# Chemical Composition and Antioxidant Activity of the Extract and Essential oil of *Rosa damascena* from Iran, Population of Guilan

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**ABSTRACT**

*Background and the purpose of study*: *Rosa damascena* Mill. (Rosaceae) has cooling, soothing, astringent, and anti-inflammatory effects, and has been used in the north of Iran as a cardiotonic agent. The aim of this study was to identify components of *R. damascena* (cultivated in Guilan Province) extract and essential oil and to study their biological activities.

*Methods:* Essential oil of *R. damascena* was prepared by hydrodistillation and analyzed with GC/MS instrument. The antioxidant activity of hydro-alcohlic extract of petals and essential oil was measured using free radical scavenging activity with 2-2-diphenyl, 1-picrylhydrazyl (DPPH) and lipid peroxidation (ferric ammonium thiocyanate) methods.

*Results:* Hydro-alcoholic extract showed strong free radical scavenging capacity compared to lipid peroxidation inhibitory effects. $IC_{50}$ values of the extract were 2.24 µg/mL and 520 µg/mL in free radical scavenging and lipid peroxidation assays, respectively. The major components of essential oil were linalool (3.8%), nerol (3.05%), geraniol (15.05%), 1-nonadecene (18.56%), n-tricosane (16.68%), hexatriacontane (24.6%) and n-pentacosane (3.37%). The bioassay-guided fractionation of extract led to the isolation of three flavonol glycosides: quercetin-3-O-glucoside, kaempferol-3-O-rhamnoside and kaempferol-3-O-arabinoside. The $IC_{50}$ value of the radical scavenging activity of kaempferol-3-O-rhamnoside which was, 530 µg/mL was weaker than the extract.

*Major conclusion*: The petal of this cultivated rose has no bitter taste and because of its potential antioxidant activity and good taste, can be used as food flavor and a preventing agent for many diseases.

**Keywords:** *Rosa damascena*, Flavonoids, Antioxidant, Essential oil

## INTRODUCTION

*Rosa damascena* Mill. (Rosaceae) is a small plant with aromatic, light pink flower which appears in spring (1). At present, *R. damascena* is the principle species cultivated for rose attar not only in Bulgaria but also in Iran and India (2). This plant is cultivated originally in Kashan Province (central part of Iran) for preparing rose water and attar. *R. damascena* and *R. gallica* var. *officinalis* Thory were widely grown in medieval times for medicinal purposes (3). *R. damascena* also has some other benefits such as cooling, soothing, astringent, and anti-inflammatory effects (4), especially it has been used in north of Iran as a cardiotonic agent. Considerable interest has developed recently concerning the alternative utilization of aromatic plants rich in essential oils as antioxidant and antibacterial agents in the food industry (5). On the basis of an investigation on *R. damascena* from Kashan Province, by GC-MS β-citronellol (14.5-47.5%), nonadecane (10.5-40.5%), geraniol (5.5-18%) and henicosane (7-14%) were the major components of the oil (6). It has been reported that the main rose oil and its components (citronellol, geraniol and nerol) have strong antimicrobial activity against some bacteria (7-9). In a study by combined use of HPLC, paper chromatography and spectral analysis 285 mg/kg total anthocyanins were isolated from fresh petals. The main anthocyanin was characterized as cyanidin 3, 5-diglucoside and identified compounds were several kaempferol and quercetin glucosides, galactoside, arabinosides, and rhamnosides (10). In another investigation hot water infusions (teas) of air- dried petals of twelve rose cultivars were assayed for antioxidant activity, total phenols, and total anthocyanin contents. The range of total phenols content in rose teas was 50.7 to 119.5 mg gallic acid equivalents (GAE) per gram of dry matter, as compared to 62.1 mg GAE/g dry weight in the green tea. No clear relationship between anthocyanin level and radical-scavenging activity

