

Chemical Composition, Antioxidant Activity and Antimicrobial Effect of *Rosa damascena* Mill. Essential Oil Against *Staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi*

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ABSTRACT: Food preservation is an action or a method of maintaining foods at a desired level of properties or nature for their maximum benefits. In general, each step of handling, processing, storage, and distribution affects the characteristics of food, which may be desirable or undesirable. Thus, understanding the effects of each preservation method and handling procedure on foods is critical in food processing. In this study, the essential oil composition, antioxidant activity and antibacterial activity of *Rosa damascena* Mill. under Kashan (Isfahan province, Iran) were evaluated. The essential oil was obtained by hydro-distillation and gas chromatography-mass spectrometry (GC-MS) analysis revealed the presence of 19 compounds with the major constituents including Nonadecane (44.74%), 9-Nonadecane (16.91%), Eicosane (10.40%) and Heptadecane (6.17%). Antioxidant activity was examined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assays. The results showed good free radical scavenging capacity and IC₅₀ value (the concentration of inhibitor) of the essential oil was 3.91±0.4 µL/mL. The disc diffusion method was employed to measure the minimum inhibitory concentration (MIC). The minimum bactericidal concentration (MBC) was measured, by using the wells in which no growth was observed. The essential oil of the *Rosa damascena* Mill. exhibited antibacterial capacity against *staphylococcus aureus* 250, and for *Escherichia coli* and *Salmonella typhi* with the MIC and MBC for *staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi* 500 and 1000 µL/mL, respectively. The obtained *Rosa damascena* Mill. essential oil is a promising source of natural antioxidant and antimicrobial agent for foods and cosmetics applications.

Keywords: *Antioxidant Activity, Antibacterial Activity, Essential Oil, GC-MS, Rosa damascena* Mill.

Introduction

Plants in response to environmental conditions produce groups of bioactive components that are defined as secondary metabolites. Firstly, the secondary metabolites exist as inactive precursors in

different organs of the plant but the stimulation of plants by extrinsic stress converts these components to constituents with bioactive properties (Leistner, 2000; Lanciotti *et al.*, 2004). The unique characteristics and health of the bioactive

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compounds caused these compounds to be the target of many studies in the recent decades. Antioxidant, anticancer, anti-diabetes, antihypertensive, anti-mutagenesis activities, inhibition of their reaction of oxidation as well as its application as flavoring agent are the important and practical properties of these compounds (Bakkali *et al.*, 2008; Raut, & Karuppaiyil, 2014).

Phenolic compounds, anthocyanins, flavonoids, glycosides, alkaloids and carotenoids are various bioactive compounds in plant tissues that all have antioxidant activity and radical scavenging property. These natural antioxidants can be used as a partial or complete replacement to synthetic antioxidants (Leistner, 2000; Contini *et al.*, 2008). The concerns about the induction of cancer and mutagenesis by these synthetic compounds such as butylatedhydroxyanisole (BHA), butylatedhydroxytoluene (BHT), etc. have increased (Barlow, 1990), and researchers are looking for methods to control these substances in food products. Maskan and Horuz (2017) have studied the antioxidant property of Za'atar (*Thymbra spicata*) essential oil as natural antioxidant for the stability of palm olein during deep-fat frying process (BHT was used as a control), and reported that Za'atar essential oil like BHT could be used to preserve palm olein. They expressed that the anisidine and iodine values as well as free fatty acid content in the samples treated with the essential oils were reduced to an acceptable level.

On the other hand, the potential use of essential oils and plant metabolites as an antimicrobial agent and bio preservative in foods is another promising application in their products. Essential oils are generally recognized as safe (GRAS) products; therefore, their direct use in foods as a preservative does not have an adverse effect on products and consumer health (Bakkali *et al.*, 2008; Turek & Stintzing, 2013). Accordingly, different studies have been carried out on the use of essential oils as an

antimicrobial agent and biopreservative in various foods (Noori *et al.*, 2014; Huang *et al.*, 2018; Ju *et al.*, 2018; Huang *et al.*, 2018). Consequently, the identification and study of new and unique sources of essential oils is very important.

