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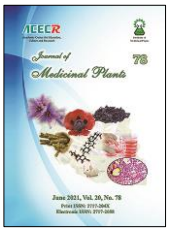

Research Article

Quality control of *Rosa* × *damascena* flowers from Layzangan of Fars province in Iran

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ABSTRACT

Background: Damask rose (DR) with the scientific name of *Rosa* × *damascena* Herrm. is a popular medicinal plant belonging to the Rosaceae family. According to the literature, DR cultivation was originally developed in the Layzangan valley, Darab region of Fars province in Iran. **Objective:** In this study, quality control and standardization of Layazangan DR flowers were investigated. **Methods:** Histological, physicochemical, and heavy metal analysis as well as the chromatographic fingerprints including GC-MS and HPLC analysis were studied. **Results:** According to the microscopic observations, secretory and simple trichromes in sepal, rosette crystals in ovule, and the tricolporate shape of pollen grain were found to be the major characteristics in DR flowers. The results of heavy metal analysis revealed that the amounts of zinc (82.5 ppm), copper (4.02 ppm), lead (0.11 ppm), and cadmium (not detected) were less than maximum permitted amounts. The essential oil of fresh petals was obtained by hydro-distillation and was analyzed by GC-MS method. Twenty-three components were identified representing 98.66 % of the total essential oil composition. Citronellol (41.44 %), nonadecane (16.44 %), and heneicosane (10.58 %) were the main components. According to HPLC analysis results, quercetin was determined as 7300.5 µg/g dried DR petals. **Conclusion:** The essential oil obtained from Layzangan DR is rich in citronellol compound. On the other hand, the diagnostic characteristics presented in this study can help in better quality control and standardization of DR samples. It could also provide information for the authentication of relevant undeclared samples as well as the detection of adulterated materials.

Abbreviations: DR, Damask rose; GC-MS, Gas chromatography-mass spectrometery; HPLC, High performance liquid chromatography; LOQ, Limits of quantitation; LOD, Limits of detection

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1. Introduction

Rosa × *damascena* Herrm. commonly known as damask rose (DR) from the Rosaceae family is a famous medicinal plant which is a hybrid derived from *Rosa gallica* L. and *Rosa moschata* Herrm. [1,2]. DR is a thorny shrub ranged from 90-180 cm in height. Its beautiful flowers have pleasant fragrance and the color of petals is ranged from light red to white. The flower is made up of around 30-40 petals and it has long erect sepals [3]. DR is commonly known as gol-e-mohammadi or gol-e-sorkh in Persian, ward-e-ahmar in Arabic, damascus rose or otto rose in English, and golab in Hindi [4]. It is widely cultivated in India, China, Libya, Morocco, South Italy, South France, South Russia, and Ukraine, but it is found especially in Bulgaria, Turkey and Iran [5]. It is considered that cultivation of *R. damascena* originally developed in the Layzangan valley of Fars in Iran [6].

Several groups of chemical compounds are isolated from DR flowers. Essential oil of DR is a valuable and expensive volatile oil obtained by distillation of its fresh flowers. Kovats has identified 127 chemical constituents in the essential oil of damask rose [7]. The oil is semisolid at ordinary temperatures because of C_{14} to C_{23} normal paraffin content [8]. The major components of rose essential oil are reported as geraniol, citronellol, nerol, *β*-phenethyl alcohol, geranic acid, and eugenol which are found in both free and bound forms. Also, other components such as terpene hydrocarbons, esters, C_{14} to C_{23} normal paraffin, and nerol oxide are detected in rose essential oil [4,9]. On the other hand, the flowers from different regions may have different compositions. For example, major compounds identified from essential oil of Kashan flowers are *β*-citronellol, nonadecane, geraniol, nerol and kaempferol [10], while major compounds of Kamfiroz flowers are nonadecane, heneicosane, docosane, citronellol and 9-nonadecene according to previous reports [8]. Flavonoids are other group of active components in DR flowers. Several kaempferol and quercetin glucosides, galactoside, arabinosides, and rhamnosides were identified in petals. Also, anthocyanins such as cyanidin 3,5-diglucoside (over 95 % of the total anthocyanins) were reported from the DR petals in previous studies [11]. In an intensive survey on flavonoids in the flowers of 120 taxa from 10 sections of subgenus Rosa, 19 flavonols and 6 anthocyanins including 6 kaempferol glycosides, 6 quercetin glycosides, 7 unidentified flavonols, 2 cyanidin, 2 peonidin glycosides, and 2 unidentified anthocyanins were detected [12]. Flowers also contain tanning matter, fatty oil, organic acids, and a bitter principle [10].