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was revealed (11). In a survey of flower flavonoids in 120 taxa from 10 sections of subgenus *Rosa*, 19 flavonols and six anthocyanins; six kaempferol glycosides, six quercetin glycosides, seven unidentified flavonols, two cyanidin, two peonidin glycosides and two unidentified anthocyanins were detected. (12). There are several reports antioxidant and antimicrobial properties of *Rosa* species (9, 11, 13, 14). The flower of *R. damascena* in Guilan has pleasant smell and taste but it is not used for preparation of Rose water and according to reports of folk uses, its petal has no laxative property (15). This differences between rose of Guilan and other origin sites especially Kashan, may be due to genetic and environmental factors, and there was not any report from essential oil and secondary metabolites of cultivated *Rosa* in Guilan. The aim of this study was to identify components of *R. damascena* extract and essential oil and to determine their biological activity.

## MATERIALS AND METHODS

*Plant material*

*Rosa damascena* was collected from Rasht (Guilan Province) in May 2004. This species was confirmed by Dr. V. Mozaffarian (Dept. of Botany, Research Institute of Forests and Rangelands, Tehran, Iran) and deposited in herbarium of Pharmacognosy (Voucher No. 6555 TEH), Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran.

*Extraction*

Fresh petals (400 g) were extracted with methanol and water (80:20).The solvent was evaporated under low temperature and low pressure to give gummy residue (crude extract).

*Isolation of the essential oil*

The fresh petals of *R. damascena* (500 g) were subjected to hydrodistillation using a Clevenger-type apparatus for 4 hrs and the distillate was dried over anhydrous sodium sulfate and stored at 4 °C in a sealed vial for analysis. Hydrodistillation of petals yielded 0.20% (v/ w) of essential oil. The yield was based on dry materials of petals.

*GC/MS analysis*

The oil was analyzed by GC/MS using a Hewlett Packard 5973 mass selective detector connected to a HP 6890 gas Chromatograph. The separation was achieved by capillary column, HP-5MS (5% phenyl methyl polysiloxane) (30 m × 0.25 mm, film thickness 0.25 µm). The column temperature was kept at 60°C for 5 min and programmed to 220°C at a rate of 6°C/min. The flow rate of helium (the carrier gas) was 1 mL/min and MS was taken at 70 eV. The relative percentage of the oil constituents was expressed as percentages by peak area normalization. The identification of individual compounds was based on comparison of their relative retention indices with those of authentic samples on the HP-5MS capillary column and by matching of their mass spectra with those obtained from Wiley libraries and published data (16-18).

*Inhibition of lipid peroxidation*

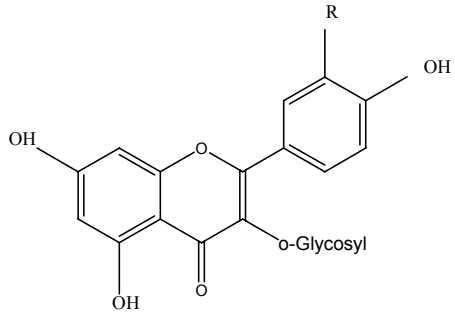
The ferric ammonium thiocyanate method was used for determination of antioxidant activity of petal extract (19, 20). One mL of different concentrations of crude extract in methanol (300, 225 and 150 µg/mL) was mixed with an emulsion of 2.51% linoleic acid in absolute ethanol (0.5 mL) and 0.05 M sodium hydrogen phosphate buffer, pH=7 (1 mL) in a screw capped tube and incubated in an oven at 40°C in the dark. The same mixture without sample extract was used as the control. During incubation (every 24 hrs), 0.1 ml of each tube was diluted with 4.7 mL of 75% ethanol and 0.05 mL of 30% ammonium thiocyanate. Precisely 3 min after addition of 0.05 mL of 0.02 M ferrous chloride in 3.5% hydrochloric acid to the reaction mixture, the absorbance was measured against blank (75% ethanol) at 500 nm every 24 hrs for 96 hrs. The degree of linoleic acid peroxidation was calculated during 96 hrs using the following formula:

AA% =100-[(absorbance of sample t /absorbance of control t) ×100]

Vitamin E and BHT were included as natural and synthetic antioxidant standards respectively for comparison.

