Antioxidant activity of *Rosa damascene flos* ethanol extracts using hydroxyl and nitrite oxide scavenging methods

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**Abstract**

**Objective:** The purpose of this study were to determine antioxidant activity of *Rosa damascene flos* ethanol extracts.

**Methods:** The ethanol extracts were extracted from the crown of rose and rose base by maceration using ethanol 70% solvent. Antioxidant activity was determined with hydroxyl and nitrite oxide scavenging methods and the IC\(_{50}\) analyzed using SPSS 23.

**Results:** The IC\(_{50}\) of crown rose ethanol extract (CREE) and rose base ethanol extract (RBEE) on hydroxyl and nitrite oxide scavenging were 7.61 ± 0.38 μg/mL, 17.55 ± 0.37 μg/mL and were 349.57 ± 0.35 μg/mL, 54.93 ± 4.49 μg/mL.

**Conclusions:** The crown of rose ethanol extract (CREE) and rose base ethanol extract (RBEE) has activity as antioxidant.

**Keywords:** Antioxidant, *Rosa damascene flos*, hydroxyl, nitrite oxide

**Introduction**

*Rosa damascene* of the family *Rosaceae*, is one of the most important commercial flower crops with over 150 species, more than 20 000 cultivars and with colour spectrum ranging from subtle whites, yellows and pinks to intense purple, orange and red tones. Its flower colour is attributed to the presence of anthocyanins and carotenoids\(^1\). The crown of rose have been consumed as a food ingredient in teas, cakes, and flavour extracts as well as medicinal remedies of various illnesses\(^2\). The rose flower is known as an astringent, stomachic, and is used traditionally as an agent for activating blood circulation to relieve blood stasis, and counteracting toxin. Being rich in anthocyanin content, rose petals are a good colorant and potentially a good source of antioxidants\(^3\).

Antioxidant activity of a plant is important because of two reasons. First the consumption of a food rich in antioxidants has been suggested to prevent or delay oxidation of major biomolecules within the cell by chelating metals or scavenging free radicals that are produced as consequences of metabolism\(^4\). Free radicals can defined as some free entities having one or more unpaired electrons which play a vital role in the development of various human diseases including aging\(^5\). Antioxidant constituents can protect the human body from free radicals such hydroxyl radicals (OH) and nitrite oxide radicals (NO) \(^6\). Secondly antioxidants are used in food preservation to prevent the food from oxidation, and increase their shelf life by retarding the process of lipid peroxidation, which is one of the major reasons for deterioration of food and pharmaceutical products during processing and storage\(^7\).

There are several ways to test the strength of antioxidant activity of natural products, including scavenging OH and NO as free radicals. OH is considered as one of the most powerful oxidizing agents that can attack biomolecules and cause irreversible damage\(^8\). While NO is a free radical that plays a role in the process of vasodilation, inflammation and the immune system. Decreasing the number of OH and NO radicals with scavenging method can inhibit cell damage\(^9\). Several constituents are present in the *R. damascena* including flavonoids, anthocyanins, terpenes and glycosides, which have useful effects on body. The
investigation confirmed flavonoids and other contents in *R. damascena* has antioxidant effect\[10\].

**EXPERIMENTAL**

**Plant and chemicals materials**

*Rosa damascena* flos were collected from Pansoburan Village, Toba Samosir, North Sumatra, Indonesia. *Rosa damascena* flos was identified in Herbarium Medanense (MEDA) University of Sumatera Utara. The chemicals materials used in this study were sodium nitroprusside (Sigma), sulphanilamide (Sigma), phosfat acid (Merck), N-(1-naphthyl) ethylenediamine dihydrochloride (Sigma), Ethanol (Merck), ferrous ammonium sulfate (Merck), hydrogen peroxide (Merck), buffer phosfat, L-ascorbic acid (Sigma), deoxyribose (Sigma), trikloroasetat (TCA) (Merck), tiobarbiturat acid (TBA) (Merck) and aquedest.

**Preparation of CREE and RBEE**

The air-dried and powdered flos of *Rosa damascena*(Lour.) (500 g) were repeatedly macerated with ethanol 70% (3x3 d, 7.5 L). The filtrate was evaporated with a rotary evaporator with a temperature of ± 40°C to give a viscous extract\[11\].

