



Comparative analysis of phenolic compounds in two samples of *Rosa damascena* by HPLC

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Abstract

Rosa damascena Mill. (Rosaceae) commonly known as Damask rose is widely grown in Kashan and Tabriz of Iran which has different therapeutic indications among Iranian traditional medicine practitioners. *R. damascena* is a rich source of phenolic compounds responsible for its medicinal properties. The objective of this study was to quantify the phenolic compounds present in *R. damascena* samples. The analytical separation and determination of phenolic compounds were performed using reversed phase HPLC with UV detector. Based on the results obtained throughout this study total phenolic and total flavonoid contents of sample from Tabriz (217.728 ± 0.13 and 22.8 ± 0.18 mg CE/g, respectively) were significantly higher than Kashan (134.568 ± 0.11 and 15.84 ± 0.23 mg CE/g respectively). Also phenolic compounds including gallic acid, syringic acid and quercetin were detected in both samples. The most abundant phenolic compound was gallic acid with amounts of 118.213 mg/g in Tabriz and 86.562 mg/g in Kashan samples ($p < 0.05$) but there was not any significant difference between two samples in the case of quercetin and syringic acid. The present study represents differences in total phenolics and gallic acid between two studied samples which might be implicated in different antioxidant activity and therapeutic applications of *R. damascena* from Tabriz and Kashan.

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Introduction

Phenolic compounds, the largest group of plant antioxidants have attracted growing interest due to their potential health benefits. Several traditional medicine suggestions and modern related studies have consistently revealed a significant positive association between intake of natural products containing phenolic antioxidants and reduced rates of disease (Baziar *et al.*, 2013). Phenolic antioxidants have an important role in protecting tissues against oxidative stress induced by reactive oxygen species (ROS). It has been proved that phenolic compounds can protect against diseases including cardiovascular and inflammatory disorders as well as the aging process (Farzaei *et al.*, 2014; Hajimahmoodi *et al.*, 2013).

Medicinal plants have gained considerable importance due to their valuable bioactive phenolic compounds. *Rosa damascena* Mill. (Rosaceae) as a well known plant has been used for a long time for its medicinal effects and aroma properties (Yassa *et al.*, 2009). In Iranian traditional medicine (ITM) literatures, *R. damascena* has been proposed to decrease dysfunction of several organs such as cardiovascular diseases, gastrointestinal tract, and liver (IbnSina, 2005). Many pharmacological effects have been reported from this plant including anti-inflammatory, hypnotic, anticonvulsant, anti-depressant, anti-anxiety, analgesic, antitussive, antidiabetic, relaxant effects on tracheal chains, laxative, prokinetic and hepatoprotective activities (Boskabady *et al.*, 2011).

Physiological functions of *R. damascena* may be partly associated to abundance of phenolics and hereby their antioxidant activity (Kumar *et al.*, 2009). Phytochemical studies have been indicated that *R. damascena* is a rich source of phenolic compounds responsible for its bitter taste and astringent trait (Kumar *et al.*, 2008; Kumar *et al.*, 2009; Haghi and Hatami, 2010). Several reports are available on the identification of phenolic acids and flavonoids by high performance liquid chromatography (HPLC) techniques on *R. damascena* (Velioglu and Mazza,

1991; Schiber *et al.*, 2005; Vinokur *et al.*, 2006; Kumar *et al.*, 2008; Haghi and Hatami, 2010; Baydar and Baydar, 2013). Velioglu and Mazza (1991) have reported several compounds in *R. damascena* petals such as phenolic acids, kaempferol, quercetin, anthocyanins, Galactoside and arabinoside (Velioglu and Mazza, 1991). Schiber *et al.* (2005) extracted flavonol glycosides from the residue of damask rose after industrial essential oil extraction and analyzed by HPLC. Among the 22 major components were analyzed, only kaempferol and quercetin glycoside compounds were identified (Schiber *et al.*, 2005). Haghi and Hatami (2010) have also reported the presence of myricetin, luteolin, quercetin, kaempferol, isorhamnetin, and chlorogenic acid in flowers of *R. damascena* by the simultaneous quantitation of flavonols, flavones, and phenolic acids via HPLC. However the study did not clarify the origin of *R. damascena* studied (Haghi and Hatami, 2010).

