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Quantification of Methanol, Ethanol, and Essential Oil Contents of Commonly Used Brands of Rosewater (*Rosa Damascena*) in Iran

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ABSTRACT

Background: *Rosa damascena* Mill. (*Rosaceae*) is a relevant ornamental and medicinal flower, whose distillation is known as Golab (Rosewater) in Iran. Due to the nature of the distillation process, methanol and ethanol are likely to be formed in the final product. Therefore, due to their toxicity, along with their frequent prescription, this study aims to investigate the concentrations of essential oil, methanol, and ethanol in commonly used brands of Rosewater available in Tehran, Iran. **Methods:** Methanol and ethanol concentrations were determined by gas chromatography, using a Flame Ionized Detector. **Results:** The mean essence level in the tested samples was 204.1 ± 18.0 ppm, which was significantly higher ($P < 0.001$) than the Iranian standard level. The mean methanol and ethanol levels in the tested samples were 1.25 ± 0.70 ppm and 700.0 ± 100.0 ppm, respectively, which were significantly lower than the maximum residues levels. **Conclusion:** The mean essence level in the tested samples well coped with the Iranian standard level. Moreover, there is no health risk to methanol and ethanol through Rosewater consumption.

Keywords: Rosewater; Essence; Methanol; Ethanol; Gas chromatography

Introduction

Rosa damascena Mill. (*Rosaceae*) is an ornamental plant with light pink flowers, generally known as “Gole Mohammadi” in Iran (Boskabady *et al.*, 2011, Mahboubi and medicine, 2016, Yassa *et al.*, 2015). It is a valuable medicinal plant in traditional and modern medicine (Mahboubi *et al.*, 2016, Nikbakht and Kafi, 2004). The anti-depressant, antimicrobial, anti-

inflammatory, antioxidant, anticancer, anti-diabetic properties, as well as anticonvulsant and hypnotic effects of damask rose, have been confirmed (Baydar *et al.*, 2013, Mahboubi and medicine, 2016, Yassa *et al.*, 2015). Various commercial products are manufactured from Rose flowers (i.e., Rosewater, Rose essential oil, dried flowers, Rosehips, Rose concrete, Rose absolute)

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(Mahboubi and medicine, 2016). Rosewater, commonly known as Golab, is the primary product of damask rose in Iran (Akram *et al.*, 2020). Due to its calming and relaxing properties, Rosewater is used in religious ceremonies. It also applied as a flavoring agent in Iranian foods (Boskabady *et al.*, 2011, Mahboubi, 2016). Moreover, because of its antiseptic properties, it was traditionally used for eye washing and mouth disinfecting. The antibacterial activity of Rosewater was proved against *E. coli*, *P. aeruginosa*, *B. subtilis*, and *S. aureus*. Besides, due to its antispasmodic properties, it is used for alleviating abdominal pains and bronchial congestions (Mahboubi, 2016, Moein *et al.*, 2014).

Besides all these beneficial aspects of Rosewater, the presence of alcohols (such as methanol and ethanol), is a matter of great concern. The fermentation of the plant's fibers (i.e., cellulose and pectin) through the manufacturing process, and also the usage of these alcohols as a solvent agent to extract natural ingredients in Rosewater, lead to their increased residual levels in Rosewater (Mousavi *et al.*, 2011, Yousefi *et al.*, 2018). Ethanol level in distillates and its toxicity is less than methanol (Yousefi *et al.*, 2018). Methanol, also named wood alcohol, is a colorless, highly toxic volatile liquid (Delirrad *et al.*, 2012). One of the main reasons for methanol toxicity is its high solubility in oil and water. Moreover, the formation of formic acid through the oxidation processes (held by alcohol dehydrogenase and aldehyde dehydrogenase) is another reason for methanol toxicity (Yousefi *et al.*, 2018). Clinical symptoms of acute methanol poisoning include abdominal pain, nausea, and vomiting, irritation, blurred vision, total blindness, ataxia, headaches, unsteadiness, dizziness, tinnitus, hearing loss, seizures, amnesia, anxiety, and phobias, weakness, malaise, fatigue, and palpitation (Delirrad *et al.*, 2012, Yousefi *et al.*, 2018). Although low-level exposures of methanol and ethanol may be asymptomatic, continual multiple exposure, especially oral intake, may lead to toxicity (Mousavi *et al.*, 2011). Hence, the diagnosis of chronic methanol and ethanol toxicity is critical.

With this assumption, the present study was done to identify the methanol, ethanol, and essential oil contents in commonly-used brands of Rosewater purchased from local markets of Tehran, Iran.

