerosis, diabetes mellitus, and hypertension. Therefore, the body needs substances that can counteract free radicals, called antioxidants. Antioxidants donate their electrons to unpaired compounds; thus, these radical compounds' activity can be inhibited [4].

In Indonesia, the demand for natural ingredients as antioxidants to reduce free radicals increases from year to year. The search for natural antioxidants derived from plants continues to be developed[4]. Indonesia has abundant biodiversity and has the potential as a source of natural antioxidants. Natural antioxidants from natural ingredients can be an option because they are safer, cheaper, and easy to obtain[5].

One plant that can be used as natural antioxidants is the rose plant (*Rosa chinensis* Jacq.). Various studies on roses' activity as an antioxidant have been investigated. Mawarni et al. showed that

the ethanol extract of rose flower (*Rosa damascena* Mill.) has antioxidant activity with an IC₅₀ value of 15.49 µg/mL[6]. Pasril et al. showed that the ethanol extract of rose flower (*Rosa damascena* Mill.) has potent antibacterial activity[7]. Francesca et al. reported that the methanol extract of the variety *Rosa canina* L, *Rosa corymbifera* Borkh, *Rosa micrantha*, and *Rosa sempervirens* L. had an antioxidant activity with IC₅₀ values of 19.6 µg/mL, 2.4 µg/mL, 7.7 µg/mL, 12.7 µg/mL, respectively[8]. In Indonesia, rose leaves are disposed of as waste. Rose leaf waste has the potential as an excellent source of antioxidants. Therefore, this study aims to determine the potential antioxidant activity of the red rose-leaf extract in several extracts, namely ethanol, methanol, ethyl acetate, and n-hexane extracts.

MATERIAL AND METHODS

Plant authentication

Plant authentication was carried out at the Herbarium Bogoriense, Research Center for Biology, the Indonesian Institute of Sciences (LIPI) Cibinong. The results showed that the plant was red roses (*Rosa chinensis* Jacq.)

Extraction

Fresh red rose leaves were weighed as much as 400 grams, then dried for five days to remove air content. After drying, the leaves are then crushed into a powder. The dry powder was weighed as much as 25 grams, then macerated with 250 mL of solvents (each in ethanol, methanol, ethyl acetate, n-hexane) for 3 × 24 hours. The macerate was filtered with Whatman No.1 filter paper and placed in a dark bottle, then continued for second round extraction using solvent as much as 250 mL. The

filtrate was collected and concentrated using a vacuum rotary evaporator at a temperature of 35-40°C to obtain a thick extract. The remaining solvent in the viscous extract was removed using nitrogen gas. The extract was then dried in a freeze dryer, and the yield was calculated (yield = weight of extract / total weight of dry powder x 100%).

Saponin identification

The sample was boiled with 20 mL of water in a water bath. The filtrate was shaken vertically and let stand for 15 minutes. The formation of a stable foam indicated the presence of saponins[9].

Flavonoid identification

The sample was mixed with 5 mL of ethanol, shaken, heated, and shaken again, then filtered. Then 0.2 g of magnesium powder and three drops of concentrated HCl were added to each filtrate. The formation of red in the ethanol layer indicated the presence of flavonoids[9].

Tannin identification

The sample was boiled with 20 mL of water and then filtered. A few drops of 1% ferric chloride was added. The formation of a greenish-brown or blue-black indicated the presence of tannins[10].

Alkaloid identification

Two mL of sample was evaporated on a porcelain dish. The residue was then dissolved with 5 mL of 2 N HCl. The solution obtained was divided into three test tubes. The first tube was added with three drops of 2 N HCl, which serves as a blank. The second tube was added three drops of Dragendorff, and the third tube was added three drops of Meyer reagent. Orange sediment was formed in the second

tube, and yellow deposits in the third tube indicated the presence of alkaloids[10].

Steroid/triterpenoid identification

The sample was dissolved in methanol and then evaporated. Then, the filtrate was dissolved with chloroform in a test tube, added with ten drops of anhydrous acetate, and then the solution was dripped with three drops of concentrated H₂SO₄ through the tube wall. A brown or violet ring is formed between the solvents, indicating the presence of triterpenoid compounds, while the appearance of green indicated the presence of steroids[9].