Two common medicinal types of *R. damascena* in herbal stores of Iran are populations of Kashan and Tabriz regions. These two types are different in therapeutic uses by Iranian traditional medicine practitioners which are needed to be investigated chemically. To the best of our knowledge no study has focused on the comparison of chemical composition of these two samples. Therefore the aim of the present study was to determine some phenolic compounds by HPLC method as well as the total phenolics and flavonoids contents in two samples of *R. damascena* from two different geographic regions of Iran.

Materials and methods

Chemical and reagents

Phenolic standards recommended for high performance liquid chromatography (HPLC) analysis of phenolics in plants. Gallic acid, caffeic acid, *p*-coumaric acid, syringic acid, ferulic acid, acetic acid, sodium bicarbonate, folin-ciocalteu reagent, ethanol, acetonitrile and methanol (HPLC gradient grade) were purchased from Merck (Darmstadt, Germany).

Plant material

Dried petals of *Rosa damascena* from two different origins (Tabriz and Kashan) were purchased from local herbal store of Tehran and authenticated by Professor G. Amin. (Department of pharmacognosy, Faculty of pharmacy, Tehran University of Medical Sciences). Voucher specimens (*Rosa damascena* Mill. [Rosaceae; No; PMP-507] have been deposited in the herbarium of faculty of pharmacy.

Preparation of the hydroalcoholic extract

The hydroalcoholic extracts of *R. damascena* (RDHE) petals from two samples were prepared with 70% ethanol at room temperature for 3 days. Each extract obtained was filtered and evaporated under vacuum (at 40°C) to yield residues about 19.65% and 20.44% on the basis of dried plant material, respectively for *R. damascena* from Kashan (k) and Tabriz (t).

Determination of total phenolics content

Total phenolics contents were determined by using Folin-Ciocalteu colorimetric method (Hajimahmoodi *et al.*, 2010). One milliliter prepared from each sample was mixed with Folin-Ciocalteu reagent (1.5mL) which previously diluted by distilled water (10-fold) and was kept at room temperature for 5 min. Then 1.5 ml of sodium bicarbonate solution (60 g/L) was added to the mixture and the absorbance was measured at 750nm by a UV-visible spectrophotometer (GBC, Cintra 40), after incubation for 90 min at room temperature. Total phenolics were quantified according to calibration curve obtained from measuring the absorbance of the known concentrations of gallic acid standard solutions. All tests were carried out in triplicate and the results were expressed as gallic acid equivalents (mg GAE/g dry extract) and reported as mean value $\pm$ SD.

Determination of total flavonoids

Total flavonoid content was determined by the aluminum chloride colorimetric method (Zhishen, 1999). In brief, each prepared sample (1mL) was added to volumetric flask containing distilled water (4mL). Then 0.3 mL NaNO₂ (5%) was added. After 5 min, 0.3 mL AlCl₃ (10%) and 6 min later, 2 mL NaOH (1 M) was added. The total volume was made up to 10

mL and the flask contents were mixed. The absorbance level was measured at 510 nm (GBC, Cintra 40). Total flavonoid contents were represented as mg catechin equivalents (CE) per one gram dry extract (mg CE/g dry extract) according to the catechin standard solutions and results reported as mean value $\pm$ SD.

High performance liquid chromatographic analysis

Each extract samples and reference standards were analyzed by a Knauer HPLC (Germany) system controlled by EuroChrom2000 software (Version 1.6, Knauer Co., Germany). The system consisted of a pump (Maxi-Star K-1000, Knauer, Germany), a degasser, an automated injector, a column oven, and a UV detector. Chromatographic conditions for determination of phenolic compounds in samples were evaluated and optimized in Eurospher-100 C18 column (5 μ m, 4.6 $\times$ 250 mm). Column temperature was maintained at 30°C. Mobile phase consisting of methanol (A) and acetic acid in water (B) (3:97 v/v), and the flow rate was set at 1 mL/min. the chromatographic detection was monitored at 280 nm. The injection volume for all samples was 10 μ L. Compounds were identified according to retention times as a comparison with the standards and also by analyzing spiked samples. The concentration of each compound was measured from peak area according to calibration curves of each corresponding standard. The amount of each compound was expressed as milligram per gram of dry extracts (mg/g).

Statistical analysis

Results were expressed as mean $\pm$ SD. The statistical difference between each two groups was calculated by using t-test. *P* values < 0.05 were considered significant.

