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Analytical Methods

# Comparison of essential oil compositions of *Salvia mirzayanii* obtained by supercritical carbon dioxide extraction and hydrodistillation methods

Yadollah Yamini [a], Mostafa Khajeh [b,*], Ensieh Ghasemi [a], Mehdi Mirza [c], Katayon Javidnia [d]

[a] *Department of Chemistry, Tarbiat Modares University, P.O. Box 14115-175, Tehran, Iran*
[b] *Department of Chemistry, University of Zabol, P.O. Box 98615-538, Zabol, Iran*
[c] *Research Institute of Forests and Rangelands, P.O. Box 13185-116, Tehran, Iran*
[d] *Department of Medicinal Chemistry, Faculty of Pharmacy, The Medical Sciences University of Shiraz, Shiraz, Iran*

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## Abstract

Essential oil of *Salvia mirzayanii* cultivated in Iran was obtained by hydrodistillation and supercritical (carbon dioxide) extraction methods. The oil was analysed by capillary gas chromatography using flame ionization and mass spectrometric detections. The compounds were identified according to their retention indices and mass spectra (EI, 70 eV). The effects of different parameters such as pressure, temperature, modifier volume and extraction times (dynamic and static) on the supercritical fluid extraction (SFE) of *S. mirzayanii* oil were investigated. The results showed that, under a pressure of 35.5 MPa, temperature of 35 °C, 6% methanol, dynamic extraction time of 50 min and static extraction time of 30 min, extraction was more selective for the linalyl acetate. Thirty four compounds were identified in the hydrodistilled oil. The major components of *S. mirzayanii* were linalyl acetate (7.6%), 1,8-cineole (8.0%), linalool (9.0%) and 8-acetoxy linalool (11.0%). However, by using supercritical carbon dioxide in optimum conditions, only three components contain more than 63% of the oil. The yield of the obtained oil based on hydrodistillation was 2.20% (v/w). Extraction yield based on the SFE varied in the range of 1.50–9.67% (w/w) under different conditions. The results revealed that, in Iranian *S. mirzayanii* oil, linalyl acetate is a major component.

*Keywords:* *Salvia mirzayanii*; Supercritical carbon dioxide; Hydrodistillation; Essential oil; Linalyl acetate

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## 1. Introduction

The *Salvia mirzayanii* is a wild-growing flowering plant belonging to the family *Salvia* (Lamiaceae) and is found in the south of Iran. It is a herbaceous biennial or perennial plant. Its height is in the range of 25–40 cm. The plant was collected from Hormozgan Province (Hormozgan, Iran), where the annual rainfall is about 521 mm and the relative humidity is 66%. The climate is hot and humid. Several species of *Salvia* are used in folk medicine as antiseptics, astringents and spasmolytics (Newall & Anderson, 1996). Many studies indicated antioxidant, antimicrobial and antiviral activities of some *Salvia* species (Javidnia, Miri, Kamalinejad, & Nasiri, 2002; Sivropoulou, Nikolaou, Papanikolaou, Kokkini, Lanaras, & Arsenakis, 1997). Salvia with extraordinary flavoring properties is a very powerful spice utilized in barbecues, fried meat, liquors, cheese, vegetable dishes and soup (http://www.aisef.org/spices/sage.htm, 2007).

Supercritical fluid extraction (SFE) has gained increasing attention over the traditional techniques, like hydrodistillation and solvent extraction, in the recovery of edible and essential oils, as the use of a non-toxic and volatile fluids in SFE, such as $CO_2$ protects extracts from thermal

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[*] Corresponding author. Tel.: +98 542 2232961; fax: +98 542 2226765.
*E-mail address:* m_khajeh@zabol.ac.ir (M. Khajeh).

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degradation and solvent contamination (Papamichail, Louli, & Magoulas, 2000).

In view of increasing environmental and health concern about the use of organic solvents in the extraction of natural products, there has been growing interest in using supercritical fluids. SFE has been demonstrated to be a valuable alternative, because it requires less solvent, has a short extraction time and a capability to extract thermally labile compounds under mild conditions. In addition, by selecting the fluid polarity and/or density, the solvating power of the fluid can be adjusted for selective extraction, and extraction fluids can be removed from the fractions by decompression into a suitable collection device. SFE has been applied to a wide range of non-polar biologically active constituents from natural products, including essential oils, other flavor and fragrance compounds, medicinal compounds, carotenes and alkaloids, but there is still a shortage of information on the extraction of more polar compounds (Cao, Tian, Zhang, & Ito, 2000; Manganiello, Rios, & Valcarcel, 2000; Tonthubthimthong, Chuaprasert, Douglas, & Luewisutthichat, 2001).