*Free radical scavenging*

The DPPH method (21) was used with some




| Compound | R | -o-Glycosyl |
|---|---|---|
| **C1** | OH | glucose |
| **C2** | H | rhamnose |
| **C3** | H | arabinose |

**Figure 1.** Structures of **C1**: quercetine-3-o-glucoside, **C2**: kaempferol-3-o-rhamnoside and **C3**: kaempferol-3-o-arabinoside

**Table 1.** $^{1}H$ and $^{13}C$-NMR (DMSO $d_6$ + $CDCl_3$) data of compounds **C1-3** (400MHz for H and 100 MHz for C; δ ppm)[a]

| *positions* | *C1 (J)* | $^{13}C$ | *C2, C3 ( J)* | $^{13}C$ |
|---|---|---|---|---|
| 2 | - | 149.00 | - | 146.90 |
| 3 | - | 136.19 | - | 135.74 |
| 4 | - | 176.19 | - | 175.84 |
| 5 | - | 160.93 | - | 160.72 |
| 6 | 6.25, *d*, (2 Hz ) | 098.05 | 6.29, *d*, (2 Hz ) | 098.18 |
| 7 | - | 163.67 | - | 163.89 |
| 8 | 6.40, *d*, (2 Hz ) | 093.37 | 6.39, *d*, (2 Hz ) | 093.35 |
| 9 | - | 156.75 | - | 156.13 |
| 10 | - | 102.56 | - | 103.01 |
| 1' | - | 131.72 | - | 121.95 |
| 2' | 7.78, *d*, (2 Hz ) | 129.52 | 8.06, *d*, (8.4 Hz ) | 115.06 |
| 3' | - | 115.45 | 7.06, *d*, (8.4 Hz ) | 145.05 |
| 4' | - | 160.44 | - | 147.70 |
| 5' | 6.94, *d*, (8.4 Hz ) | 115.45 | 7.06, *d*, (8.4 Hz ) | 115.60 |
| 6' | 7.64, *dd*, (8.4, 2 Hz ) | 129.52 | 8.06, *d*, (8.4 Hz ) | 119.97 |

[a] $C_5$-OH appeared at 12.35, 12.30 ppm for **C1** and **C2, C3** respectively. Other OH signals appeared between 8.5 -10.5 ppm.

modification in our laboratory. Different samples from hydro-alcoholic extract (90, 60, 30, 3 µg/mL) and essential oil (10, 5, 1 µl/mL) in methanol were added to 2 ml of DPPH solution ($4\times10^{-5}$ g/mL MeOH). Control consisted of sample which was added to methanol up to 3 mL; Blank consisted of 1 mL methanol which was added to 2 mL of DPPH solution. The absorbance of samples was measured at 517 nm after 30 min. The percentage of inhibition activity was calculated as follows:

Inhibition% = 100 - [(Sample absorption - control absorption)/Blank absorption] ×100.

*Statistical analyses*

Analyses of at least three samples were carried out in triplicates. Student's *t*-test was carried out to compare the data and results were considered statistically significant at $P<0.05$. $IC_{50}$ was calculated with Curve Expert 1.3.

*Isolation procedure*

Extract (3 g) was submitted to paper chromatography. The chromatograms were developed descending in the long direction of Whatman No. 3 chromatographic papers in the chromatocab using 15% acetic acid as eluent for 5 hrs (22) to obtain four fractions (A, B, C and D).