Results

Total phenolic and flavonoids content

The results regarding the total phenolics and flavonoid contents from two *R. damascena* extracts are depicted in Table 1. This results expressed variability in the content of phenolic compounds and indicated that total phenolic and total flavonoid

contents of the RDHEt (217.728 ± 0.13 mg GAE/g and 22.8 ± 0.18 mg CE/g, respectively) were significantly higher than those of RDHEk (134.568 ± 0.11 and 15.84 ± 0.23 mg CE/g respectively).

Determination of phenolic compounds

The characterization of the phenolic compounds present in the studied samples was performed by HPLC analysis. Fig.1 indicates the chromatogram of phenolic compounds in RDHEt [a], RDHEk [b] and mixture of standards [c]. HPLC quantitative analysis

of phenolic compounds is represented in Table 2. Although gallic acid, syringic acid and quercetin have been found in the sample, ferulic acid, *p*-coumaric and caffeic acids were not detected. Compared to the other phenolic compounds, the most abundant phenolic compound in RDHEt and RDHEk was gallic acid with 118.213 and 86.562 mg/g amounts, respectively (Table 2). As can be seen in Table 2 the amount of quercetin and syringic acid were approximately the same in both extracts.

Table 1. The amount of total phenolics and total flavonoids in each extracts of *Rosa damascena* from of Tabriz (RDHEt), and from Kashan (RDHEk).

Plant extract	Total phenolics (a)	Total flavonoids (b)
RDHEt	$217.728 \pm 0.13^*$	$22.800 \pm 0.18^*$
RDHEk	134.568 ± 0.11	15.840 ± 0.23

Each value represents the mean $\pm$ SD (n = 3)

(a): Total phenol content was expressed as mg Gallic acid equivalent/g dried extract (mgGAE/g)

(b): Total flavonoid content was expressed as mg Catechin equivalent/g dried extract (mg CE/g)

*Values are significantly different (t-test, *p* value <0.05).

Table 2. Qualitative analyses of the *R. damascena* hydroalcoholic extracts [of Tabriz (RDHEt) and Kashan (RDHEk)] by HPLC.

Plant extract	Phenolic compounds					
	Gallic acid	Ferulic acid	<i>p</i> -coumaric acid	Syringic acid	Caffeic acid	Quercetin
RDHEt	$118.213 \pm 0.021^*$	ND	ND	3.48 ± 0.019	ND	12.86 ± 0.31
RDHEk	86.562 ± 0.016	ND	ND	3.45 ± 0.027	ND	12.82 ± 0.1

Each value represents the mean $\pm$ SD (n = 3). The amount of each phenolic compound was expressed as mg/g.

ND: not detected.

*Values are significantly different (t-test, *p* value <0.05).

Discussion

Phenolic constituents have been shown to possess major contribution to the antioxidant activity in medicinal plants (Abbasian *et al.*, 2013). Phenolic acids and flavonoids are the major class of phenolics, widely distributed in plant kingdom (Cai *et al.*, 2004). Plant parts rich in phenolics as food or medicine are progressively of interest in the fields of health and medicine, due to their potential to retard lipid peroxidation in viable cells and also to prevent oxidative injuries (Kim *et al.*, 2003). Several lines of evidences indicate the antioxidant potential of *R. damascena* which is related to its phenolic contents

(Achuthan *et al.*, 2003; Özkan *et al.*, 2004; Shahriari *et al.*, 2007; Baydar and Baydar, 2013). It is worthy to note that total phenolic content of *R. damascena* has been variously determined in different extracts which were mostly prepared by methanol (Özkan *et al.*, 2004; Naithani *et al.*, 2006; Kumar *et al.*, 2008; Sharafi *et al.*, 2010; Moein *et al.*, 2012; Abdel-Hameed *et al.*, 2012; Baydar and Baydar, 2013; Ginova *et al.*, 2013). As it has been shown in this study, total phenolics and flavonoids content in hydroalcoholic extract of *R. damascena* from Tabriz was significantly higher than Kashan sample which it might lead to more potent antioxidant activity of *R.*

damascena of Tabriz. Moreover it could be used for more therapeutic indications in ITM. According to substantial difference in phenolic compounds contents, it can be assumed that different levels of total phenolics in these two samples are attributed to gallic acid amount. Additionally the amount of gallic acid in RDHEt and RDHEk was about 54% and 64% of total phenol content, respectively. Vinokur *et al.* (2006) has previously found that the levels of gallic acid in some samples of teas from rose petals are 35–55% of the total phenol content (Vinokur *et al.*, 2006).

