



Comparison of Two Different Traditional Methods of Rose Oil Preparation in Terms of Physicochemical Factors

Leila Mohammad-Taghizadeh Kashani¹, Mohammad Reza Memarzadeh², Alireza Hatami³,
Meysam Shirzad⁴, Mohammad Mahdi Ahmadian-Attari¹

¹ Department of Traditional Medicine, Medicinal Plants Research Center of Barij, Kashan, Iran

² Department of Formulation Medicinal Plants Research Center of Barij, Kashan, Iran

³ Department of Phytochemistry, Medicinal Plants Research Center of Barij, Kashan, Iran

⁴ Department of Traditional Medicine, School of Traditional Medicine, Tehran University of Medical Sciences, Tehran, Iran

Received: 8 Jun 2015

Revised: 12 Aug 2015

Accepted: 20 Aug 2015

Abstract

Rose oil (Rowghan-e Gol) is an Iranian traditional medicine used both topical and systemic in gastritis, inflammatory bowel disease, headache, and hemorrhoids. Traditional manuscripts have reported two different methods of preparation for this medicine; the first is macerating rose petals in sesame oil for 25 days under sunlight (R1), and the second is extracting rose petals by squeezing and then boiling the mixture of the extract with sesame oil to evaporate aqueous part (R2). The aim of this article was to study both traditional methods of rose oil preparation in terms of physicochemical factors to evaluate which method is best for industrializing. For this purpose, total phenolics (based on gallic acid), total essential oils (based on citronellol), thin layer chromatography (TLC) profile of the constituents, and oil rancidity indices, i.e., acid and peroxide values were determined through spectrophotometer, gas chromatograph, TLC, and titration, respectively. R1 had greater amounts of total phenolics (0.05% vs. 0.01%). The amount of its essential oil was 15.5 times higher than R2. TLC profiles showed that R1 had one more spots ($R_r = 0.04$) representing flavonoids (according to natural product indicator). About oil rancidity indices, both samples were in standard ranges but all indices of R1 were greater than R2. It could be due to long exposure of R1 to sunlight. According to the results, R1 had more amounts of flavonoids and essential oils. These compounds are considered as therapeutic agents of rose oil. Therefore, R1 is a more preferable than R2. Appropriate antioxidants should be utilized to protect R1 against sunlight oxidation.

Keywords: Rose Oil, Iranian Traditional Medicine, Essential Oil, Acid Value, Peroxide Value, Total Flavonoids, Citronellol

Citation: Mohammad-Taghizadeh Kashani L, Memarzadeh MR, Hatami A, Shirzad M, Ahmadian-Attari MM. Comparison of Two Different Traditional Methods of Rose Oil Preparation in Terms of Physicochemical Factors. Trad Integr Med 2016; 1(2): 69-74.

Corresponding Author: Mohammad Mahdi Ahmadian-Attari
Email: ahmadian.attari@gmail.com

1. INTRODUCTION

Damask rose (*Rosa damascena*), which is known in Iranian traditional medicine (ITM) as Garden rose (Gol-e Sorkh-e Bostani), has a long history of use in Iranian cuisine and traditional medicine [1]. Rhazes (9th and 10th century), Avicenna (10th and 11th century), and more recent traditional physicians like Mo'men Tonekaboni (17th century) and Aghili Khorasani (18th century) have mentioned numerous therapeutic indications for petals and stamens of this kind of rose including general tonic, exhilarant, laxative, and stringent [2], [3], [4], [5]. Rose water, which is the hydrosol portion of the distillate of rose petals, has been prescribed by these physicians and has been also used in Iranian cooking [6]. Rose petals have been the main and pharmaceutically active part of many traditional pharmaceutical products such as Golangabin, rose syrup, and rose tablets [6]. Rose oil (Rowghan-e Gol) has been another pharmaceutical product of rose petals [6], [7], [8].

In ITM, rose oil is referred to the oil extract of rose petals. Rose oil has been traditionally considered as an anti-inflammatory drug and therefore, it is prescribed in inflammatory diseases including gastritis, inflammatory bowel disease (IBD) (Sahaj ul-Am'a), and proctitis (Zahir). Topical application of rose oil is recommended for treatment of wounds and burning. Applying rose oil to forehead is also prescribed for a headache and insomnia [2], [6].