For further purification, fraction **C** was subjected to paper chromatography with TBA (t-butyl alcohol: acetic acid and water; 3:1:1) as eluent (22) to separate compounds C**1, C2, C3** (16 mg, Rf= 0.57; 54 mg, Rf= 0.71; 12 mg, Rf= 0.48 respectively). Sephadex $LH_{20}$ was used for the last purification of **C1**, **C2** and **C3.**

*Separation of aglycon and identification of sugars*

About 10 mg of each compound was hydrolyzed with 2N HCl and solution was extracted with diethyl ether (aglycon Portion). Aqueous layer was chromatographed on Whatman No. 3 paper chromatography, alongside with some of the more common sugars such as glucose, rhamnose, xylose, arabinose and galactose. The sugars were identified according by literature methods (23).

*Identification of aglycons*

Comounds **C1 (***Quercetin aglycon)*, **C2** and **C3** (*Kaempferol aglycon)* were identified using spectroscopic methods and comparison with authentic samples. $^{1}H$ and $^{13}C$-NMR data are in Table 1.

*Free radical scavenging activity of kaempferol-3-O-rhamnoside (*C2*)*

Among isolated flavonoids, since only the amount of kaempferol 3-O-rhamnoside was enough, its antioxidant activity was determined by DPPH method.

## RESULTS AND DISCUSSION

Fresh petals of *R. damascena* were collected from north of Iran (Guilan Province). Its essential oil was prepared by hydrodistillation and was analyzed with GC/MS. Seventeen out of twenty one compounds were identified (97.42% v/w). The oil of *R. damascena* was rich of nonterpenoid hydrocarbons comprising 73.38% of the oil. There were not sesquiterpene compounds in the oil. Linalool (3.68%), nerol (3.05%), geraniol (15.5%),

**Table 2.** Eessential oil composition of *R. damascena* from Iran Population of Guilan.

| ***Compounds Name*** | ***Percent*** | ***[a]RRI*** | ***[b]KI*** | Method of identification |
|---|---|---|---|---|
| β-myrcene | 0.13 | 990 | 991 | MS-RRI |
| linalool | 3.68 | 1092 | 1097 | MS-RRI |
| phenylethyl alcohol | 0.86 | 1106 | 1110 | MS-RRI |
| rose oxide | 0.1 | 1125 | 1127 | MS-RRI |
| nerol | 3.05 | 1234 | 1230 | MS-RRI |
| geraniol | 15.5 | 1254 | 1253 | MS-RRI |
| linalyl acetate | 0.3 | 1260 | 1257 | MS-RRI |
| eugenol | 0.18 | 1365 | 1359 | MS-RRI |
| geranyl acetate | 0.24 | 1386 | 1381 | MS-RRI |
| n-heptadecane | 2.84 | 1732 | 1700 | MS-RRI |
| 1-nonadecene | 18.56 | 1445 | - | MS |
| n-octadecane | 2.8 | 1822 | 1800 | MS-RRI |
| n-eicosane | 0.32 | 1980 | 2000 | MS-RRI |
| n-heptacosane | 2.47 | 2110 | 2100 | MS-RRI |
| n-tricosane | 16.68 | 2326 | 2300 | MS-RRI |
| n-pentacosane | 5.11 | 2545 | 2500 | MS-RRI |
| n-hexatriacontane | 24.6 | 3552 | 3600 | MS-RRI |
| Monoterpen hydrocarbons | 0.13 | | | |
| Monoterpen oxygenated | 22.87 | | | |
| Sesquiterpen hydrocarbons | 0.00 | | | |
| Sesquiterpen oxygenated | 0.00 | | | |
| Phenylpropanoids | 0.18 | | | |
| Nonterpenoids | 73.38 | | | |
| Unknown | 3.44 | | | |
| Total identified | 97.42 | | | |

a: Relative Retention Indices as determined on a HP-5MS column using the homologous of n-alkanes. b: Kovats Indices

1-nonadecene (18.56%), n-tricosane (16.68%), n-pentacosane (5.11%), n-hexatriacontane (24.6%) were the major components of essential oil (Table 2). The oil contained Phenylethyl alcohol (0.86%), rose oxide (0.1%), linalyl acetate (0.3%), eugenol (0.18%) and geranyl acetate (0.24%) that could be observed in essential oils of *R. damascena* from other regions (24 -26). Result of investigation on *R. damascena* essential oil from Kashan showed some similarity with oil of *the R. damascena* from Guilan, but there were some variations in amount of their components (6).