A limitation of supercritical $CO_2$ is that it often fails in quantitative extraction of polar analytes from solid matrices, because of the poor solvating power of this fluid and the insufficient interaction between supercritical $CO_2$ and matrix (Careri et al., 2001; Rostagno, Araujo, & Sandi, 2002). The addition of an organic modifier can greatly improve the extraction efficiency by increasing solubility of the analytes, by reducing their interaction with the sample matrix or by inducing matrix modification, in this way; release of the analytes from the matrix can be advantageously enhanced (Hawthorne & Miller, 1987, 1994; Langenfeld, Hawthorne, Miller, & Pawliszyn, 1994).

The aim of the present work is the investigation of the effects of different parameters such as pressure, temperature, modifier volume and dynamic and static extraction time on the supercritical fluid carbon dioxide extraction of *S. mirzayanii*. The essential oil obtained by hydrodistillation was used for comparison. An experimental design procedure was used to investigate the effects of five parameters on the SFE performance: temperature and pressure of the supercritical fluid, dynamic and static extraction time and percentage of methanol added as the modifier to the $CO_2$. To the best of our knowledge, no report has yet appeared on the SFE of *S. mirzayanii.*

## 2. Materials and methods

### 2.1. Plant material

A lot of plant materials were collected from Bastak in Hormazgan province (Hormozgan, Iran) during the flowering stage. The sample was collected in June 2005 when the ambient temperature was about 40–45 °C. The aerial parts were air-dried at ambient temperature in the shade and mixed well. Immediately prior to SFE, the sample was ground in a blender to produce powder.

### 2.2. Reagents

HPLC grade dichloromethane and methanol were purchased from Merck (Darmstadt, Germany). Carbon dioxide (99.99% purity), contained in a cylinder with an eductor tube, was obtained from Sabalan Co. (Tehran, Iran).

### 2.3. Hydrodistillation

The plant (40 g of dried material) was submitted to hydrodistillation for 5 h, using a Clevenger-type apparatus, according to the *European Pharmacopoeia* (European Pharmacopoeia).

A simple Clevenger-type apparatus contained a 1000 ml flask, a condenser and a measuring tube with stopcock. A return tube for the aqueous part of the distillate connects the bottom of the measuring tube and the vertical tube. A 40 g shade dried aerial part of the plant was exposed to the assembly described above. The flask was filled with 700 ml of distilled water and heated with a heating mantle. The volatile distillate was collected over anhydrous sodium sulphate and refrigerated till time of analysis. The yield of the oil (based on three replicate extractions) was $2.20 \pm 0.05\%$ (v/w) based on the dry plant weight.

### 2.4. Supercritical fluid extraction

A Suprex MPS/225 system (Pittsburgh, PA) in the SFE mode was used for all the extractions. The extraction vessel was a 10 ml stainless steel vessel. Supercritical fluid extractions were conducted at pressures of 10.1, 20.3, 25.3, 30.4 and 35.5 MPa and temperatures of 35, 45, 55, 65 and 75 °C for durations of 15, 20, 25, 30 and 35 min static, followed by 10, 20, 30, 40 and 50 min dynamic. A Duraflow manual variable restrictor (Suprex) was used in the SFE system to collect the extracted analytes. To prevent sample plugging, the restrict point was warmed electrically. The supercritical $CO_2$ flow rate through the Duraflow restrictor was approximately 0.3–0.4 ml/min (compressed). Plant powder (1.5 g) mixed well with 2 mm diameter glass beads, and then was charged into the 10-ml extraction vessel. The essential oil was extracted from the plant using supercritical $CO_2$ under various conditions according to the Taguchi method (Roy, 1990). Table 1 shows the experimental conditions for each of the SFE runs extract. The extracted analytes were collected in dichloromethane in a 5.0 ml volumetric flask. The final volume of the extract was adjusted to 5.0 ml with dichloromethane at the end of the extraction. To improve the collection efficiency, the 5.0 ml volumetric flask was placed in an ice bath during the dynamic extraction stage. For all the modifier studies, methanol was spiked directly into the extraction vessel with the charged sample prior to the extraction.

