

Antioxidant Activities of Methanolic Extracts from Four Different Rose Cultivars

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Abstract Rose petals have been used as a food ingredient in teas, cakes, and flavor extracts. The objectives of this study were to determine antioxidant components and antioxidant activities of methanolic extracts of four different rose cultivars (Macarena, Onnuri, Oklahoma, Colorado). The contents of total polyphenolics, flavonoids, and anthocyanins in rose methanol extracts (RMEs) were measured by spectrophotometric methods. 2,2'-Azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, reducing power, chelating activity and inhibition of lipid peroxidation have been used to investigate the antioxidant activities of RMEs. Results showed that Oklahoma had the highest polyphenol (129.8 ± 7.3 mg/1 g RMEs), flavonoids (23.7 ± 0.2 mg/1 g RMEs), and anthocyanins (18.699 ± 0.354 mg/1 g RMEs) contents, Oklahoma also exhibited the highest DPPH, ABTS radical scavenging activity, reducing power, and inhibitory activity of lipid peroxidation except metal chelating effect. This study provides basic information useful to determine the best rose cultivar with high antioxidant components and activities.

Keywords: rose, antioxidant components, antioxidant activities, cultivar

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1. Introduction

Reactive oxygen species (ROS) include both oxygen radicals and some non-radical oxidizing agents [1], and their formation is a natural process. Excessive production of ROS above the cell's defenses results in oxidative stress, and oxidative stress is widely recognized as a contributing factor in the development of chronic diseases [2,3,4]. Regular supplement of antioxidants can assist the endogenous defense systems to counterbalance the harmful effects of excessive ROS [5]. Antioxidants include synthetic and natural antioxidants. Several synthetic antioxidants, such as butylated hydroxytoluene and butylated hydroxyanisole are largely used in food industry and included in human diet. However, the synthetic antioxidants are suspected of being responsible for some side effects. Therefore, many researchers have focused on the safety and functional characteristics of natural antioxidants on human health [6]. The natural antioxidants, as the better alternative, from vegetables, fruits and other medicinal plants are used in food industry in recent years [7,8].

Rose is a woody perennial of the genus *Rosa*, within the family Rosaceae. They are known as edible flowers and have been consumed for many years as food components, such as cakes and beverages [9]. They contain a variety of phytochemicals and are also used in medicinal practices for remedy of various illnesses [10]. The consumption of rose shows positive regulatory effects in human health

[11,12,13]. The physiological function of Rosaceae may be partly attributed to their abundance of phenolics [14]. Phenolic compounds, flavonoids and anthocyanins are widely distributed in plants and have been reported to possess a wide spectrum of biochemical activities [11,14], such as antioxidants, free-radical scavengers [15,16], anticancer [17], anti-inflammatory [18], antimutagenic [19] and antidepressant [20].

The main objectives of the present study were to determine the antioxidant compounds and antioxidant activities of methanolic extracts from four different edible rose cultivars (Macarena, Onnuri, Oklahoma, and Colorado) and to provide basic information useful to breeders and biotechnologists who are planning to breed rose genotypes with higher antioxidant activity.

2. Materials and Methods

2.1. Materials

Gallic acid, Folin-ciocalteu's reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), diammonium salt of 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), potassium persulphate, sodium phosphate, potassium ferricyanide, ferric chloride, ferrozine, ethylenediaminetetraacetic acid (EDTA), Trolox, beta-carotene, linoleic acid, Tween 40 were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents and solvents used were of analytical and HPLC grade.

2.2. Methods

2.2.1. Sample Preparation

Approximately 3.0 g of dried samples were extracted with 100 mL of methanol in a shaker (Eyela Model MMS-300, Tokyo Rikakikai Co., Japan), at room temperature for 24 h. The supernatants were filtered through a Toyo No.2 filter paper. The filtrate was evaporated at 37°C. The dried extract was dissolved in 50 mL of methanol and stored at refrigerator until analysis.

2.2.2. Determination of Total Phenolic Content

Total phenolic contents in each extract were measured by the Folin-Ciocalteu method of Meda et al. [21] with a slight modification. Briefly, 100 μ L of extract solution was mixed with 2.0 mL of 2% sodium carbonate (Na_2CO_3) and 100 μ L of 50% Folin-Ciocalteu's reagent. After incubation at room temperature for 5 min, the absorbance of the reaction mixtures were measured at 750 nm by using a spectrophotometer. Gallic acid was used as a standard and total phenolic contents of RMEs were expressed in milligram gallic acid equivalents (mg GAE/g extract).