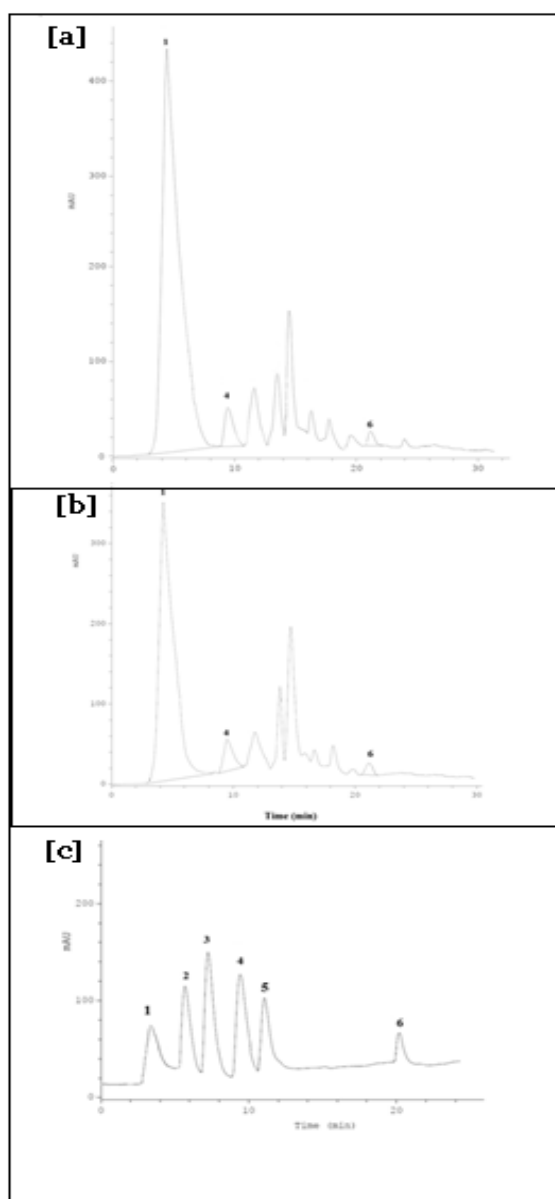


Fig. 1. HPLC chromatogram of RDHEt [a], RDHEk [b] and standard phenolic compounds mixture [c]: 1: Gallic acid, 2: Caffeic acid, 3: *p*-Cumarcic acid, 4: Syringic acid, 5: Ferulic acid, 6: Quercetin.

The main phenolics responsible for antioxidant properties of *R. damascena* are gallic acid and quercetin (Yassa *et al.*, 2009; Haghi and Hatami, 2010). In our study, no significant difference was demonstrated between quercetin amounts in RDHEt and RDHEk. Quercetin was reported 11.10 ± 0.31 mg/g after *R. damascena* petals extraction and hydrolysis of flavonoids (Haghi and Hatami, 2010). The Quercetin amount was 0.189 mg/g by using HPLC in samples prepared from *R. damascena* distilled petals (Schiber *et al.*, 2005). Abdel-Hameed *et al* (2012) identified quercetin in *R. damascena* methanolic extract and ethyl acetate fraction (7.75 mg/g, and 65.27 mg/g respectively) while gallic acid was reported as trace in methanolic extract and 10.01 mg/g in ethyl acetate fraction (Abdel-Hameed *et al.*, 2012). Quercetin and syringic acid were detected in Baydar study in the methanolic extract of fresh and spent (distillation residues) rose flower while they reported low amounts of gallic acid ranged from 1.91 to 28.18 mg/g (Baydar and Baydar, 2013). In this investigation, gallic acid amounts in RDHEt and RDHEk were 118.213 and 86.562 mg/g, respectively and amounts of syringic acid were similar in both samples. High content of phenolic compounds, in particular free gallic acid mostly causes the antioxidant property in rose petal (Vinokur *et al.*, 2006). The differences among the rose species regarding the phenolics compounds could be due to genetic derivation. It was reported that the plant genotype, cultivation site and technique affect the total phenolic content.

This investigation further supports the view that studied *R. damascena* samples are promising sources of natural antioxidants as common samples used by traditional medicine practitioners in Iran but additional studies are needed to characterize the active compounds and biological activities of this active plant extracts so that they may be included more in nutraceutical formulations.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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