Rosa damascena Mill. (*R.damascena*), known as Damask rose, a perennial bushy shrub, is the most well-known decorative plant of the Rosacea family ubiquitous, in terms of perfumery and food industries. This aromatic plant is cultivated in Iran, Turkey, Bulgaria, India, Morocco, South France, China, South Italy, Libya, South Russia and the Ukraine (Sharma & Kumar, 2016). The petals of this plant contain odorous and volatile compounds, which have many uses in food, pharmaceutical and sanitary industries. Studies in different parts of the world have identified several compounds in the structure of *R. Damascena* essential oil. Koksals *et al.* (2015) evaluated the effect of storage at temperatures of 4 and 25 °C on Damask Rose petals. The study also concentrated on dehydration at different temperatures (40, 50 and 60 °C) and on essential oil composition. They reported that the storage increased the phenyl ethyl alcohol, geraniol and nonadecane; however, convective drying resulted in the production of essential oils with less citronellol and geraniol and higher phenyl ethyl alcohol and nonadecane as compared to essential oil of the fresh petals. Therefore, volatile compounds of the *Rosa damascena* Mill. has been studied and various characteristics of these compounds such as antidiabetic (Gholamhoseinian & Fallah, 2009), analgesic and anti-inflammatory (Hajhashemi *et al.*, 2010), antihyperlipidemic (Joukar *et al.*, 2013) and anticancer (Rezaie-Tavirani *et al.*, 2013) effects as well as antioxidant and antimicrobial activities (Olgunsoy *et al.*, 2017; Sengul *et al.*, 2017), have been reported. In this study, it is aimed to investigate the antiradical activity and antioxidant capacity, antibacterial activity

and composition of the essential oil isolated from the *Rosa damascena* Mill. provided in Kashan (Isfahan province, Iran).

Materials and Methods

- Chemical reagents

The chemical reagents DPPH (2, 2-diphenyl-1-picrylhydrazyl) was purchased from Sigma aldrich chemicals (USA). Macconkey Broth, Muller Hinton Agar, Blood Agar, Nutrient Broth and Tryptic Soy Broth were purchased from Merck chemical company. (Germany).

- Plant material

About 25 kg fresh *Rosa damascena* Mill. was purchased from a local market in Kashan (Isfahan province, Iran). The species of the plant was identified and confirmed by herbarium, the herbal systematic laboratory of Islamic Azad University, Science and Research Branch. After separating *Rosa damascena* Mill. petals, they were dried in shade at ambient temperature (20-25 °C) for one week. Before the extraction of essential oil the samples were powdered by laboratory grinder (Figure 1).

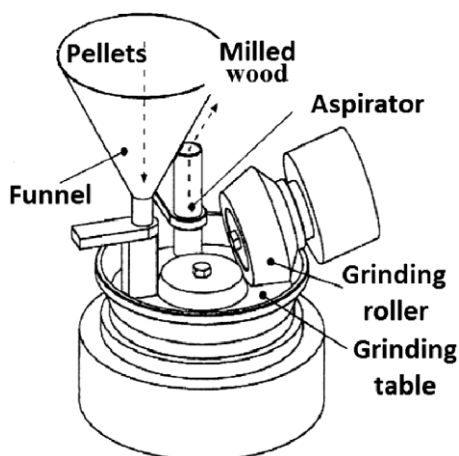


Fig. 1. Schematic of laboratory Grinder

- Essential oil extraction

The dried samples from *R. damascena* Mill. were submitted to hydro distillation for 3-4 hours (Figure 2). By using a Clevenger

apparatus the essential oil was isolated and stored in sealed dark vials, at 4 °C till further use (Darderafshi *et al.*, 2014; Ghoshoonizade *et al.*, 2015).

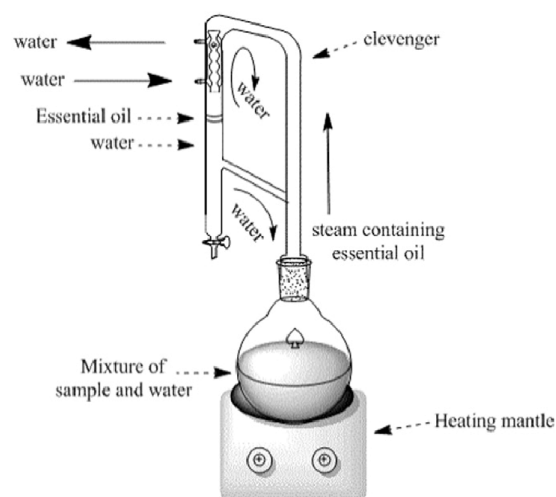


Fig. 2. Hydro distillation system

- Extraction efficiency

The *R. damascena* Mill. Essential oil efficiency (%) was determined using the following equation (Zhang *et al.*, 2015):

$$\text{Extraction efficiency (\%)} = (m_1/m_0) \times 100$$

Where, m_1 is the essential oil obtained (g) and m_0 is mass of dry matter (g).