DR is widely used in traditional Persian medicine preparations. Indeed, it has been a popular medicinal plant through the history (Fig. 1) [2]. The expansion of using DR in modern commercialized products reveals the necessity of identification and standardization of this popular flower. Furthermore, determination of the content of heavy metals in medicinal plants growing in nature is also considered to be an important issue [14]. A literature survey revealed that DR has no monograph in the Iran herbal pharmacopoeia. Also, no previous attempts for the quality control and standardization of Layzangan DR was done. The present study provides information on various parameters including histological characteristics, physicochemical properties, chromatographic profiles, and standardization of the petals.

Fig. 1. Illustration of damask rose in Dioscorides Alhashayesh book: "Der Wiener Dioskurides: Codex medicus Graecus" manuscript [13]

2. Materials and methods

2.1. Plant materials and chemicals

DR was purchased from Layzangan valley (Darab, Fars, Iran). It was identified as *Rosa damascena* Herrm. by an expert botanist in Shiraz School of pharmacy. The sample was deposited in the Herbarium Center of Shiraz Traditional Pharmacy Department (Shiraz School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran) under the voucher number of 783, and the plant material code of PM 1157. Furthermore, all the chemicals, reagents, and solvents were purchased from Sigma-Aldrich, Inc.

2.2. Histological and physicochemical analysis

In order to carry out the histological analysis of flowers, the microscopic transection of ovary, petal, sepal, and pollen grain were studied. The sections were cut manually and were simple and double stained (by methylene blue 0.5 mg/ml and Congo red 10 mg/ml) [15]. Light microscopy was performed by a Canon microscope and images were taken by Canon camera under bright field light. On the other hand, the physicochemical properties including total ash, water soluble ash, acid-insoluble ash, sulfated ash, and loss on drying were determined as previously described [16].

2.3. Analysis of heavy metals

Heavy metal content (lead, cadmium, zinc, and copper) in the petals were measured by a polarographic processor model 746 VA combined with a polarographic stand model 747 VA according to Metrohm method described by Sobhanardakani in 2011. Firstly, 1.5 g powder was weighed and oven-dried at 70 °C for 3 hours until the weight of sample was fixed. Secondly, 250 mg of the sample was put in an Erlenmeyer flask and 4 ml HNO_3, 0.5 ml $HClO_4$, 0.1 ml H_2SO_4, and 1 ml H_2O_2 30 % were added, respectively. Then, the Erlenmeyer was transferred to a heater at 200 °C until the mixture was dried. In the next step, 1 ml H_2O_2 30% was added and the mixture was heated to be dried (this step was repeated twice). Then 10 ml of

distilled water was added and the mixture was stirred with the magnet for 5 minutes. Finally, the mixture was passed through a filter paper and the obtained clear liquid was collected in the test tube [17].