There are two main methods for preparation of rose oil in ITM: the first is to macerate rose petals in sesame oil under sunlight. In this method, rose petals are changed with fresh ones every 5 day for 25 days [6]. The second method is to extract rose petal juice by squeezing and then to boil the mixture of the extract with sesame oil (1:1) until evaporation of aqueous part [8]. Although the indication of use of both kinds of rose oil is mentioned the same, compared with the other, the quality of each kind and the preference of each method is controversial. Most of the ITM manuscripts introduce the first method (extraction under

sunlight) as a routine method [2], [6], [7], [8] but a few one prefer the second method (extraction via boiling) [9]. On the other hand, Iranian traditional pharmaceuticals should be moved toward industrialization to survive in competition with chemical ones. In this way, selecting the best method for producing traditional formulations is critical. This method should be cost-benefit, simple, and repeatable. The aim of this article was to study both traditional methods of rose oil preparation via physicochemical factors to evaluate which method is best for industrializing.

2. METHODS

Sesame oil was obtained from Barij Essence Pharmaceutical Co. Rose petals were obtained from the Research Farm of Golkaran Agribusiness.

2.1 Rose Oil Preparation

2.1.1 Method I

A total of 250 g of fresh rose petals in five 50 g batches were macerated in 300 g sesame oil in for 25 days under sunlight. After each 5 days, the previously soaked petals were brought out from the oil and pressed until all of the remaining oil is extracted. Then, the oil was weighted and if it was < 300 g, a diminution was substituted by fresh oil. This process was done in a closed cubic glass container under the sunlight of April. After the last maceration step, the oil was filtered and kept in a closed container in a cool dark place.

2.1.2 Method II

About 250 g of fresh rose petals was grinded and extracted by pressure to obtain 100 rose petals extract. This extract was added to 100 g sesame oil and was heated slowly until the mixture boiled and the aqueous part was evaporated. The remaining oily part was then filtered and kept in a closed container in a cool dark place.

2.2 Determination of Total Phenolic Content (TPC)

1.5 g of each kind of rose oil samples was transferred into a test tube with cap and added 2.5 ml of 70 ethanol. After 10 minutes in

water bath condition, the mixtures were centrifuged at 4000 rpm for 5 minutes to separate ethanolic phase from oily one. The oily phase was extracted again by another 2.5 ml of 70 ethanol with the same protocol. Both ethanolic phases were unified and then, 2 ml of distilled water and 0.25 ml of Folin–Ciocalteu reagent were added to 0.2 ml of this ethanolic phase and the mixture was shaken well. After 2 minutes, 0.5 ml of sodium carbonate solution (20% w/v) was also added to the mixture and the final volume was adjusted to 5 ml by adding distilled water. The samples were mixed thoroughly and allowed to stand at ambient temperature for 2 hours until the characteristic blue color developed. The absorbance of reaction mixture was measured at 760 nm. Quantification of TPC was based on a standard curve generated with gallic acid at 760 nm using the following equation:

$$\text{Abs} = 0.0011C + 0.0215$$

Where, Abs is absorbance and C is the concentration ($\mu\text{g/ml}$) of gallic acid. All tests were conducted in triplicate and averaged. The results were expressed as percentage of TPC in sample as gallic acid equivalents.

2.3 Quantification of Citronellol

Citronellol as one of the main components of rose essential oil was determined through gas chromatography (GC). 50 mg of rose oil was mixed with 300 ml of distilled water in a one-liter balloon. 0.5 ml of xylon in octan mixture (10 μl in 2 ml) was added to the top of Clevenger apparatus. The volatile fraction was extracted by steam-distillation during 4 hours. Then, solvent-containing oil was collected and injected 3 times to GC (Column: cp8; oven: 50-230° C, 3° C/minutes; injector: 230° C; detector: 250° C; split: 50; injector volume: 1 μl) and the ratio of area under curve of citronellol to xylene was calculated.