- GC-MS analysis and identification of compounds

The composition of the *R. damascena* Mill. essential oil was analyzed by Hewlett-Packard gas chromatography-mass spectrometry operating at 70eV ionization energy, equipped with a HP-5MS capillary column phenyl methyl siloxane (30 m 0.25 mm, 0.32 μ m film thickness) with Helium as the carrier gas at a split ratio of 1:20. Oven temperature program was adjusted to be increased from 60 °C to 200 °C in increments of 5 °C/min and subsequently held for 10 min. The retention times for all the fractions were determined according to the Van Den Dool method using n-alkanes as standard (Van den Dool & Kratz, 1963). The fractions

were identified by comparison of retention indices (RRI- HP-5) with those reported in the literature and by comparison of their mass spectra with the Wiley and mass finder 3 libraries or with the published mass spectra (Adams, 2001).

- Antioxidant activity

DPPH (Figure 3) radical scavenging test was analyzed using method of Tohidi *et al.* 2017, with slightly modifications. Briefly, 0.1 mL of the *R. damascena* Mill. essential oil was mixed at various concentrations (50, 100, 200, 300 and 500 ppm) with 2 mL of 0.1 mL methanol DPPH solution. The solution was shaken strongly and allowed to halt at ambient temperature for 30 min. The absorbance of the samples was recorded using the UV-visible spectrophotometer at 517 nm. Methanol was used as blank and all calculations were performed in triplicate. The inhibition activity of the samples was determined according to the following equation:

$$\text{Inhibition activity (\%)} = \frac{[(\text{absorbance control} - \text{absorbance sample}) / \text{absorbance control}] \times 100}{}$$

The inhibition activity was plotted versus the sample concentration and 50% of the inhibitory concentration (IC₅₀) of the DPPH values was defined by linear regression analysis.

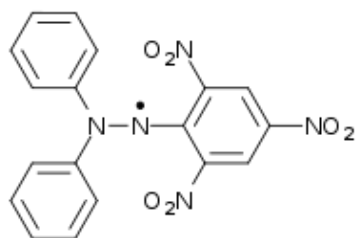


Fig. 3. DPPH (2, 2-Diphenyl-1-picrylhydrazyl)

- Antibacterial activity

Bacteria used (*staphylococcus aureus*, ATCC:2912139, *Escherichia coli*, PTCC:1609, *Salmonella typhi*, ATCC:2592)

in this study were obtained from the Faculty of Veterinary, Tehran university and the Persian Type Culture Collection. Bacteria were subcultures from stocks maintained in nutrient broth containing 20% glycerol at -80°C (Ghoshoonizade *et al.*, 2015; Abdollahzadeh *et al.*, 2013).

MIC and MBC values were carried out as earlier reported (Shohayeb *et al.*, 2014), with some modification. Briefly, the *R. damascena* Mill. essential oil was serially diluted two-folds in nutrient broth medium in microtiter plates. Each dilution was inoculated with 1×10⁷ CFU/ml of the test bacterial strain. The plates were incubated at 37°C for 18 hours. MIC was taken at the highest dilution (Least concentration) of essential oil showing detectable growth. Sub inhibitory concentrations were sub-cultured onto nutrient agar plates to determine the minimum bactericidal concentrations.

The statistical analyses were carried out using SPSS 22.0 (Akhavan *et al.*, 2016).

Results and Discussion

- Chemical Composition of the *R. damascena* Mill. Essential oil

The results of the GC-MS analysis of the *R. damascena* Mill. essential oil are presented in Table 1. A total of 19 compounds accounted 97.2% of the oil. The indexes of the chromatogram horizontal axis illustrate the retention time of the fractions of the *R. damascena* Mill. essential oil and the vertical axis gives the relative abundance of each component (Figure 4). The main fraction was nonadecane (44.74%). Moreover, other major compounds included 9-nonadecane (16.91%), eicosane (10.40%) and heptadecane (6.17%). The color of the *R. damascena* Mill. essential oil was yellowish and its extraction efficiency was equal to 0.14 v/w (Yassa *et al.*, 2009).