2.2.3. Determination of Total Flavonoid Content

According to the method of Kim et al. [22], total flavonoid contents were determined by preparing 250 μ L of extracts in 1.25 mL of distilled water and 75 μ L of 5% NaNO_2 . After 6 min, 150 μ L of 10% AlCl_3 was added. After 5 min, 500 μ L of 1 M NaOH was added to the mixture. Absorbance of the mixture was determined at 510 nm. Results were expressed as catechin equivalents (mg CTE/g extract).

2.2.4. Determination of Total Anthocyanin Content

The total anthocyanins content was measured with the pH differential absorbance method, as described by Cheng and Breen [23]. Briefly, absorbance of the extract was measured at 510 and 700 nm in buffers at pH 1.0 and 4.5. The anthocyanin content was calculated as milligrams of cyanidin-3-glucoside per gram of RMEs, using molar extinction coefficient of 26900 and a molecular weight of 449.2.

2.2.5. DPPH and ABTS Radical Scavenging Activities

DPPH radical scavenging activity of RMEs was determined according to the method described by Kim et al. [24] with some modifications. In brief, aliquots of 0.8 mL of 0.2 mM solution of DPPH methanol were mixed with 0.2 mL of the extracts. The mixtures were vigorously shaken and left to stand for 10 min under subdued light, the absorbance was measured at 520 nm against blank samples. Results were expressed as Trolox equivalent antioxidant activity (TEAC) defined as mg Trolox[®] equivalents per 1 g residue.

ABTS assay was performed according to the method reported by Re et al. [25] with some modifications. The ABTS radical cation was generated by adding 7 mM ABTS to 2.45 mM potassium persulphate solution, and the mixture was left to stand overnight in the dark at room temperature. The ABTS radical cation solution was diluted with ethanol to obtain an absorbance of 1.0 at 734 nm. Diluted ABTS radical cation solution (1 mL) was added to 20 μ L of sample fractions or Trolox[®] standard solution. The absorbance was measured at 734 nm after 30

min. The ABTS radical cation scavenging activity was expressed as TEAC.

2.2.6. Ferric-reducing Activity

The reducing activity of RMEs was determined by the method of Oyaizu [26] with slight modifications. In brief, 250 μ L of sample solution was mixed with 250 μ L of sodium phosphate buffer (200 mM, pH 6.6) and 250 μ L of 1% potassium ferricyanide. Reaction mixture was incubated at 50°C for 20 min. After incubation, 2.5 mL of trichloroacetic acid (10%) was added and centrifuged for 1 min. From the upper layer, 500 μ L solution was mixed with 500 μ L distilled water and 100 μ L FeCl_3 (0.1%). Absorbance of all the sample solutions was measured at 700 nm. Trolox is used as a positive control.

2.2.7. Metal-chelating Activity

The ferrous ion chelating activity was determined by the method of Dinis et al. [27] with slight modifications. The extract (1 mL) was reacted with 100 μ L of ferrous chloride (2mM) and ferrozine (5mM) for 10 min, and the absorbance of the mixture was measured at 562 nm. EDTA is used as a positive control.

2.2.8. β -Carotene Bleaching Inhibition Activity

Beta-carotene bleaching activity of RMEs was determined by the method of Hajar et al. [28] with slight modifications. Briefly, 3 mL of beta-carotene solution were added to 40 mg of linoleic acid and 40 mg of Tween 20. The mixture was dried by concentration. Immediately, 100 mL distilled water were added to the dried mixture to form a beta-carotene-linoleic acid emulsion. In order to determine the beta-carotene bleaching activity of the extract, 300 μ L of emulsion were added to 20 μ L of RMEs. Immediately, absorbance was measured at 470 nm. After these mixtures were incubated in water bath at 50°C for 60 min, the absorbance was measured at 470 nm.

2.2.9. Statistical Analysis

Results are reported as mean \pm standard deviation and are representative of three independent experiments. Significant differences were tested using a one-way ANOVA and Duncan's test using SAS version 9.0 (SAS Institute, Cary, NC, USA). A value of $P < 0.05$ was considered significant.