2.4 Determination of Acid Value

The acid value is the number of mg of potassium hydroxide required to neutralize the free fatty acids in 1 g of the substance. To

do this titration, equal volumes of ethanol 96° and diethyl ether were merged and the mixture was neutralized by potassium hydroxide (0.1 N) in the presence of 0.5 ml of phenolphthalein indicator. 50 ml of this solution was poured on 10 g of each sample, shaken well, and titrated by potassium hydroxide (0.1 N) until a 15 seconds persistent pink color is observed. The acid value was calculated from the following formula:

$$\text{Acid value} = 56.11 V \times N/W$$

Where, V is the volume of potassium hydroxide (ml), N is the normality of potassium hydroxide, and W is the weight of the oil (g).

2.5 Determination of Peroxide Value

About 5 g of each oil was poured into a 250 ml glass-stoppered conical flask, and was mixed by 30 ml of a mixture of glacial acetic acid and chloroform (2:3) until the sample were completely dissolved. 0.5 ml of saturated potassium iodide solution was added to this solution and shaken occasionally for 1 minute. Then, 30 ml of water was added, and the mixture was gradually titrated by 0.01 M sodium thiosulfate until the yellow color disappears. 5 ml starch solution was added in this step and while the mixture was shaking vigorously, titration was continued until the blue color disappears. These steps were also done for blank.

The peroxide value was calculated from the following formula:

$$\text{Peroxide value} = [1000(V_T - V_B) N]/W$$

Where, W is weight of the oil (g), N is the normality of sodium thiosulfate, V_T is the ml of 0.01 M sodium thiosulfate used for the sample, and V_B is the ml of 0.01 M sodium thiosulfate used for blank.

2.6 Thin Layer Chromatography (TLC)

1 g of each oil was extracted by 2.5 ml of methanol in two steps. The extracts were evaporated and dissolved again in 1 ml of methanol. Then, each of the samples was spotted on aluminum TLC plate 60F-254 (Merck, Germany) and run with the mobile

phase of toluene: ethyl acetate (9:1). TLC profiles were detected under ultraviolet (UV) 254 nm and 366 nm and also under the visible light by the spraying of 4% solution of sulfuric acid in ethanol. To detect flavonoid compounds, natural product (NP), reagent was used.

3. RESULTS

The results showed that in comparison with boiling rose petal juice in sesame oil (Method II), macerating rose petals in sesame oil under the sunlight (Method I) resulted in a product with more phenolic compounds (0.05% vs. 0.01%). GC revealed that the rose oil produced by Method I contains more volatile compounds than the other (0.0062% vs.

0.0004%). It means that the essential oil in rose oil of Method I is 15.5 times higher than that of rose oil from Method II. These results along with organoleptic properties of both samples are presented in table 1. TLC showed that rose oil which prepared by Method I has one spot more in its TLC profiles with R_f of 0.04. Using NP indicator revealed the nature of this spot as flavonoid. Figure 1 presents the TLC profile of both samples in comparison with the extractor, sesame oil. About oil rancidity indices, as shown in table 2, both samples were in standard ranges but all indices of rose oil of Method I were worse than rose oil of Method II. Long exposure to sunlight is probably responsible for this happening.

Table 1. Comparing two kinds of rose oil via detecting organoleptic and physicochemical properties

Tested samples	Appearance	Odor	Phenolic compounds* (%)	Essential oils [†] (%)
Rose oil produced via Method I	Yellow	Rose scent	0.05	0.0062
Rose oil produced via Method II	Light yellow	Rose scent	0.01	0.0004
Sesame oil	Light yellow	Sesame oil odor	-	-

*Phenolic compounds are measured based on gallic acid, [†]Essential oils are measured based on citronellol

