

Effects of dried Rose Petals (*Rosa damascena*) on the antioxidant capacity of Green and Black Tea

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Date of submission: 10 Jun 2018, **Date of acceptance:** 06 Oct 2018

ABSTRACT

The health benefits of green and black tea are mainly associated with their antioxidant potential and phenolic compounds. The present study aimed to evaluate the effects of dried rose petals (*Rosa damascena*) on the antioxidant capacity of green and black tea. Antioxidant capacities of tea and rose infusions were assessed using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and 2, 2-azinobis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical scavenging. In the DPPH method, various concentrations of rose increased the radical scavenging activity of green tea, while the higher concentrations (2 g) negatively influenced the radical scavenging activity of black tea. In the ABTS assay, lower concentrations of rose (0.5 and 1 g) significantly increased the antioxidant activity of green tea. Moreover, various concentrations of rose enhanced the ABTS radical scavenging activity of black tea. According to the results, higher concentrations of rose decreased the DPPH radical scavenging activity of black tea, while the lower concentrations exerted synergistic antioxidant effects on the ABTS radical scavenging activity of green tea.

Keywords: Rose, Antioxidant Activity, Tea

Introduction

Tea (*Camellia sinensis* L.) is the most widely consumed beverage in the world next to water. Depending on the manufacturing process, several varieties of tea could be produced, including white, yellow, green, red, and black tea.¹⁻⁴

Today, green tea has gained popularity across the world owing to its numerous health benefits, including antioxidant, antimicrobial, anti-carcinogenic, and anti-inflammatory properties.^{5, 6} Green tea is obtained from fresh tea leaves and is often the preferred beverage in Japan, China, and Western countries. Green tea reduces the risk of free radicals and oxidative stress^{7, 8}, which in turn decreases the potential risk of life-threatening diseases such as cancer,

coronary heart disease, stroke, and obesity.⁹⁻¹¹ Furthermore, green tea is an abundant source of chemical components, such as polyphenols, which are known to have potent antioxidant properties.^{12, 13} The beneficial effects of black and green tea are associated with the antioxidant activities of their phenolic compounds.¹⁴

Black tea consumption constitutes approximately 80% of the total tea beverage industry.¹⁵ Among various types of tea, black tea has a higher consumption rate compared to green tea in different regions across the world. Black tea is an abundant source of polyphenolic compounds, which have numerous health benefits considering their antioxidant, anti-inflammatory, and antitumor properties.¹⁶ Tea leaves contain various polyphenols and flavonoids. Catechins are the main polyphenols found in tea.¹³ Non-toxicity is another major benefit of tea.¹⁷ Antioxidants play a key role in regulating defense against oxidative stress.¹⁸

Rose (*Rosa damascena*) belongs to the *Rosaceae* family and is an important ornamental

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Citation: Aliakbarlu J, Mohammadi Sh, Dehestaniathar S, Khalili Sadaghiani S. Effects of dried Rose Petals (*Rosa damascena*) on the antioxidant capacity of Green and Black Tea. J Adv Environ Health Res 2018; 6(4): 240-245

plant used in foods and traditional medicines.¹⁹ Rose contains large amounts of phenolic compounds, which are associated with remarkable antioxidant capacity.^{9,20} Addition of dried rose petals to tea is a routine practice in many regions in Iran, which provides a plausible taste and a pleasant aroma. In other parts of the world, it is common to drink tea with milk or lemon. Reports are indicative of the effects of whole and skimmed milk,²¹ milk and sugar,²² soy milk,²³ and ascorbic acid¹⁴ on the antioxidant potential of tea.

The present study aimed to investigate the effects of dried *R. damascena* petals on the antioxidant activity of green and black tea.

Materials and Methods

Preparation of Plants

In this study, green tea, black tea, and dried *R. damascena* petals were purchased from a local market in Urmia, Iran (The origin of that is unknown).

Experimental Chemicals

Potassium per sulfate, 2,2-azinobis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and butylated hydroxytoluene (BHT) were purchased from Sigma-Aldrich Chemie (Steinheim, Germany). In addition, the analytical grades of ethanol and methanol were obtained from Merck (Germany).