Phenolic total content determination

The total phenolic test was modified from Ismail et al[11]. Briefly, 68 µL of samples (1 mg/mL) and standard gallic acid with a concentration range of 0.03125-0.2500 mg/mL, then added 68 µL of Folin-Ciocalteu reagent 50% and vortexed for 1 minute. To the solution, 1,364 µL of sodium carbonate 2% was added, incubated for 30 minutes. The absorbance of the extract solution was measured at a wavelength of 750 nm with a UV-VIS spectrophotometer. The results were expressed as mg GAE/g of extract.

Flavonoid total content determination

The total flavonoid test was modified from Zou et al [12]. Briefly, samples (1 mg/mL) and standard quercetin with a concentration range of 0.03125-0.2500 mg/mL in ethanol p.a. were taken 150 µL, added with aquadest 600 µL, 45 µL NaNO₂ 5%, incubated for 6 minutes, then added 45 µL AlCl₃ 10%, vortexed and incubated again for 6 minutes. Added 600 µL of 1 M NaOH and a distilled-water to a maximum volume of 1.5 mL. The solution was measured using a spectrophotometer with a wavelength of

510 nm. The results were expressed as mg QE/g of extract.

Qualitative antioxidant activity by thin layer chromatography – bioautography

Antioxidant activity was screened against DPPH free radicals by using TLC-Biautography (Dot-Blot). The red rose-leaf extract was dotted on a 60 F254 silica gel plate with a concentration of 10 μ L, cold dried, and sprayed with 0.2% DPPH in methanol. A yellow zone around the extract characterized antioxidant activity, while catechins were used as positive controls and solvents as negative controls[13].

Quantitative antioxidant activity by using the DPPH assay

DPPH solution was prepared in a concentration of 61.50 μ g/mL in a 50 mL Erlenmeyer. Sample and standard catechins were prepared in concentrations of 20480 μ g/mL and 10240 μ g/mL in DMSO, respectively. 1 mL of methanol pro analysis (p.a) was put into the tube (number 1-5), and add 50 mL of sample or standard. 1 mL of methanol p.a was added to tube number 1. A serial dilution was made by homogenizing, then was picked 1 mL, and discarding 1 mL. 1 mL of DPPH with a concentration of 61.50 μ g/mL was added to all tubes. DPPH control contain 100% compound in 1 mL methanol p.a. Then, tubes were incubated for 90 minutes in a dark room at room temperature of 22-24°C. Finally, the solutions were measured

by spectrophotometry at a wavelength of 517 nm[14].

Data analysis

The extract's antioxidant activity was determined based on the IC₅₀ value and the Antioxidant Activity Index (AAI) value. IC₅₀ is the concentration needed to reduce 50% of DPPH free radicals [IC₅₀ = (delta DPPH – sample absorbance)/delta DPPH x 100%]. AAI is a value that indicates the strength of the antioxidant activity. AAI is expressed as 1/2 DPPH concentration used / IC₅₀ value. AAI value is interpreted as a very strong (>2), strong (1-2), moderate (0.5-1), and weak (<0.5)[15].

RESULTS

Phytochemical screening of red rose-leaf extract

Phytochemical screening was carried out to identify several secondary metabolites in the red rose-leaf extracts. Based on **Table 1**, the ethanol and methanol extracts contain flavonoids, tannins, saponins. Meanwhile, ethyl acetate and n-hexane extracts showed only a small proportion of secondary metabolites were extracted in non-polar solvents. Based on this study, secondary metabolite compounds found in the rose-leaf extract are extracted in polar solvents such as ethanol and methanol.

Table 1. Phytochemical screening of red rose-leaf extracts

Extracts	Yield (%)	Saponin	Flavonoid	Tannin	Alkaloid	Steroid
Ethanol extract	2.4	++	+	++	-	nd
Methanol extract	7.6	+	++	++	-	nd
Ethyl acetic extract	11.5	-	+	-	-	nd
n-hexane extract	4	-	-	-	-	++

Note: (-) = absent, (+) = present (low), (++) = present (high), nd = not determined