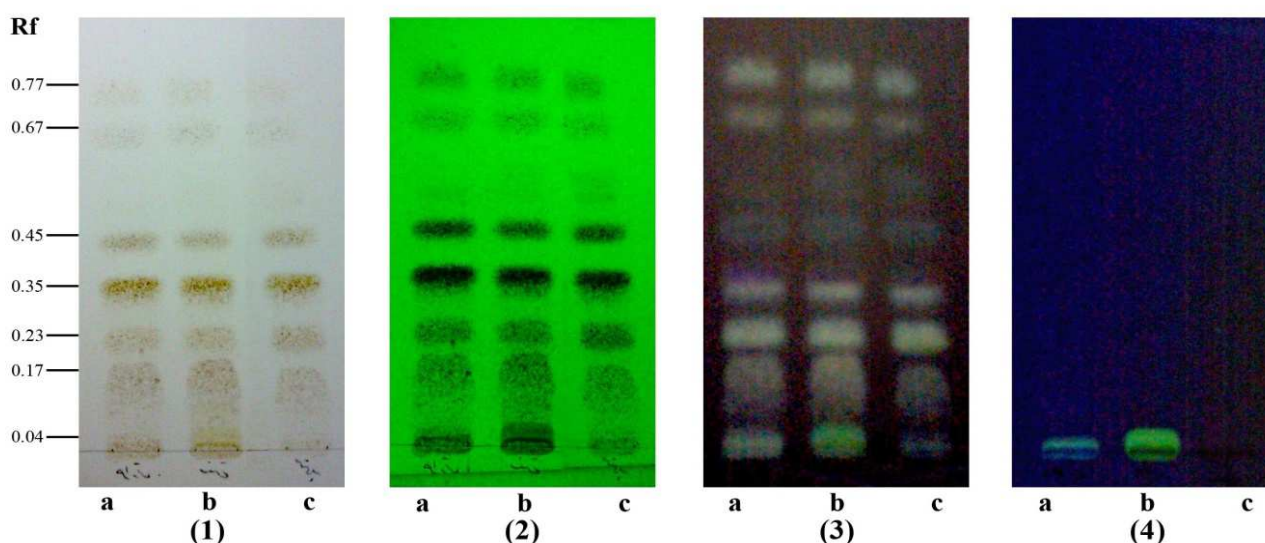


Figure 1. Thin layer chromatography (TLC) profile of different samples of rose oil and sesame oil as the extractor: (1) TLC under visible light, (2) under ultraviolet (UV) 254 nm, (3) under UV 366 nm, (4) under UV 254 nm after spraying natural product (NP) reagent; (a) Rose oil produced via Method I, (b) rose oil produced via Method II, (c) sesame oil, In TLC 4, (b) has one spot more at R_f 0.04 which was identified as flavonoids via NP reagent

Table 2. Acid and peroxide value of two kinds of rose oil and the extractor, sesame oil

Tested samples	Acid value	Peroxide value
Rose oil produced via Method I	2.35	11.26
Rose oil produced via Method II	1.56	3.18
Sesame oil	1	2.5

4. DISCUSSION

Before discussing about the results, we should clarify the differences of our meaning of rose oil – which is the literal translation of Persian term Roghan-e Gol - with the common term of “Rose oil” in western articles. The western usage of the term rose oil is usually referred to volatile parts of rose petals especially essential oils. This kind of rose oil is always produced via steam distillation [10]. The method of preparation and the major components of Iranian rose oil, however, are completely different from western kind. The main active compounds of Iranian rose oil are both flavonoids and essential oils. Flavonoids are well-known herbal metabolites which are responsible for most of the anti-inflammatory effects of herbs [11]. Extensive prescription of Iranian rose oil in inflammatory conditions such as gastritis, alimentary ulcers, IBD, proctitis, wounds, and burning is vindicated by presenting flavonoids in this kind of rose oil. However, based on recent investigations, western rose oil – which contains essential oils of rose petals-, has no anti-inflammatory activities [12].

Rose oil is one of the important medicines of ITM with wide spectrum medical properties in inflammatory conditions [2], [6]. Combinations of rose oil with other herbs extent its therapeutic activities. For example, a combination of rose oil with leek extract makes a pharmaceutical product with anti-hemorrhoid activity [13]. Extensive indications of rose oil encourage Iranian herbal pharmaceutical industries to industrialize this medicine. In this study, we tried to compare the advantages and disadvantages of two traditional methods of rose oil preparation.

Based on our results, macerating rose petals in sesame oil under sunlight can extract more amounts of flavonoids and essential oils and therefore, this kind of rose oil is therapeutically more potent than the other. The reasons of this fact are related to

sufficient exposing time of rose petals to the extractor (sesame oil), warmth of the sunlight, and closed system of extraction which prevents evaporation of volatile parts.