Preparation of Tea and Rose Infusions

Rose infusions were prepared using various concentrations of tea and dried rose petals, which were similar to those commonly consumed with tea. Green and black teas (2 g) and dried rose petals (0.5-2 g) were added to hot distilled water (500 ml) and brewed in an Erlenmeyer flask at the temperature of 80°C for 10 minutes. Seven groups of samples were prepared; one group had only two grams of tea, three groups contained 0.5, one, and two grams of dried rose petals, and three groups were infusions of tea and dried rose petals (two grams of tea, 0.5 gram of rose petals, two grams of tea with one gram of rose petals, and two grams of tea with two grams of rose petals). The infusions

were cooled at room temperature and were filtered with Whatman grade 42 filter paper. The filtrates were immediately used for the antioxidant assays.

DPPH Radical Scavenging Assay

Scavenging activity of the samples was determined using DPPH radical scavenging assay.²⁴ To do so, 50 microliters of each infusion (dilution: 1:5) were added to two milliliters of DPPH methanol solution (24 µg/ml). After shaking, the samples were preserved at room temperature in the dark for one hour. Afterwards, the absorbance of the samples was recorded against a blank at 517 nm using a spectrophotometer (LKB Novaspec II; Pharmacia, Sweden). Radical scavenging activity of the samples was calculated based on the following formula:

$$\text{Radical Scavenging Activity (\%)} = (A_{\text{blank}} - A_{\text{sample}}) / (A_{\text{blank}}) \times 100$$

where A_{blank} is the absorbance of the blank (DPPH solution), and A_{sample} represents the absorbance of the samples. In this process, BHT (2 mg/ml) was used as the positive control.

ABTS Radical Scavenging Assay

The ABTS radical stock solution was prepared by blending the aqueous solutions of ABTS (7 mM) and potassium persulfate (2.45 mM), and the combination was preserved in the dark for 16 hours.²⁵ Following that, the green-blue solution was diluted with ethanol in order to obtain the absorbance of 0.7 ± 0.02 at 734 nm using a spectrophotometer (LKB Novaspec II; Pharmacia, Sweden). In addition, 200 microliters of each sample (diluted with distilled water 1:20) was mixed with two milliliters of ABTS solution. After incubation at room temperature for six minutes, the absorbance was measured at 734 nm. ABTS radical scavenging activity was calculated using the following equation:

$$\text{ABTS Radical Scavenging Activity (\%)} = (A_{\text{blank}} - A_{\text{sample}}) / (A_{\text{blank}}) \times 100$$

In this process, BHT (2 mg/ml) was used as the reference compound.

Statistical Analysis

In this study, all the measurements were performed in triplicate. Data analysis was performed in SPSS version 9.1 (SAS Institute, Cary, NC) using Tukey's multiple range test to determine the significant differences between the treatments at the significance level of $P \leq 0.05$.

Results and Discussion

DPPH assay has been widely used to examine the antioxidant activity of various herbal extracts. This assay is a simple and quick method for the assessment of radical scavenging activity. In DPPH assay, the purple color of the DPPH solution becomes yellowish due to receiving hydrogen atoms or electrons from an antioxidant.

The DPPH radical scavenging activity of green tea, rose, and their infusions are depicted in Figure 1. As can be seen, various concentrations of rose (0.5, 1, and 2 g) increased the radical scavenging activity of green tea. The infusion of green tea with two grams of rose (GT2R2) showed the strongest radical scavenging effect (50.18%), while this rate was estimated at 19.38% in green tea alone (GT2), and the combination containing two grams of rose (R2) had 28.07% radical scavenging activity. In addition, the rate of radical scavenging activity was estimated at 8.30% and 16.35% in the infusions with one and 0.5 gram of rose (R0.5 and R1), respectively.