Different authors have evaluated the chemical composition the *R. damascena* Mill. grown in Iran and other countries. The chemical composition of the *R. damascena*

Mill. (grown in Mashhad, Iran) is consisted of β -citronellol (23%), nonadecane (16%), geraniol (16%) and heneicosane (5%) as the main components. Moein *et al.*, (2016) also reported that nonadecane (40.38%), heneicosane (26.17%), 1-nonadecene (15.33%) and heptadecane (10.33%) are the main components of the *R. damascena* Mill. essential oil in Fars province of Iran (Kumar *et al.*, 2017). Sharma and Kumar (2015) identified citronellol+nerol (32.76%), *trans*-geraniol (19.07%), nonadecane (12.16%), and heneicosane (4.57%) as the major oil components of fresh the *R. damascena* Mill. collected in western Himalayas. The great difference in the main chemical composition of the *R. damascena* Mill. essential oil of different regions of the world might be attributed to the conditions and environmental changes. Concentration, type and amount of fractions in essential oils of different plants changes depending on growth stage, atmospheric conditions, geographical conditions (longitude, latitude and altitude), planting and harvesting time and extraction system (Behbahani *et al.*, 2017; Sefidkon *et al.*, 2006).

- Antioxidant activity

Antioxidants are mainly known as compounds that are capable of hydrogen donation and prevent the occurrence of oxidation chain reactions. There are several methods to evaluate the antioxidant activity of bioactive compounds. The DPPH scavenging activity assay is one of the most common antioxidant activity evaluation methods. This test can be distinguished by changing the violet color of the DPPH solution to reddish brown (Ohkita & Tubokawa, 1972; Baharfar *et al.*, 2015). Based on the results of antioxidant activity evaluation, IC₅₀ index for the *R. damascena* Mill. essential oil was determined. The index was equal to $3.91 \pm 0.4 \mu\text{L}/\text{mL}$. The IC₅₀ is defined as the minimum concentration of

bioactive components capable of inhibiting 50% of the DPPH free radical activity. Martucci *et al.* (2015) and Moein *et al.* (2016) studied the extract of Iranian medicinal plants to compare their antioxidant effects with gallic acid. They reported that ethanolic extract of the *R. damascena* Mill. exhibited IC₅₀ = $287.9 \pm 5.675 \mu\text{g}/\text{mL}$ that it was much higher than gallic acid (IC₅₀ = $25.32 \pm 5.593 \mu\text{g}/\text{mL}$). Kalim *et al.* (2010) also reported that the IC₅₀ index of methanolic (50%) extract of the *R. damascena* Mill. for scavenging DPPH was $10.36 \pm 0.02 \mu\text{g}/\text{mL}$. On the other hand, Yassa *et al.* (2009) Stated that the IC₅₀ of the *R. damascena* Mill. essential oil was $3.54 \mu\text{g}/\text{mL}$, which was less than IC₅₀ of the BHT ($110.98 \mu\text{g}/\text{mL}$) and kaempferol 3-O-rhamnoside ($531 \mu\text{g}/\text{mL}$). Therefore, they expressed that the *R. damascena* Mill. essential oil has more radical scavenging activity than the synthetic antioxidant BHT.

Phenolic compounds are a group of bioactive compounds that play the role of antioxidant and antiradical compounds in many medicinal plants (Zheng & Wang, 2001; Aaby *et al.*, 2004). Due to their reducing property and free radical scavenging ability, these compounds can quench free radicals and various radical species (Koleva *et al.*, 2002; Trueba *et al.*, 2004). Free radicals may provide conditions for many chronic diseases such as cancer and oxidative stress. Therefore, free radicals cause oxidative damages to cells as well as fatty substances such as oils (Kris-Etherton *et al.*, 2002). Hence, the use of herbal extracts and essential oils that contains phenolic and antioxidant compounds is recommended. The *R. damascena* Mill. essential oil and extract contain numerous phenolic compounds that their antioxidant properties have been proven (Kovatcheva-Apostolova *et al.*, 2008; Boskabady *et al.*, 2011; Verma *et al.*, 2011). Therefore, the antioxidant ability of the essential oil in this study is probably related to the phenolic compounds present in it (Akhavan *et al.*, 2016).