Materials and Methods

A total of 28 bottles of the most commonly used brands of Rosewater (28 different brands) were randomly selected and purchased from the local markets of Tehran, Iran, in summer 2020. Based on ISO (Iranian Standardization Organization, No. 2836), the production and expiration date of all samples were verified and recorded. In addition, all samples were collected in 200 ml sterile polyethylene containers and sealed with paraffin. Then, they were transferred to the cooperator laboratory of the Food and Drug Organization located in Tehran, Iran.

Methanol and ethanol concentrations of each sample were measured by Gas Chromatography (GC), according to ISO (No. 22448) (Shirani *et al.*, 2018, Shirani *et al.*, 2016). The GC instrument was Agilent Technologies (7890) equipped with a HP-5MS capillary column (length: 30 m, film thickness: 0.5 μm , inside diameter: 0.32 mm) and a flame ionization detector (FID). The split ratio of injections was 10:1. The FID temperature was 280 $^{\circ}\text{C}$. The injection port temperature was 250 $^{\circ}\text{C}$, and the oven was programmed from 50 to 250 $^{\circ}\text{C}$ at a rate of 2 $^{\circ}\text{C}/\text{min}$. The carrier gas was helium with a flow rate of 1 ml/min and 1 μl of each sample was injected into the device after adding the internal standard.

Chemical tests to determine the essence value were carried out according to the Pantan method (ISO No. 1487) (Asemi *et al.*, 2006). Also, 50 g of sodium chloride and 33 ml of pentane solution were added to 250 ml of the sample and stirred for 15 minutes. After the exhaust gases were released, the mixture was left stilled until two phases of water and oil were well separated. Then, the water phase discharged, and the amount of essence was calculated according to the weight difference, as shown in the following equation:

$$S = (A-B) \times 0.53 \times 1000$$

A: weight of Erlenmeyer with essence (g), B:

weight of Erlenmeyer without essence (g), *S*: The amount of essence (mg / 199ml).

Data analysis: The student's *t*-test (SPSS software Ver. 16, Chicago, IL, USA) was carried out to compare the mean values of methanol, ethanol, and essential oil contents with the Iranian standard content, which were considered statistically significant at P -value < 0.001 .

Results

The concentrations of the essence, methanol, and ethanol of 28 Rosewater samples were represented in **Table 1**.

The mean contents of essence, methanol, and ethanol in Rosewater samples, and their

comparison with the ISO standards (No. 1487), are displayed in **Table 2**. Based on the findings, the mean essence level in the tested samples was 204.1 ± 18.0 ppm, which was significantly higher ($P < 0.001$) than the Iranian standard level. The mean methanol and ethanol levels in the tested samples were 1.25 ± 0.7 ppm and 700.0 ± 100.0 ppm, respectively, which were significantly ($P < 0.001$) lower than the Iranian standard level. Additionally, the highest and the lowest amounts of methanol were 20 and ND ppm, respectively. Moreover, the maximum and minimum levels of ethanol were observed as 2400 and 50 ppm, respectively.

Table 1. The concentrations of essence, methanol, and ethanol of Rosewater samples.

| No. of samples | Essence content (ppm) | Methanol concentration(ppm) | Ethanol concentration (ppm) |
|----------------|-----------------------|-----------------------------|-----------------------------|
| 1 | 176.4 | ND | 140 |
| 2 | 131.0 | ND | 120 |
| 3 | 121.0 | ND | 400 |
| 4 | 55.0 | 20 | 1200 |
| 5 | 132.5 | ND | 800 |
| 6 | 205.6 | ND | 140 |
| 7 | 280.0 | ND | 250 |
| 8 | 159.0 | ND | 600 |
| 9 | 501.0 | ND | 50 |
| 10 | 121.0 | ND | 400 |
| 11 | 121.0 | ND | 80 |
| 12 | 120.0 | ND | 140 |
| 13 | 150.0 | ND | 460 |
| 14 | 120.0 | ND | 140 |
| 15 | 179.0 | ND | 550 |
| 16 | 309.2 | 8 | 250 |
| 17 | 217.0 | ND | 200 |
| 18 | 402.8 | ND | 2400 |
| 19 | 196.0 | 10 | 400 |
| 20 | 159.0 | ND | 115 |
| 21 | 120.0 | ND | 2000 |
| 22 | 243.0 | 7 | 750 |
| 23 | 301.0 | ND | 700 |
| 24 | 196.0 | ND | 1500 |
| 25 | 286.0 | ND | 1200 |
| 26 | 279.0 | ND | 2400 |
| 27 | 181.0 | ND | 2400 |
| 28 | 255.0 | ND | 250 |