2.4. Gas Chromatography-Mass Spectrometry (GC-MS)

The essential oil of fresh petals (500 g) was obtained with 2700 ml water in Clevenger apparatus. The procedure was done during 4.5 hours. Then the acquired essential oil was subjected to GC-MS for analysis of the ingredients, using Agilent Technologies 7890 linked with mass specific detector (Agilent 7000 Series Triple Quadrupole Mass detector). The device was equipped with a fused silica capillary column (Agilent DB-1 capillary; 30 m × 0.25 mm i.d. and film thickness 0.25 µm). Samples were injected in the split mode with a split ratio of 1:30. Helium was as the carrier gas (at 1.2 mL/min flow rate). Oven temperature program was adjusted to be increased from 70 °C (0 min) to 280 °C in increments of 3 °C/min and subsequently held for 4 min. Mass spectrometer regulated in EI mode (70eV) and 30-600 m/z mass range with 250 °C interface temperature. The Constituents of sample was identified by determination of its relative retention indices with reference to the injected normal alkanes (C_9-C_{30}). To confirm the identification, the derived mass spectra and calculated retention indices for each compound were compared to those of the internal reference libraries, NIST Database, Wiley (275), and Adams libraries spectra. Retention indices were determined relative to retention times of a series (C_9-C_{30}) of *n*-alkanes [18, 19].

2.5. High Performance Liquid Chromatography (HPLC)

HPLC analysis was carried out on a Knauer technologies model apparatus attached to Eurospher 100-5 C18 column (250 × 4.6 mm with precolumn) and connected to a photodiode array (PDA) detector. Mobile phase: [0-20 min] gradient of (15 %-35 %), [20-25 min] 35 % and [25-30 min] gradient of 35-15 % acetonitrile in water adjusted to pH 3 with phosphoric acid [20]. HPLC analysis was performed by gradient elution with flow rate 1 ml/min. All solvents were filtered through a 0.45 mm Millipore filter before use and degassed in an ultrasonic bath. Quantification was effected by measuring at the 365 nm. The chromatographic run time was 30 minutes.

2.5.1. Standard Working Solution and Calibration Curve

Quercetin (2 mg) was accurately weighed into a 2 ml volumetric flask and dissolved in methanol up to 2 ml. Different concentrations were made by serial dilution of the main sample. Triplicate 20 µl injections were made for each standard solution to see the reproducibility of the detector response at each concentration level. The peak area of each solution was plotted against the concentration to obtain the calibration graph. The five concentrations of the compound (25, 50, 75, 250, 500 µg/ml for quercetin) were subjected to regression analysis to calculate calibration equation and correlation coefficients.

2.5.2. Sample Preparation

Sample of damask dose petals: One g of air dried petal powder was extracted with 50 ml methanol and it was heated for 30 min at water

bath. Two ml of this solution was dissolved in 2 ml of 1.1 M HCl-methanol (60:40 v/v) mixture and heated in a water bath for 30 min, dried in a desiccator, dissolved in 2ml methanol and filtered. Then it was extracted via methanol (1:1 volumes) in decanting apparatus with 3 times extraction [21].

3. Results

3.1. Histological and physicochemical analysis

The results of microscopic transection of DR are demonstrated in Fig. 2. The secretory and simple trichomes in sepal, rosette crystals in ovule, and the tricolporate shape of pollen grain were found to be the major characteristics in DR flowers. On the other hand, the results of physicochemical analysis including the total ash (4 %), water soluble ash (2.5 %), acid-insoluble ash (9 %), sulfated ash (6 %), and loss on drying (3.2 %) of Layzangan DR flowers were determined.

3.2. Analysis of heavy metals

The heavy metals (zinc, copper, lead, and cadmium) contamination of DR petals was analyzed. The results revealed 82.5 ppm zinc, 4.02 ppm copper, 0.11 ppm lead, and 0 ppm cadmium in the petals of Layzangan DR petals.

3.3. Gas chromatography-mass spectrometry (GC-MS)

The hydro-distillation essential oil composition of DR petals was identified by GC-MS method (Fig. 3).

Twenty-three components were identified representing 98.66 % of the total essential oil composition of fresh DR petals; citronellol (41.44 %), nonadecane (16.44 %), heneicosane (10.58 %), were the main components (Table 1).