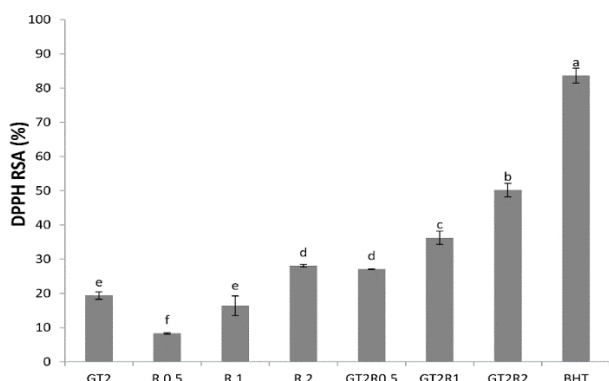


Fig. 1. DPPH Radical Scavenging Activity of Green Tea, *Rosa damascena*, and their infusions (Small letters show significant differences [$P < 0.05$] among the DPPH scavenging activity of the samples; BHT (2 mg/ml) as the positive control)

The DPPH radical scavenging activity of black tea, rose, and their infusions are illustrated in Figure 2. According to the findings, lower concentrations of *R. damascena* (0.5 and 1 g) enhanced the radical scavenging activity of black tea. On the other hand, the infusion of black tea with two grams of rose (BT2R2) showed the strongest radical scavenging effect (51.20%), while two grams of black tea alone (BT2) had a radical scavenging activity of 29.35%, and two grams of rose (R2) had a radical scavenging activity of 28.07%. Therefore, it could be inferred that the higher concentrations of *R. damascena* negatively influenced the radical scavenging activity of black tea.

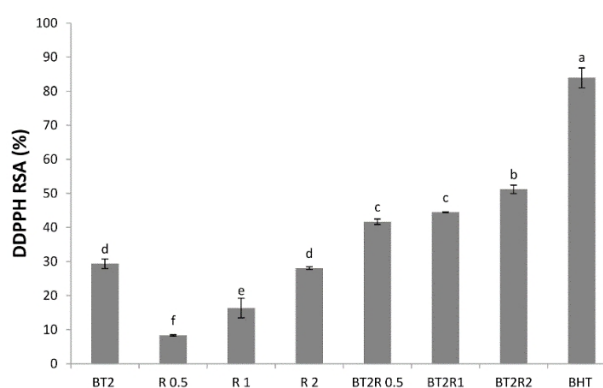


Fig. 2. DPPH Radical Scavenging Activity of Black Tea, *Rosa damascena*, and their infusions (Small letters show significant differences [$P < 0.05$] among the DPPH scavenging activity of the samples; BHT (2 mg/ml) as the positive control)

In the ABTS assay, the green-blue color of the ABTS solution became yellowish or colorless due to receiving hydrogen atoms or electrons from an antioxidant. The antioxidant activity of lipid- and water-soluble compounds could be measured using the ABTS assay.²⁶ Figure 3 shows the ABTS radical scavenging activity of green tea, *R. damascena*, and their infusions. Various concentrations of rose (0.5, 1, and 2 g) increased the radical scavenging activity of green tea.

According to the results, GT2R2 had the most significant radical scavenging effect (98.63%), while this rate was 60.47% in GT2 and 69.77% in R2. Moreover, lower concentrations of *R. damascena* (R0.5 and R1) had the radical scavenging activity of 7.16% and

24.75%, while GT2R0.5 and GT2R1 exhibited 78.77% and 91.77% of radical scavenging activity, respectively. These values were higher compared to the antioxidant activities of the combination of green tea and *R. damascena* (R0.5 and R1). Therefore, it could be concluded that the lower concentrations of *R. damascena* (0.5 and 1 g) exerted synergistic antioxidant effects on the radical scavenging activity of green tea.

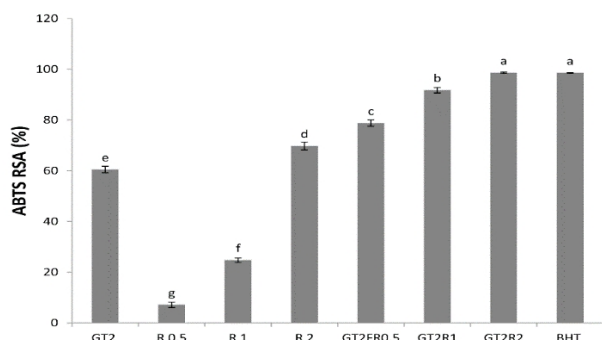


Fig. 3. ABTS Radical Scavenging Activity of Green Tea, *R. damascena*, and their infusions (Small letters show significant differences [$P < 0.05$] among the ABTS scavenging activity of the samples; BHT (2 mg/ml) as the positive control)

The ABTS radical scavenging activity of black tea, *R. damascena*, and their infusions are illustrated in Figure 4. In general, various concentrations of *R. damascena* (0.5, 1, and 2 g) enhanced the radical scavenging activity of black tea. In addition, BT2R2 showed the highest radical scavenging effect (97.38%), while this rate was estimated at 56.71% in BT2 and 69.77% in R2.