ND; not detected

Discussion

Rosewater is a liquid obtained by hydro-distillation from fresh Rose flowers (Agarwal *et*

al., 2005). It can be extracted using traditional (boiling petals) or industrial (steam distillation) methods (Nikbakht and Kafi, 2004). Rosewater

frequently contains 10–50% Rose oil (Moein *et al.*, 2014). Based on ISO (No. 1487), the minimum acceptable amount of Rosewater essence is 120 ppm. The mean essence level in the current study was 204.1 ± 18.0 ppm. In the study conducted by Rao *et al.*, (Rajeswara Rao *et al.*, 2000) on Rose flowers in three regions in India, the essential oil values were reported as 320, 340, and 500 ppm, respectively. In general, Rosewaters are classified into three types, including light (1 - 119 ppm), middle (120 - 350 ppm), and high (> 350 ppm) essence (Asemi *et al.*, 2006). In this study, one sample out of 28 samples (or 3.5%) was categorized in light essence group (55 ppm), two samples (or 0.14%) were among the high essence group (501 and 402.8 ppm), while others (or 89.28%) were in the middle essence group.

Table 2. Comparison of the mean contents of essence, methanol, and ethanol with ISO standards.

| Variables | Mean content | Standard error | ISO (No. 1487) |
|----------------|--------------|----------------|----------------|
| Essence (ppm) | 201.4 | 18 | > 120 |
| Methanol (ppm) | 1.25 | 0.7 | < 100 |
| Ethanol (ppm) | 700 | 100 | < 2500 |

ISO; Iranian standard organization

Due to the nature of the distillation process used in the production of herbal distillates, methanol and ethanol are likely to be formed in the final product (Delirrad *et al.*, 2012). Fermentation of cellulose and demethylation of pectin (cell wall components) may lead to methanol production (Yousefi *et al.*, 2018). Thus, omitting the wooden structure of the plant and enzyme deactivating by the distillate's pasteurization would lead to lessen the methanol and ethanol concentrations in the end-product (Mousavi *et al.*, 2011). Other important factors in their production are the maceration method (i.e., soaking in water), soaking time, plant health, plant varieties, storage duration, and temperature (Kazaz *et al.*, 2009, Mousavi *et al.*, 2011, Yousefi *et al.*, 2018). Methanol is a poisonous volatile

liquid that can potentially cause symptoms like optic nerve damage, abdominal pain, diarrhea, metabolic acidosis, hypotension, seizures, and coma (Shirani *et al.*, 2018). Owing to its significant toxicity, as well as frequent production and prescription of herbal distillates, quality control of these products is greatly recommended (Shirani *et al.*, 2018). ISO (No. 1487) sets 100 ppm and 2500 ppm as the maximum acceptable level for methanol and ethanol in Rosewater, respectively (Shirani *et al.*, 2016). In the current study, the mean methanol and ethanol levels in the tested samples were, respectively, 1.25 ± 0.7 ppm and 700.0 ± 100.0 ppm, which were significantly ($P < 0.001$) lower than the Iranian standard level. Delirrad *et al.* determined that the lowest mean content of methanol among the tested herbal distillates produced in Urmia (Iran) was found in Rosewater (72.4 ± 32.1 ppm) (Delirrad *et al.*, 2012). Karimi *et al.* also measured the methanol contents in 10 types of herbal distillates, including Rosewater, in Mashhad, Iran (Karimi *et al.*, 2008). They declared that the methanol contents were lower than legal limits in 18 samples. Yousefi *et al.* reported that the maximum ethanol level was for Rosewater (3900 ppm) in Mashhad (Iran), which excess from the standard level, and is not in line with the present study (Yousefi *et al.*, 2018). Mousavi *et al.* also quantified methanol values in traditional herbal waters of various brands in Mashhad, Iran (Mousavi *et al.*, 2011). They found that methanol contents in all herbal distillates, particularly in Rosewater, was very high that may cause toxicity in human using these products frequently, for a long time. The highest methanol content was measured in concentrated Rosewater (1017.41 ± 59.68 ppm).

Conclusion

Considering the fact that methanol and ethanol are highly toxic, regular consumption of Rosewater, particularly those with high concentrations, can cause significant complications. It is thus required to improve all processing steps, from sorting of plants to the

distillation, to reduce their concentrations. The finding revealed that there is no health risk to methanol and ethanol by Rosewater consumption, since their mean concentrations were significantly lower than the Maximum Residues Levels.

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Conflict of interest

The authors declare that there is no conflict of interest.

Authors' contribution

Yazdanfar N and Zienali T designed the study and guided the analyses; Sadighara P conducted the experimental research and data analysis. Mohamadi S prepared the manuscript's first draft. All authors reviewed the paper and confirmed it.

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