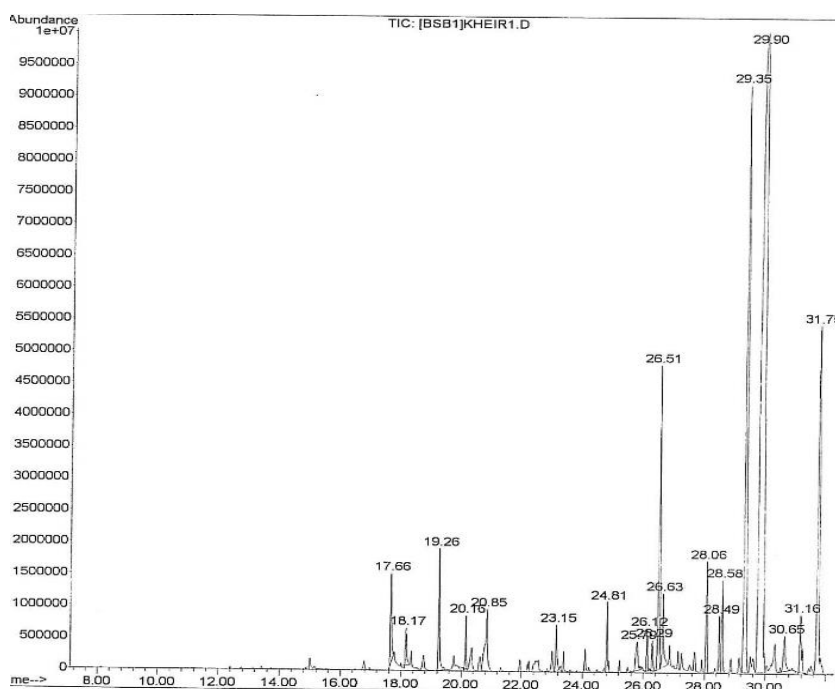


Fig. 4. Spectroscopy GC-MS chemical composition of *R. damascena* mill essential oil

Table 1. Chemical composition of the *R. damascena* mill essential oil

No.	Retention time (min)	Composition	(%)
1	17.66	Citronellol	1.80
2	18.17	Geraniol	0.66
3	19.26	Phenol, 2-methyl-5-(1-methylethyl)- OR Carvacrol	2.32
4	20.16	Eugenol	0.71
5	20.85	Benzene, 1,2-dimethoxy-4-(2-propenyl)- OR Methyleugenol	2.44
6	23.15	Pentadecane	0.98
7	24.81	Tetradecanal OR Myristic aldehyde	1.15
8	25.79	Decanoic acid, decyl ester	1.11
9	26.12	8-Heptadecene	0.65
10	26.51	Heptadecane	6.17
11	26.63	2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-, (Z,E)- OR Farnesol	1.79
12	28.06	Tetradecanal OR Myristic aldehyde	2.27
13	28.49	2-Phenylethyl octanoate OR Octanoic acid, 2-phenylethyl ester	0.86
14	28.58	2-Phenylethyl benzoate OR Benzoic acid, 2-phenylethyl ester	1.71
15	29.35	9-Nonadecane	16.91
16	29.90	Nonadecane	44.75
17	30.65	Hexadecanoic acid OR Palmitic acid	1.27
18	31.16	(E)-5-Octadecene	1.54
19	31.75	Eicosane	10.4