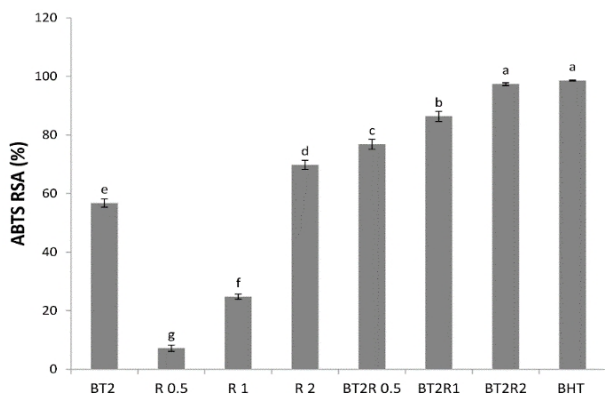


Fig. 4. ABTS Radical Scavenging Activity of Black Tea, *R. damascena*, and their infusions (Small letters show significant differences [$P < 0.05$] among the ABTS scavenging activity of the samples; BHT (2 mg/ml) as the positive control)

The present study aimed to assess the effects of dried rose petals on the antioxidant activities of green and black tea infusions. According to the findings, *R. damascena* increased the DPPH radical scavenging activity of green tea, while the higher concentrations decreased the radical scavenging activity of black tea. Moreover, *R. damascena* improved the ABTS radical scavenging activity of green and black tea.

Several studies have investigated the effects of various additives (e.g., ascorbic acid, bovine milk, soy milk, and sugar) on the antioxidant potential of tea. The findings have demonstrated that the addition of ascorbic acid could increase the total antioxidant activity of black and green tea.¹⁴ In a study, Ryan and Petit stated that the addition of various volumes of whole milk and semi-skimmed or skimmed bovine milk to tea infusions could reduce the antioxidant capacity of black tea.²¹

Another research in this regard confirmed that the addition of various volumes of soy milk could decrease the total antioxidant capacity of a black tea infusion.²³ In the mentioned study, the addition of milk and sugar was observed to reduce the DPPH radical scavenging activity of black tea.²² It has been argued that the addition of whey proteins could decrease the antioxidant activity of green tea.²⁷ Previous studies have also investigated the effects of various brewing methods on the antioxidant properties of green tea. According to the obtained results, water temperature has a significant effect on the extraction of antioxidant compounds, so that the efficiency was higher with the use of hot water extraction.²⁸

Although the antioxidant activity of rose flowers has been previously reported, there are no data on the effects of rose infusions on the antioxidant activity of tea. In a study in this regard, Sagdic *et al.* claimed that the fresh flower extract of *R. damascena* caused the antiradical activity of 75.51% at 100 ppm.²⁹ In another research,³⁰ the methanolic extract of fresh *R. damascena* flowers was associated with the antiradical activity of 89.44%.

In another study in this regard, the antioxidant capacity of the methanolic extracts

of three rose species (*R. damascena*, *R. bourboniana*, and *R. brunonii*) was compared using the DPPH assay. According to the findings, the extract of *R. brunonii* exhibited the maximum radical scavenging activity ($64.5 \pm 0.38\%$), followed by *R. bourboniana* ($51.8 \pm 0.46\%$) and *R. damascena* ($43.6 \pm 0.25\%$).¹⁹

Conclusion

The present study aimed to investigate the effects of dried rose petals (*R. damascena*) on the antioxidant capacity of green and black tea. According to the results, higher concentrations of *R. damascena* decreased the DPPH radical scavenging activity of black tea, while the lower concentrations exerted synergistic antioxidant effects on the ABTS radical scavenging activity of green tea.

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