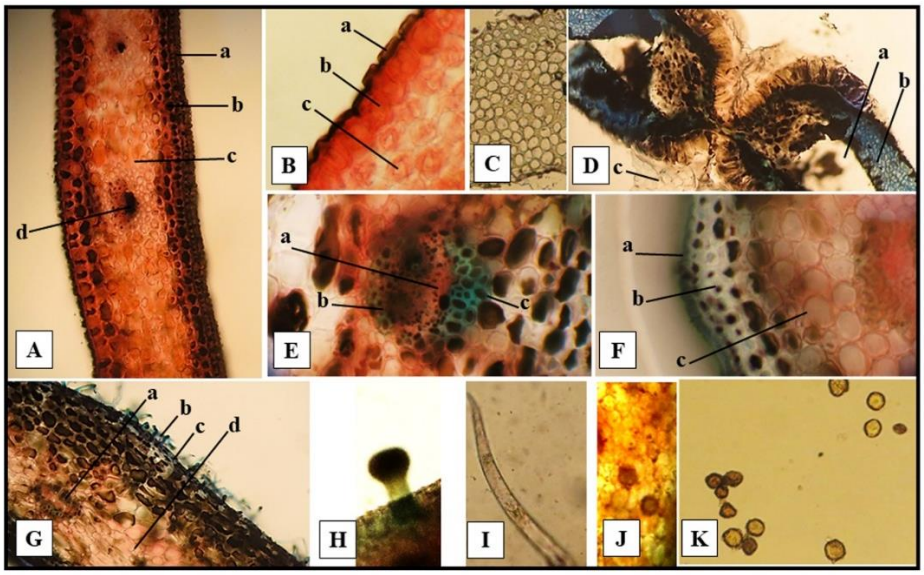

Fig. 2. Microscopic transection of damask rose. A: mesophyll tissue of petal with vessels (×100): a: epidermis, b: collenchyma, c: spongy parenchyma , d: vessel bundle, B: mesophyll tissue of petal (×400): a: cuticle , b: epidermis, c: spongy parenchyma, C: petal epidermis (×100), D: cross section of anther (×400): a: pollen sac, b: endothelium, c: epidermis, E: petal (×400): a: phloem , b: xylem, c: fiber, F: petal(×400): a: epidermis, b: collenchyma, c: spongy parenchyma, G: sepal (×100): a: vascular bundle, b: trichome, c: epidermis, d: spongy parenchyma, H: secretory trichome of sepal (×400), I: epidermal trichome of the ovule (×400), J: transection of ovule with rosette shaped crystals (×400), K: pollen grain (×100).