3. Results and Discussion

3.1. Total Phenolic, Total Flavonoid and Anthocyanin Contents

Rose species have been demonstrated to possess antioxidant potential. Plant phenolics have been shown to inhibit the formation of superoxide anion radicals generated by various enzyme [29]. The total phenolic contents in RMEs were expressed as milligram of gallic acid equivalents per gram of residue (mg GAE/g residue). The order of phenolic contents was as follows: Oklahoma; 129.8 \pm 7.3, Onnuri; 111.3 \pm 13.1, Colorado; 108.0 \pm 2.5, and Macarena; 64.8 \pm 3.5 mg GAE/g residue. The results showed that Oklahoma was higher in phenolic content compared to other samples (Table 1). Flavonoids as the most common group of phenolic compounds are also

responsible for antioxidant activity. In this study, the total flavonoid contents in RMEs were expressed in milligram of catechin equivalents per gram of residue (mg CE/g residue). The total flavonoid content in RMEs was varied considerably from 3.6 to 23.7 mg CE/g residue. The data presented in Table 1 indicates that the highest flavonoid content of 23.7 mg CE/g residue was observed in Oklahoma and the lowest content was observed in Macarena (3.6 mg CE/g residue). Total flavonoid content of rose extracts is arranged in the following sequence: Oklahoma > Onnuri > Colorado > Macarena. The total content of anthocyanin varied from 0.025 mg/g (Macarena) to 18.699 mg/g (Oklahoma) residue. Oklahoma contained obviously higher anthocyanin content than other samples, and almost 750-fold of Macarena.

Table 1. Antioxidant components of methanol extracts from four different cultivars

Sample	Polyphenolics ¹⁾	Flavonoids ²⁾	Anthocyanin ³⁾
Macarena	64.8 ± 3.5	3.6 ± 0.1	0.025 ± 0.009
Onnuri	111.3 ± 13.1	14.5 ± 1.6	1.643 ± 0.051
Oklahoma	129.8 ± 7.3	23.7 ± 0.2	18.699 ± 0.354
Colorado	108.0 ± 2.5	12.9 ± 0.2	0.297 ± 0.025

¹⁾ Mean of triplicate determinations expressed as mg gallic acid equivalents per 1 g of rose petal extracts.

²⁾ Mean of triplicate determinations expressed as mg catechin equivalents per 1 g of rose petal extracts.

³⁾ Mean of triplicate determinations expressed as mg cyanidine-3-glucoside equivalents per 1 g of rose petal extracts.

3.2. DPPH and ABTS Radical Scavenging Activities

DPPH is a stable free radical which has a maximum absorption at 515 nm. Antioxidants are thought to have DPPH radical scavenging activity due to hydrogen donating ability [30]. The DPPH radical scavenging activity of RMEs is shown in Figure 1. Oklahoma showed the strongest DPPH radical scavenging activity, followed by Onnuri, Colorado, and Macarena. It was reported that antioxidant activities of various vegetables, fruits, and natural plants are attributed to the contents of phenolic compounds [31]. ABTS, as a stable free radical cation, was also used to evaluate the antioxidant activity of RMEs. The ABTS radical is commonly used to measure the radical scavenging activity of hydrogen donating and chain breaking antioxidants in many plant extracts [32]. As shown in Figure 1, the ABTS radical scavenging activity of RMEs was expressed as mg Trolox equivalent antioxidant capacity (TEAC)/g residue. Oklahoma showed the most effective ABTS scavenging activity, followed by Onnuri, Colorado, and Macarena. The results indicated that the Oklahoma extract contained relatively higher amounts of antioxidant compounds which may be responsible for higher ABTS radical scavenging activity compared to other cultivars.