**Scavenging OH Assay of CREE and RBEE**

Enter 2 µl of the sample into well blank and well sample. Add 10 µl of FeCl₃-EDTA to the sample well and control well. Add H₂O₂ of 5 µl to the sample well and control well. Add 5 mL of L-Ascorbic Acid 1 mM to the sample well and control well. Add 10 µl of deoxyribose to the sample well and control well. To well blank, add 120 µl buffer. To the well control, add 70 µl buffer. To the sample well, add 69 µl buffer. Incubation plate for 30 minutes at 37°C. Add 25% 25% TCA solution to the sample well and control well. Add a 25% 25% TBA solution to the sample well and control well. The plate for incubated 30 minutes at 80°C. Absorbance was measured using a microplate reader at λ = 532 nm\[12\]. The equation to determine scavenging activity:

\[
\% \text{Scavenging activity} = \frac{\text{Absorbance of control} - \text{absorbance of sample}}{\text{Absorbance of control}} \times 100\%
\]

**Scavenging NO Assay of CREE and RBEE**

Enter 10 µl of the sample into well blank and well sample. Add 40 µl SNP to the sample well and control well. To well blank, add 140 µl ethanol. To the well control, add 10 µl ethanol. The plate incubated for 2 hours at room temperature. Add 100 µl Greiss solution to sample well and control well. Absorbance was measured using a microplate reader at λ = 546 nm\[13\]. The equation to determine scavenging activity:

\[
\% \text{Scavenging activity} = \frac{\text{Absorbance of control} - \text{absorbance of sample}}{\text{Absorbance of control}} \times 100\%
\]

**Statistical Analysis**

The results were presented as means ± SD. The statistical analysis was carried out by using SPSS edition 23.

**RESULT AND DISCUSSION**

Antioxidant activity of CREE and RBEE, it is begin from extraction *Rosa damascena* flos. The extraction results can be seen in Table 1. Crown rose and base rose is a part of Description: The difference in superscript letters (a, ab, b, c, d) in the same column shows a significant difference at P <0.05 (Tukey HSD post hoc test).

CREE has better antioxidant OH scavenging activity compared to BREE. CREE with concentrations of 0.83 µg/mL, 1.67 µg/mL, 3.33 µg/mL, 6.67 µg/mL, 13.33 µg/mL.
µg/mL and 26.67 µg/mL each have antioxidant activity 36.32 ± 5.27%, 36.34 ± 3.80%, 42.63 ± 1.12%, 50.37 ± 1.75%, 61.73 ± 1.80%, and 85.59 ± 0.65%. BREE compounds at the same concentration each had antioxidant activity of 19.60 ± 1.85%, 23.79 ± 1.30%, 27.34 ± 3.32%, 33.05 ± 0.97%, 44.00 ± 1.01% and 64.29 ± 1.14%.

The content of chemical compounds in roses is a strong reason that roses are antioxidants. Rose contains various active compounds including tannin, geraniol, nerol, citronellol, geranic acid, terpenes, flavonoids, polyphenol pectin, vanillin, carotenoids, stearopen, farnesol, eugenol, pheniletilakohol, and vitamin C in the fight against free radicals. According to the results of the present study, the fresh and spent flower extracts obtained from *Rosa damascena* could be a good natural antioxidant source. Based on the data above, it can be calculated the IC₅₀ value of each test sample. IC₅₀ results can be seen in Table 4.

| Sample | IC₅₀ (µg/mL)          | NO scavenging | OH scavenging |
|--------|-----------------------|---------------|---------------|
| CREE   | 39.29 ± 0.47          | 7.61 ± 0.38   |               |
| BREE   | 54.93 ± 4.49          | 17.55 ± 0.37  |               |

IC₅₀ value was obtained through the calculation of the absorbance value using the linear regression equation \( y = a + bx \) by comparing the extract concentration with the NO and OHscavenging values. In the table 4, it can be seen that the IC₅₀ value of NO scavenging activity shows that the CREE has an IC₅₀ value was 39.29 ± 0.47 µg/mL smaller than the BREE which has an IC₅₀ value was 54.93 ± 4.49 µg/mL. IC₅₀ CREE was also smaller when compared to BREE in OH scavenging testing. IC₅₀ Value of CREE was 7.61 ± 0.38 µg/mL while BREE was 17.55 ± 0.37 µg/mL. IC₅₀ values indicate the sample concentration needed to inhibit 50% of free radical activity. The smaller the IC₅₀ value produced, the better the ability of a compound in free radical scavenging activities.

**CONCLUSION**

Based on the results we obtained ethanol extract of *Rosa damascena* flower had a potentially antioxidant activity.

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