The components of petal extracts of the other *Rosa* species (e.g., *R. persica*, *R. damascena*, *R. chinensis* and *R. rugosa*) are closely related and also demonstrated good antioxidant activity (27). There is a report describing that forty landraces of Damask rose were collected from 28 provinces in Iran (13 origin sites Os1- Os13) and evaluated to determine the diversity among them. The cluster analysis confirmed the distinctiveness of the Os12 which included Guilan, Mazandaran, Golestan provinces with a specific climate condition of humid temperate from other origin sites as a whole. This distinctiveness was already confirmed by the bi-plot of the principal components (28). The results of mentioned report may confirm different properties of cultivated rose in Guilan (North Iran) and other regions of Iran.

In this investigation the rose petals were extracted with 80% methanol and evaporated to dryness under vacuum (45g). Since most investigations show that *Rosa* species have good antioxidant activity, the extract was subjected to evaluation for antioxidant activity using 2-2-diphenyl-1-picrylhydrazyl (DPPH) for measurement of free radical scavenging activity and with ferric ammonium thiocyanate method for evaluation of lipid peroxidation properties. The $IC_{50}$ of the extract with DPPH method and FTC method are reported in Table 3. Based on the statistical analyses, there were significant differences ($P<$ 0.02) between results of extract, vitamin E and BHT were; the results showed that the extract has powerful free radical scavenging activity compared

**Table 3.** $IC_{50}$ of Samples from *R.damascena* with DPPH and FTC methods.

| *Samples* | *FTC/72 h µg/mL*[a] | *DPPH/30 min µg/mL*[a] |
|---|---|---|
| Extract | 520 ± 9.68 | 2.24 ± 0.98 |
| essential oil | ND | 3.54 µL ± 1.22 |
| kaempferol 3-O-rhamnoside | ND | 531 ± 11.02 |
| BHT | 153.9 ± 7.24 | 110.98 ± 8.25 |
| Vitamine E | 20.98 ± 2.98 | 22.72 ± 1.91 |

[a]Values are mean ± SD (n=3). ND: not determined

to standards. Therefore the extract was fractionated by paper chromatography and isolated substances were purified by Sephadex LH20 to obtain three compounds (**C1, C2, C3**). Isolated compounds were identified by UV, MS and NMR instruments. All these components were glycosides and the results obtained from acidic hydrolysis showed the presence of glucose, rhamnose, and arabinose as the sugar part of each compound respectively. The spectral data of compounds showed good agreement with those of literature data (29, 30) and confirm the structure of compound **C1** as: 3, 5, 7, 3′, 4′-tetrahydroxy flavone glycosylated at position 3 with glucose and the structure of compounds **C2** and **C3** as: 3, 5, 7, 4′-trihydroxy flavone glycosylated at position 3 with rhamnose and arabinose respectively. The three compounds have been reported previously from *R. damascena* and some species of other origin (10, 12, 31). Additionally the antioxidant activity of the essential oil and kaempferol 3-O-rhamnoside were determined by DPPH procedure. The results showed radical scavenging effect of essential oil was stronger than BHA and vitamin E ($P< 0.03$) with IC50= 3.54 µL ± 1.22. $IC_{50}$ of kaempferol 3-O-rhamnoside in comparison to $IC_{50}$ of extract showed weak antioxidant activity. The potential antioxidant activity of extract could be due to existence of quercetin 3-O-glucoside and other flavonoids in extract with more hydroxyl groups in B ring (32).

In conclusion the petal of *Rosa damascena* from Gilan Province has gentle smell without bitter taste, and powerful antioxidant activity. Therefore it can be used as a food flavor, natural antioxidant and a preventing agent for many diseases which are caused by free radicals.

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