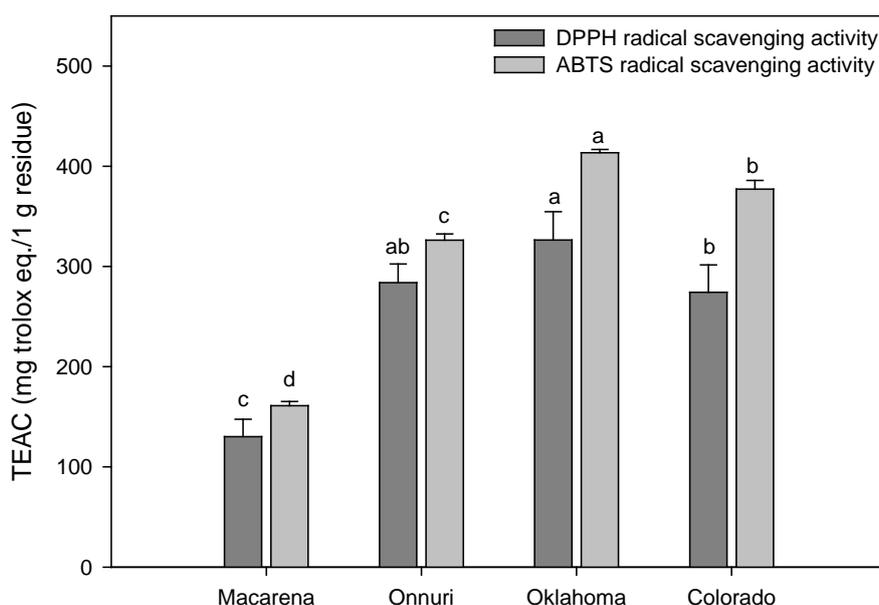


Figure 1. DPPH and ABTS free radical scavenging activity of methanolic extracts (1 mg/mL) from four different cultivars. Values are expressed as a mean ± SD (n = 3). ^{a-d} Mean values followed by different superscripts above bars are significantly different by Duncan's multiple range test at p < 0.05

3.3. Reducing Power and Chelating Activity

Reducing power is one action mechanism of antioxidants which may possess potential antioxidant activity [33]. The reducing power of RMEs is presented in Figure 2. Ferric-ferricyanide complex is reduced to the ferrous form due to the presence of antioxidants in the method. Trolox as positive control showed the highest reducing power than other sample extracts. Colorado and Oklahoma showed higher reducing power than Onnuri and Macarena, and there was no significant difference between Colorado and Oklahoma.

Ferrous ions as the effective pro-oxidants were used to determine the chelating activity of RMEs. In the assay, ferrozine and Fe²⁺ can quantitatively form complexes, however, due to the present of chelating agents, the complex formation is disturbed, and as a result, the red color of the complex is decreased [34]. EDTA was used as the standard metal chelator in this study. Chelating activities of EDTA on ferrous ions are shown in Figure 3. EDTA showed the strongest chelating activity. Colorado and Onnuri possessed the stronger chelating activity, followed by Oklahoma and Macarena. Although Oklahoma showed relatively higher phenolic, flavonoid and anthocyanin contents than other samples, it did not show higher chelating activity than the others.

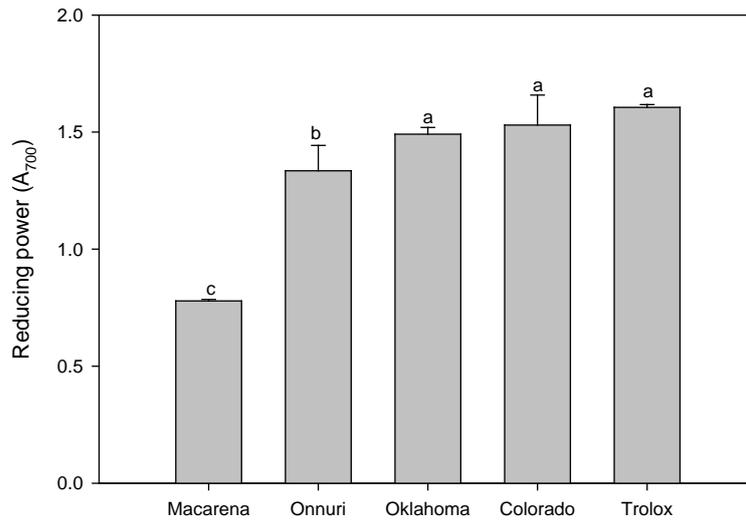


Figure 2. Reducing power of methanolic extracts (1mg/ml) from four different cultivars. Values are expressed as a mean \pm SD (n=3). ^{a-c}Mean values followed by different superscripts in a column are significantly different by Duncan's multiple range test at p<0.05. Trolox at the concentration of 1 mg/mL were used as a positive control