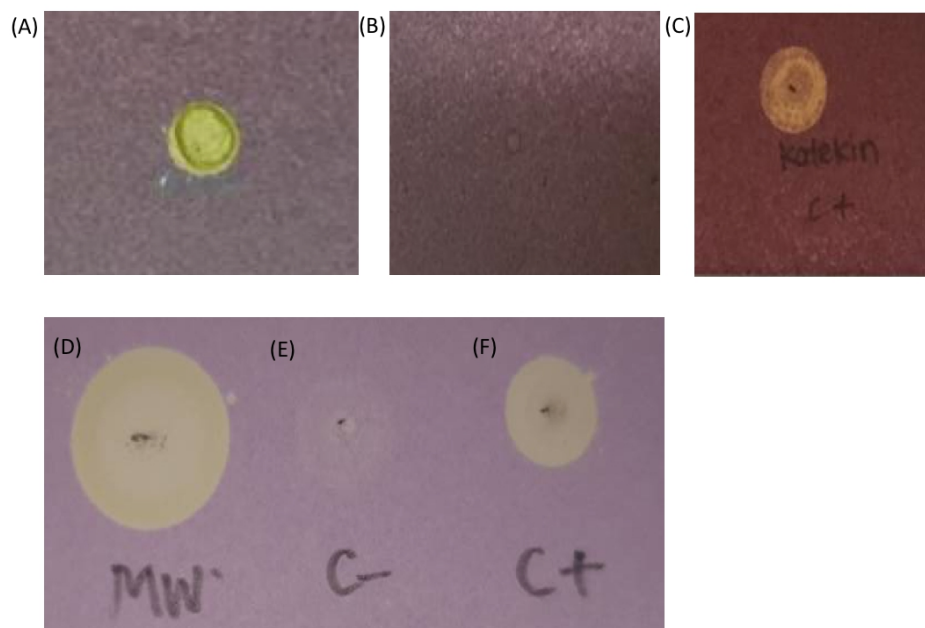


Figure 1. Antioxidant activity test using the dot-blot method of red rose leaves (MW). (A) Rose-leaf ethanol extract, (B) ethanol, (C) positive control (catechins), (D) rose-leaf methanol extract, (E) methanol, (F) positive control (catechins)

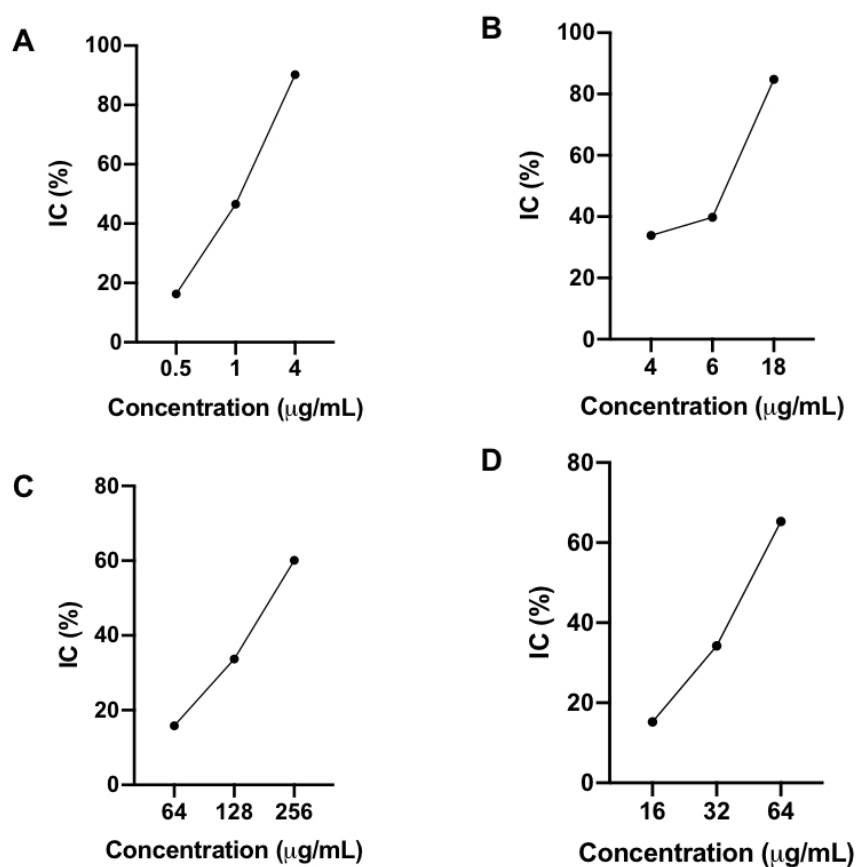


Figure 2. Curves of antioxidant activity of the red rose-leaf extract. (A) ethanol extract, (B) methanol extract, (C) ethyl acetate extract, (D) n-hexane extract

Phenolic total and flavonoid total content

The phenolic total in red rose-leaf ethanol extract was 197 mg gallic acid equivalent or GAE/g extract, which means each gram extract equivalent to gallic acid of 197 mg/g extract. Whereas the total flavonoid total in red rose-leaf ethanol extract was 163 mg quercetin equivalent or QE/g extract, which means each gram of extract contains flavonoids equivalent to 163 mg of quercetin. Here, we found that flavonoid total in methanol extract was the highest rather than all extracts. The content phenolic and flavonoid total in other extracts is presented in **Table 2**.