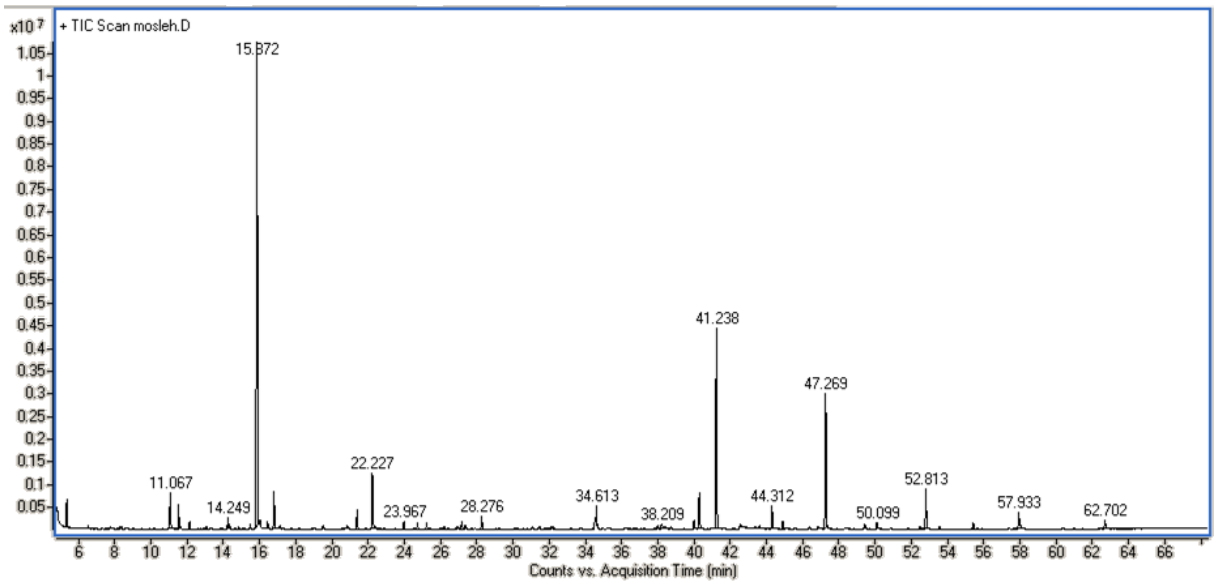

Fig. 3. GC-MS chromatogram of the essential oil obtained from Layzangan damask rose petals

Table 1. Chemical composition of essential oil obtained from Layzangan damask rose petals

No.	Components	% Composition	RT	RI	Methods of identification	Ref.
1	Heptanal	1.38	5.343	879	ABC	[22]
2	Linalool	3.24	11.067	1084	ABC	[22]
3	*cis*-Rose oxide	1.58	11.527	1098	ABC	[22]
4	*trans*-Rose oxide	0.56	12.140	1114	ABC	[22]
5	Citronellol	41.44	15.872	1212	ABC	[22]
6	Phenyl ethyl acetate	0.65	16.431	1225	ABC	[23, 24]
7	Geraniol	3.12	16.800	1234	ABC	[23]
8	Methyl eugenol	4.86	22.227	1388	ABC	[22]
9	(*E*)-Caryophyllene	0.63	23.967	1412	ABC	[25]
10	*α*-Guaiene	0.52	24.715	1432	ABC	[22]
11	*α*-Humulene	tr	25.229	1446	ABC	[22]
12	*α*-Bulnesene	0.66	27.176	1497	ABC	[26]
13	*n*-Heptadecane	1.06	34.613	1700	ABC	[22]
14	Phenethyl benzoate	0.53	38.209	1807	ABC	[7]
15	9-Nonadecene	3.13	40.292	1872	ABC	[8]
16	Nonadecane	16.44	41.238	1901	ABC	[8]
17	Eicosane	1.81	44.312	2000	ABC	[22]
18	Unknown	0.62	44.898	2019	-	-
19	Heneicosane	10.58	47.269	2099	ABC	[22]
20	Docosane	0.52	50.099	2200	ABC	[22]
21	Tricosane	3.40	52.813	2300	ABC	[22]
22	Tetracosane	0.51	55.427	2400	ABC	[22]
23	Pentacosane	1.82	57.933	2497	ABC	[22]
24	Heptacosane	0.91	62.702	2700	ABC	[22]
	Identification (%)	98.66				

tr = trace (< 0.5 %)

RI: Linear retention indices were calculated using a homologous series C_9-C_{30} *n*-alkanes.

A: Comparison of mass spectra and/or retention indices with literature. B: Comparison of the fragmentation patterns with those of the computer mass libraries NIST library; C: Comparison of % Identification obtained from NIST and Adams 2007.

3.4. High performance liquid chromatography (HPLC)

3.4.1. Peak selectivity control via photodiode array

In advance, we checked peaks of quercetin in the samples and standards via photodiode array detector for peak purity control. It was controlled as seen in the Fig. 4.A. Also, the peak of quercetin in the sample was approved by comparing the peak of quercetin in the sample before and after adding standard quercetin to the sample. The peak of quercetin became taller after adding the standard quercetin to the sample.

3.4.2. Calibration Curves

The method produced linear responses throughout the quercetin concentrations with R^2 (0.9999), adjusted R squared (0.9999) and slope (4.39×10^{-7} with *p-value* of 0.8241). A typical chromatogram of the method is shown in Fig. 4.B. For accuracy, the mean absolute recovery values of the method throughout the linear range as the intra- day variations are shown in Table 2. The limits of quantitation (LOQ) and detection (LOD) for quercetin were 0. 012 µg/ml and 0.004 µg/ ml.