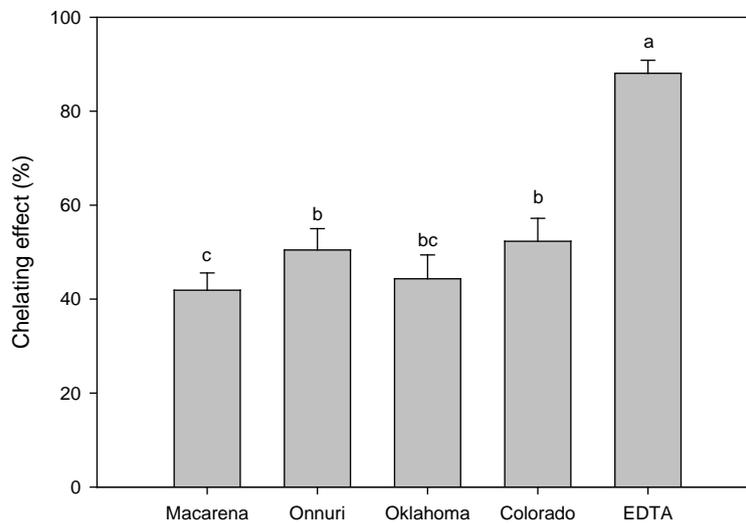


Figure 3. Chelating effect of methanolic extracts (1 mg/mL) from four different cultivars. Values are expressed as a mean \pm SD (n=3). ^{a-c}Mean values followed by different superscripts in a column are significantly different by Duncan's multiple range test at p<0.05. EDTA at the concentration of 1 mg/mL were used as a positive control

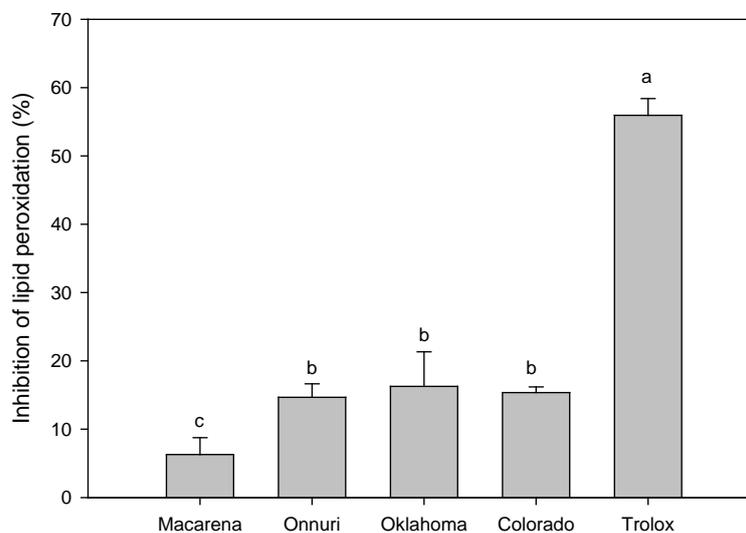


Figure 4. Inhibition of lipid peroxidation of methanolic extracts (1 mg/mL) from four different cultivars. Values are expressed as a mean \pm SD (n=3). ^{a-c}Mean values followed by different superscripts in a column are significantly different by Duncan's multiple range test at p<0.05. Trolox at the concentration of 1 mg/mL were used as a positive control

3.4. Inhibition of Lipid Peroxidation

Antioxidant activity of RMEs was measured by bleaching of β -carotene. In the assay, during incubation at 50°C for 60 min, linoleic acid generated hydroperoxides which can cause rapid discoloration of β -carotene [35]. Figure 4 shows that Trolox exhibited the highest hydroperoxides inhibitory activity. All samples significantly inhibited the generation of hydroperoxides in the linoleic acid emulsion system, but all samples showed relatively lower inhibition activity than Trolox.

In conclusion, high antioxidant components and activities were observed in methanolic extracts of Oklahoma as compared to other tested extracts. Although further research is necessary, our study shows that a supplementation with RMEs could at least help in preventing or decreasing the damages caused by oxidative stress.

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