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

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A B S T R A C T

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 were7.61 ± 0.38µg/mL, 17.55 ± 0.37 µg/mL and were 349,57 ±0,35µg/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

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INTRODUCTION

Rosa damescene 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 spectre 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 reactive spesies, it 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. damascene* has antioxidant effect[10].

EXPERIMENTAL

Plant and chemicals materials

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

Preparation of CREE and RBEE

The air-dried and powdered flos of *Rosa damascene*(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 $FeCl_3$-EDTA to the sample well and control well. Add H_2O_2 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 µl 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 damascene 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).

Rosa damascene flos were extracted by maceration methods using ethanol 70%.

Table: 1 Extract content of CREE and RBEE

No	Sample	Gram
1	CREE	40.50
2	RBEE	53.67

Description: The samples were extracted by maceration method. Each samples weight used was 250 gram.

CREE and RBEE effect as antioxidant was carried out by OH and NO scavenging methods. The comparison of antioxidant test results through NO scavenging by CREE and RBEE was showed in Table 2. There was a statistically significant difference followed by the Post Hoc test using the Tukey HSD method. The average percentage of NO scavenging antioxidant activity by CREE was higher than by BREE. The results showed an increase that was in line with the high concentration.

Table: 2 NO scavenging activity of CREE and BREE

Concentration (ug/mL)	NO scavenging activity (%)	
	CREE	BREE
2.08	28.33±4.30^{a}	24.10±1.39^{a}
4.17	32.83±1.96^{ab}	27.74±2.32^{ab}
8.33	34.46±2.23^{ab}	28.67±2.84^{ab}
16.67	38.59±1.79^{b}	31.75±2.49^{b}
33.33	46.62±2.90^{c}	40.27±0.84^{c}
66.67	64.30±0.90^{d}	55.45±1.47^{d}

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).

At concentrations of 2.08 µg/mL, 4.17 µg/mL, 8.33 µg/mL, 16.67 µg/mL, 33.33 µg/mL and 66.67 µg/mL CREE each has an antioxidant activity of 28.33 ± 4.30%, 32.83 ± 1.96%, 34.46 ± 2.23%, 38.59 ± 1.79%, 46.62 ± 2.90%, and 64.30 ± 0.90%. BREE at the same concentration each had antioxidant activity of 24.10 ± 1.39%, 27.74 ± 2.32%, 28.67 ± 2.84%, 31.75 ± 2.49%, 40.27 ± 0.84% and 55.45 ± 1.47%. While antioxidant activity of CREE and BREE using OH scavenging method showed the same results. The was showed in Table 3.

Table: 3 OH scavenging activity of CREE and BREE

Concentration (ug/mL)	NO scavenging activity (%)	
	CREE	BREE
0.83	36.32±5.27^{a}	19.60±1.85^{a}
1.67	36.34±3.80^{a}	23.79±1.30^{ab}
3.33	42.63±1.12^{ab}	27.34±3.32^{b}
6.67	50.37±1.75^{b}	33.05±0.97^{c}
13.33	61.73±1.80^{c}	44.00±1.01^{d}
26.67	85.59±0.65^{d}	64.29±1.14^{e}

CREE has better antioxidant OH scavening 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 and 26.67 µg/mL each have antioxidant antivitivity 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, stearopten, 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 *Rose damascena* could be a good natural antioxidant source[14].Based on the data above, it can be calculated the IC_{50} value of each test sample. IC_{50} results can be seen in Table 4.

Table: 4 IC_{50}values of CREE and BREE

Sample	IC_{50} (µg/mL)	
	NO scavenging	OH scavenging
CREE	39.29 ± 0.47	7.61± 0.38
BREE	54.93 ± 4.49	17.55± 0.37

IC_{50} 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 IC_{50} value of NO scavenging activity shows that the CREE has an IC_{50} value was 39.29 ± 0.47 µg/mL smaller than the BREE which has an IC50 value was 54.93 ± 4.49 µg/mL. IC_{50} CREE was also smaller when compared to BREE in OH scavenging testing. IC_{50}valueof CREEwas 7.61± 0.38 µg/mLwhile BREE was 17.55± 0.37 µg/mL. IC_{50} values indicate the sample concentration needed to inhibit 50% of free radical activity.The smaller the IC_{50} value produced, the better the ability of a compound in free radical scavenging activities[15].

CONCLUSION

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

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