3.4.3. Quercetin Content of the Acid Hydrolyzed Damask Rose Petals

Based on the validated HPLC method, the content of quercetin in the acid hydrolyzed extraction of damask rose petals was 146.01 µg quercetin per 20 mg (7300.5 µg/g) dried petals. The chromatogram obtained by injecting the acid hydrolyzed extraction of DR petals is given in Fig.4.C-right.

Table 2. Variation of the HPLC method for quantitation of quercetin

Nominal Concentration (µg/ml)	Sample Number	Measured concentration (µg/ml)	Mean ± SD	CV%	Accuracy	Mean ± SD
500	1	478.14	498.92 ± 21.18	4.24	95.63	99.78 ± 4.24
	2	520.48			104.10	
	3	498.13			99.63	
250	1	235.38	252.85 ± 15.13	5.98	94.15	101.14 ± 6.05
	2	261.67			104.67	
	3	261.49			104.60	
75	1	70.76	72.94 ± 2.15	2.94	94.34	97.26 ± 2.86
	2	73.03			97.37	
	3	75.05			100.06	
50	1	45.53	48.98 ± 3.19	6.52	91.07	97.95 ± 6.39
	2	51.84			103.69	
	3	49.54			99.09	
25	1	26.17	25.28 ± 1.40	5.53	104.68	101.13 ± 5.60
	2	26.01			104.04	
	3	23.67			94.68	