Despite the advantages, the maceration method has some disadvantages, too. One of the main problems of this method is related to sunlight. In fact, in this case, sunlight acts as a double-edged sword. On one hand, it provides mild heat which increases the yield of extraction, and on the other hand, it oxidizes sesame oil and elevates the oil rancidity indices. Therefore, elimination of sunlight by using opaque containers is suggested.

Another solution for preventing oxidation is to add appropriate antioxidants. Sesame oil is naturally resistant against oxidation because it contains some natural antioxidant compounds like sesamol and sesamolol [14]. Recent investigations have indicated that some compounds like vitamin E synergistically increase the anti-oxidant effects of sesamol and sesamolol [15]. Thus, the second suggestion is to add these kinds of the compound to sesame oil before starting the extraction to prevent spoilage of the oil.

As a conclusion, macerating rose petals in sesame oil under sunlight makes this rose oil preparation better than the other in term of flavonoids and essential oils contents but a long period of exposure to sunlight elevates acid and peroxide indices in this preparation. Adding an antioxidant, like vitamin E, to sesame oil before starting the extraction and extracting in the dark container are suggested.

5. CONFLICT OF INTERESTS

Authors have no conflict of interests.

6. ACKNOWLEDGMENTS

This study was done by the financial support of Medicinal Plants Research Center of Barij, Kashan, Iran. The authors would like to thank the late Dr. Nima Shariatpanahi, Former Head of Department of Traditional Medicine, Medicinal Plants Research Center of Barij, for his contribution.

REFERENCES

- [1] Daryabandari N. *Ketab -e- mostatab e ashpazi: az sir ta piaz*. Karnameh, Iran: Karnameh Publications; 2011. [In Persian].
- [2] Zakariya al-Razi M. *Al-Hawi fi Al-Tibb*. Tehran, Iran: Academy of Medical Sciences of the Islamic Republic of Iran; 2005. [In Persian].
- [3] Avicenna. *Canon of medicine*. New Delhi, India: Jamia Hamdard; 1998.
- [4] Momen Tonekaboni SM. *Tohfa Tul Momineen*. Tehran, Iran: Shahr Publications; 2007. [In Persian].
- [5] Aghili MH. *Makhzan Al-Advieh*. Tehran, Iran: Secretariat of the Council of the Cultural Revolution; 1992. [In Persian].
- [6] Aghili Khorasani MH. *Qarabadin-e Kabir*. Tehran, Iran: Mahmoudi Publications; 1999. [In Persian].
- [7] Ghaieni Heravi MS. *Gharab al-Din Salehin*. Tehran, Iran: Chogan Publications; 2013. [In Persian].
- [8] Nazem Jahan MA. *Qarabadin-e A'zam*. Tehran, Iran: Research Institute for Islamic and Complementary Medicine (RICM); 2004. [In Persian].
- [9] Sekandarpouri A. *Yaghoti*. Tehran, Iran: Research Institute for Islamic and Complementary Medicine (RICM); 2003. [In Persian].
- [10] Ody P. *The complete guide to medicinal herbs*. Toronto, Canada: Key Porter Books; 1993.
- [11] Gonzalez-Gallego J, Sanchez-Campos S, Tunon MJ. Anti-inflammatory properties of dietary flavonoids. *Nutr Hosp* 2007; 22(3): 287-93.
- [12] Hajhashemi V, Ghannadi A, Hajiloo M. Analgesic and anti-inflammatory effects of *rosa damascena* hydroalcoholic extract and its essential oil in animal models. *Iran J Pharm Res* 2010; 9(2): 163-8.
- [13] Aghili Khorasani MH. *Kholasat al-Hikmah*. Qom, Iran: Esmaelian Publications; 2006. [In Persian].
- [14] Lee J, Lee Y, Choe E. Effects of sesamol, sesamin, and sesamolin extracted from roasted sesame oil on the thermal oxidation of methyl linoleate. *LWT - Food Science and Technology* 2008; 41(10): 1871-5.
- [15] Ghafoorunissa, Hemalatha S, Rao MV. Sesame lignans enhance antioxidant activity of vitamin E in lipid peroxidation systems. *Mol Cell Biochem* 2004; 262(1-2): 195-202.