Antioxidant activity

Firstly, the antioxidant activity was tested using the thin layer chromatography method, a preliminary qualitative test. The results showed that the ethanol and methanol extract of red rose-leaf has antioxidant activity, indicated by the formation of a yellow color against a purple background **Figure 1**. The results showed a yellow change in methanol and ethanol extracts, equivalent to catechins as a positive control.

Table 2. Phenolic and flavonoid total content in red rose-leaf extracts

Extracts	Phenolic total (mg GAE/g extract)	Flavonoid total (mg QE/g extract)
Ethanol extract	197	163
Methanol extract	118	256
Ethyl acetic extract	16	46
n-hexane extract	1.6	30

The IC_{50} value of antioxidant activity was determined by using the

DPPH method. The quantitative testing results of red rose-leaf extract and catechins' antioxidant activity can be seen in **Table 3** and **Figure 2**.

Table 3. Antioxidant activity of red rose leaves

Samples	IC_{50}	AAI	Note
Ethanol extract	1.8	17.2	very strong
Methanol extract	8.7	3.5	very strong
Ethyl acetate extract	210.3	0.1	weak
n-hexane extract	48.7	0.6	moderate
Cathechin	9.6	3.2	very strong

The ethanol extract of red rose leaves has the IC_{50} value of 1.78 $\mu\text{g/mL}$ with an AAI value of 17.28, which can be categorized as a very strong antioxidant. Meanwhile, the methanol extract of red rose leaves has an IC_{50} value of 8.69 $\mu\text{g/mL}$ with an AAI value of 3.53, categorized as a very strong antioxidant. Whereas the IC_{50} values of ethyl acetate and n-hexane extract showed weak antioxidant activity.

DISCUSSION

Here, we found that the use of solvents in the extraction process can affect the compound's content in the extract. The present study demonstrates that the red-rose leaves ethanol and methanol extract's antioxidant capacity exhibited a very strong antioxidant activity. The IC_{50} value of the antioxidant activity of the ethanol and methanol extract of red rose leaves were 1.78 $\mu\text{g/mL}$ and 8.69 $\mu\text{g/mL}$, respectively. IC_{50} 1.78 $\mu\text{g/mL}$ means that amount of 1.78 $\mu\text{g/mL}$ of the ethanol extract was required to scavenge 50% of free radicals DPPH. These results suggested that ethanol and methanol

extracts of red rose leaves have very strong antioxidant activity. It may have phenolic compounds such as flavonoids in the role of antioxidant activity. The phenolic compounds exert their redox ability to absorb and neutralize free radicals, bind to singlet and triplet oxygen, and decompose peroxides[16].

Based on the source, antioxidants are divided into endogenous and exogenous antioxidants. In biological systems, the body produces its antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. Exogenous antioxidants, such as natural antioxidants, comes from plants and animals. The molecular structure of natural antioxidants generally has a hydroxy group. Natural antioxidants derived from plants are phenolic compounds in flavonoids, cinnamic acid derivatives, coumarin, tocopherols, and polyfunctional organic acids[17]. Further research in the use of fractionation from ethanol and methanol extract to identify the specific compound is needed.

This study proves that red rose leaves have a very strong antioxidant activity similar to catechin, which can be seen from qualitative and quantitative antioxidant activity testing. Flavonoids can convert or reduce free radicals and act as free radicals scavenger so that they can be useful as antioxidants[18]. Since red-rose leaves currently are treated as a waste; therefore, they may be used as a natural source of antioxidants, offering the possible ability to provide high-value goods, helping to escape numerous conditions associated with oxidative stress.

CONCLUSION

The ethanol and methanol extract of red rose leaves (*Rosa chinensis* Jacq.)

showed the most potent antioxidant activity.

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