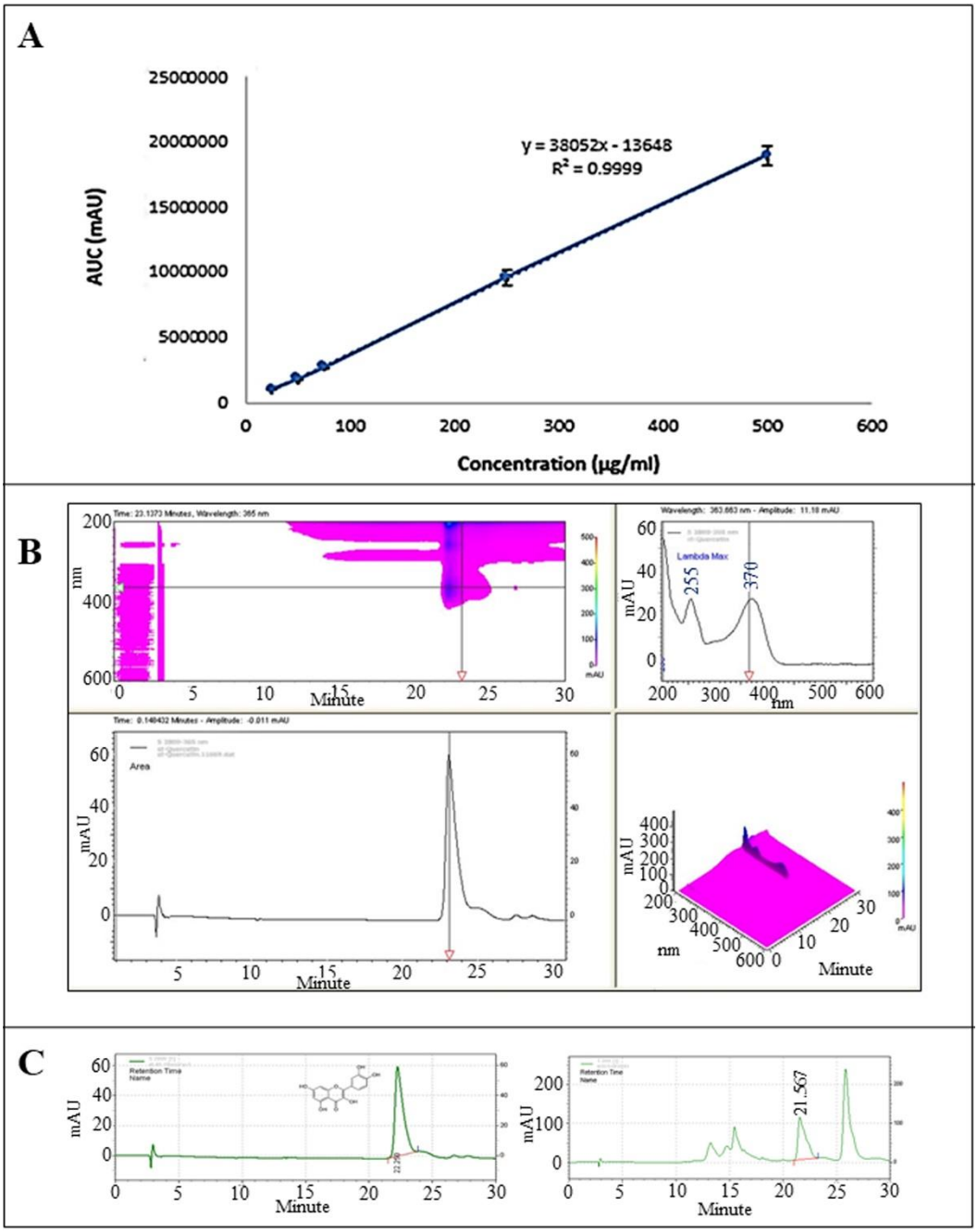

Fig. 4. A: Quercetin standard curve using HPLC method (n = 3); B: Photodiode array graph of standard quercetin (75 µg/ml) using HPLC method C: Left: A typical chromatogram, R.T = 22.250: Quercetin (75 µg/ml), Right: Chromatogram of acid hydrolysis residue of damask rose petals, R.T. = 21.57: Quercetin.

4. Discussion

Reproducibility is a necessity for producing standardized drugs. Although the popularity and expansion of commercialized DR products in Iran, the herb has no monograph in Iran herbal pharmacopeia. Layzangan is one of the most important resources of DR in Iran. So, this study performed quality controls including microscopy, physicochemical, and GC-MS techniques for Layzangan DR flowers. Also,

HPLC was conducted to standardize the DR sample. In the current study, raw herbal material was identified according to the botanical descriptions by an expert botanist. The pharmacopoeia tests including ash tests and loss on drying test as well as microscopic transection and powder microscopy characteristics were studied. The secretory and simple trichomes in sepal, rosette crystals in ovule, and the tricolporate shape of the pollen grain of DR flower were major characteristics identified by microscopic tests. These findings can help identification of undeclared herbal samples of herbal markets and also detection of probable adulterations of DR flowers samples in markets. Heavy metal analysis indicated that the content of toxic heavy metals was below the permissible levels [14]. The low content of lead is may be due to the organic cultivation of DR in the Layzangan valley. According to GC-MS analysis, twenty-three components were identified representing 98.66% of the total essential oil composition of fresh DR petals; citronellol (41.44 %), nonadecane (16.44 %), heneicosane (10.58 %), were the main components. Previous studies on essential oil content of fresh DR petals from Guilan region (in the north of Iran) has shown 24.60 % *n*-hexatriacontane, 16.68 % *n*-tricosane, and 18.56 % 1-nonadecene as their major components [12]. Furthermore, essential oil sample of dried flowers from Kamfirouz region (in the south of Iran) has shown 39.73 % nonadecane, 32.38 % heneicosane, and 7.34 % docosane as their major components [8]. Comparing the essential oil profile of Layzangan flowers in present study with the reported essential oil profile of DR in other regions of Iran, citronellol was found to be higher in our Layzangan samples. In addition, HPLC was performed to quantify the quercetin content in the acid hydrolyzed DR petals which was calculated as 7300.5 µg/g dried petals.

5. Conclusion

This study reveals the diagnostic characteristics of Layzangan damask rose petals. Also, presented quality control and standardization of damask rose samples could provide information for authentication of undeclared samples as well as finding the adulterated materials.