- Antimicrobial Activity

The antimicrobial activity of the essential oil from the *R. damascena* Mill. against *staphylococcus aureus*, *Escherichia coli* and *Salmonella typhi* was assessed by the MIC and MBC values using the disc diffusion method. The results of these tests are shown in Table 2, 3 and 4. Based on the results of this study, it was found that the MIC concentration (500 µL/mL) was the same for both the bacteria. Similarly, the MBC concentration for *E. coli* and *S. typhi* was 1000 µL/mL, also the result of *staphylococcus aureus*, was the two gram negatives or even better. Accordingly, it can be concluded that *R. damascena* Mill. essential oil has the same effect on inhibition of the two of gram negative and the gram positive studied bacteria. Plants are rich in secondary metabolites and active ingredients. Essential oils and extracts as sources of plant metabolites are a mixture of plant volatile fractions that contain bioactive, antioxidants and antimicrobials compounds (Calsamiglia *et al.*, 2007). The phenolic compounds constitute most of these metabolites; therefore, antimicrobial activity of essential oils has a similar mechanism to phenolic compounds. The hydrophobicity properties of essential oils cause their ability to react with the lipids in the cell membrane of the

bacteria (Kalemba & Kunicka, 2003; Burt, 2004). This condition increases the permeability of the membrane, disruption of cellular and bacterial enzyme activities, as well as homeostasis disorders. As a result of these factors, part of the intracellular contents may leak out into the surrounding environment and subsequently a bacterial cell death occurs (Devi *et al.*, 2010; Bajpai *et al.*, 2013). Similar reports with these justifications have been previously reported by Cetin-Karaca and Newman (2015) that they found good correlations between the results from phenolic analysis and the antimicrobial efficacy.

In a study conducted by Shohayeb *et al.* (2014), different extracts (aqueous, hexane and ethanol extracts) and essential oil of *R. damascena* Mill (Taif, Saudi Arabia) showed the MIC value ranged between 0.125 and 8 mg/mL against tested bacteria (gram-negative bacteria and gram-positive bacteria). Ulusoy *et al.* (2009) also, researched on *R. damascena* Mill. essential oil as an antibacterial agent, and reported that these compounds have strong antibacterial activity against *E. coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Staphylococcus aureus*, *Chromobacterium violaceum* and *Erwinia carotovora* strains.

Table 2. The MIC and MBC of the *R.damascena* Mill. essential oil against *Staphylococcus aureus*

Concentration (µL/mL) / Value	0	15.625	31.25	62.5	125	250	500	1000
MIC	+	+	+	+	+	-	-	-
MBC	+	+	+	+	+	+	+	-

Table 3. The MIC and MBC of the *R. damascena* Mill. essential oil against *Escherichia coli*

Concentration (µL/mL)/ Value	0	15.625	31.25	62.5	125	250	500	1000
MIC	+	+	+	+	+	+	-	-
MBC	+	+	+	+	+	+	+	-

Table 4. The MIC and MBC of the *R. damascena* Mill. essential oil against *Salmonella typhi*

Concentration (µL/mL) / Value	0	15.625	31.25	62.5	125	250	500	1000
MIC	+	+	+	+	+	+	-	-
MBC	+	+	+	+	+	+	+	-

Conclusion

In this study, the essential oil of *R. damascena* Mill. was extracted by hydrodistillation method and the fractions were analyzed and identified by GC-MS and the components were identified. The predominant compounds of the essential oil were nonadecane, 9-nonadecane, eicosane and heptadecane with 44.74, 16.91, 10.4 and 6.17% concentration respectively. Evaluation of the antioxidant and radical scavenging activities showed that the *R. damascena* Mill. has good antioxidant properties. Also, antibacterial activity of the *R. damascena* Mill. revealed that the essential oil had good effect on the studied bacteria (*S. aureus*, *E. coli* and *S. typhi*).

The microbial safety and stability as well as the sensory and nutritional qualities of most foods are based on the application of combined preservative factors called hurdles (Leistner, 2000; Leistner, 1996; Leistner, 1997; Shafiur, 2007; Cleveland *et al.*, 2001; Claude Cheftel, 1995).

Aromatic and volatile products of plant secondary metabolism are used in the pharmaceutical, chemical, cosmetic, and food industries. In recent years, there has been an increasing interest in the use of natural substances due to concerns about the safety of some synthetic compounds, which have encouraged more detailed studies on originated substances (Gourine *et al.*, 2010; Hosni 2011; Ginova *et al.*, 2012; Seify *et al.*, 2018).

Flower of *Rosa damascena* Mill is widely used in Iran for gastrointestinal (GI) disorders (Sadraei *et al.*, 2013).

Therefore, one of the alternatives to replace the artificial preservatives is the application of essential oil of *Rosa damascena* Mill. to varieties of food as well as its application to various industries (Laranjo *et al.*, 2017).

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