Author contributions

G.M contributed as Ph.D. student and the main study investigator; P.B, A.A, Z.A, and A.M contributed as supervisors of the whole project. F.AS, A.I, and S.K contributed in laboratory investigations. All authors approved the final draft of the manuscript.

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Conflicts of interest

The authors have no competing interests to declare.

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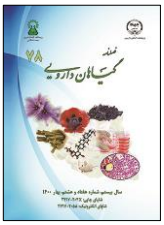

مقاله تحقیقاتی

کنترل کیفیت گل محمدی (*Rosa × damascena*) منطقه لایزنگان استان فارس (ایران)

غزاله مصلح[۱]، پرمیس بدر[۲،۱]، امیر آزادی[۳]، فروغ افسری سرداری[۴]، آیدا ایرجی[۵]، صدیقه خادمیان[۴]، زهره ابوالحسن‌زاده[۶]، عبدالعلی محققزاده[۲،۴،*]

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اطلاعات مقاله

گل‌واژگان:
گل محمدی
Rosa × damascena
آنالیز بافتی
کروماتوگرافی گازی متصل به طیف‌سنجی جرمی
کروماتوگرافی مایع با کارایی بالا

چکیده

مقدمه: گل محمدی با نام علمی *Rosa × damascena* Herrm. گیاه دارویی بسیار شناخته شده از خانواده گل سرخ می‌باشد. بر اساس متون نگاشته شده، منشأ کشت گل محمدی، در دره لایزنگان منطقه داراب از استان فارس در ایران است. **هدف:** در این مطالعه، کنترل کیفیت و استانداردسازی گل محمدی لایزنگان مورد بررسی قرار گرفت. **روش بررسی:** آنالیز بافت‌شناسی، فیزیکوشیمیایی و فلزات سنگین و همچنین کروماتوگرافی اثر انگشت شامل GC-MS و تجزیه و تحلیل HPLC مورد بررسی قرار گرفت. **نتایج:** طبق مشاهدات میکروسکوپی، تریکوم‌های ترشحی و ساده در کاسبرگ، کریستال‌های رزت شکل در تخمک و دانه گرده سه وجهی از ویژگی های اصلی گل محمدی بودند. نتایج آنالیز فلزات سنگین نشان داد که مقادیر روی (۸۲/۵ ppm)، مس (۴/۰۲ ppm)، سرب (۰/۱۱ ppm) و کادمیم (یافت نشد) کمتر از حداکثر مقدار مجاز تعیین شده بود. اسانس گلبرگ‌های تازه گل‌ها به کمک تقطیر با آب استخراج و ترکیب شیمیایی آن با روش کروماتوگرافی متصل به طیف‌سنج جرمی شناسایی شد. تعداد ۲۳ جزء بیانگر ۹۸/۶۶ درصد از کل ترکیبات اسانس مشخص شد. سیترونلول (۴۱/۴۴ درصد)، نونادکان (۱۶/۴۴ درصد) و هنیکوزان (۱۰/۵۸ درصد) از ترکیبات اصلی اسانس بودند. بر اساس آنالیز HPLC میزان کوئرستین ۷۳۰۰/۵ میکروگرم در هر گرم گلبرگ خشک اندازه‌گیری شد. **نتیجه‌گیری:** اسانس گل محمدی لایزنگان غنی از ترکیب سیترونلول است. ویژگی‌های تشخیصی ارائه شده در این مطالعه می‌تواند به کنترل کیفی بهتر و استانداردسازی نمونه‌های گل محمدی کمک کند. همچنین می‌تواند اطلاعاتی را برای تأیید اعتبار نمونه‌های مجهول و همچنین تشخیص موارد تقلبی فراهم نماید.

مخففها: DR، گل محمدی؛ GC-MS، کروماتوگرافی متصل به طیف‌سنج جرمی؛ HPLC، کروماتوگرافی مایع با کارایی بالا؛ LOQ، حد تعیین مقدار؛ LOD، حد تشخیص

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