Four milliliters of solution was poured into a 20 ml beaker. Bubbling of the solution was done by using argon gas to evaporate the solution. Then the weight of the essential

Table 1
SFE experimental conditions and extraction yields for *Salvia mirzayanii*

| No. | $T$ (°C) | $P$ (MPa) | $t_d$ (min) | $t_s$ (min) | Modifier volume (μl) | Extraction yield (w/w) |
|---|---|---|---|---|---|---|
| 1 | 35 | 10.1 | 10 | 15 | 0 | 1.51 |
| 2 | 75 | 20.3 | 20 | 15 | 3 | 2.52 |
| 3 | 45 | 25.3 | 10 | 30 | 3 | 5.13 |
| 4 | 35 | 20.3 | 30 | 20 | 4.5 | 5.28 |
| 5 | 35 | 35.5 | 20 | 30 | 6 | 9.67 |
| 6 | 55 | 10.1 | 30 | 25 | 3 | 3.00 |
| 7 | 75 | 25.3 | 40 | 20 | 0 | 4.37 |
| 8 | 75 | 35.5 | 10 | 25 | 4.5 | 5.02 |
| 9 | 65 | 35.5 | 50 | 20 | 3 | 7.94 |
| 10 | 55 | 20.3 | 50 | 30 | 0 | 5.15 |
| 11 | 65 | 20.3 | 10 | 35 | 1.5 | 2.99 |
| 12 | 45 | 20.3 | 40 | 25 | 6 | 6.69 |
| 13 | 55 | 35.5 | 40 | 15 | 1.5 | 5.35 |
| 14 | 65 | 10.1 | 40 | 30 | 4.5 | 1.58 |
| 15 | 75 | 10.1 | 50 | 35 | 6 | 1.63 |
| 16 | 55 | 30.4 | 10 | 20 | 6 | 4.97 |
| 17 | 45 | 30.4 | 50 | 15 | 4.5 | 7.07 |
| 18 | 35 | 25.3 | 50 | 25 | 1.5 | 6.23 |
| 19 | 65 | 25.3 | 30 | 15 | 6 | 8.23 |
| 20 | 35 | 30.4 | 40 | 35 | 3 | 6.37 |
| 21 | 45 | 35.5 | 30 | 35 | 0 | 3.79 |
| 22 | 45 | 10.1 | 20 | 20 | 1.5 | 2.37 |
| 23 | 65 | 30.4 | 20 | 25 | 0 | 4.52 |
| 24 | 55 | 25.3 | 20 | 35 | 4.5 | 6.20 |
| 25 | 75 | 30.4 | 30 | 30 | 1.5 | 7.75 |

oil was measured. Finally, the extraction yield was calculated.

### 2.5. GC and GC/MS analyses

GC analyses were performed using a Hewlett–Packard 5890 series II gas chromatograph equipped with an FID and a DB-5 fused silica column (20 m × 0.53 mm i.d., film thickness 1.5 μm). Oven temperature was programmed to 50 °C for 2 min, and then increased to 260 °C at a rate of 3 °C/min. Injector and detector temperatures were 260 °C and 270 °C, respectively. The carrier gas, helium, was adjusted to a linear velocity of 30 cm/s. The SFE samples (1 μl) were injected into the GC (without any further dilution) using the splitless mode. Hydrodistilled extracts were diluted 30 times and 1 μl of the diluted solution was injected into the GC. The GC/MS analysis was carried out on a Varian 3400 equipped to a DB-5 column with the same characteristics as the one used in GC. The transfer line temperature was 260 °C. The ionization energy was 70 eV with a scan time of 1 s and mass range of 40–300 amu. The percentages of compounds were calculated by area normalization method, without considering response factors. The components of oil were identified by comparison of their mass spectra with those of a computer library (Wiley) or with authentic compounds. Data obtained were confirmed by comparison of their retention indices, either with those of authentic compounds or with the data published in the literature (Sandra & Bicchi, 1987).

## 3. Results and discussion

### 3.1. General

The hydrodistillation process has been traditionally used in the extraction of essential oils at a laboratory scale. In this research, we intend to compare the efficiency of this process with its relationship to the volatile composition of the extracts from *S. mirzayanii* obtained by SFE.

### 3.2. Optimization of the SFE experimental conditions

Since various parameters potentially affect the extraction process, optimization of the experimental conditions would represent a critical step in the development of an SFE method. In fact, pressure and temperature of the fluid, percentage of the modifier and extraction times were generally considered as the most important factors. Optimization of the method can be carried out step-by-step or by using an experimental design. Table 1 shows different conditions of experiments carried out with SFE for extractions of *S. mirzayanii* according to the Taguchi experimental design. All the selected factors were examined using a five-level orthogonal array design with an $OA_{25}$ $(5^5)$ matrix. In this study, the main effects of the five most important factors on the extraction efficiency of SFE were investigated. The results of the SFE experiments based on the extraction yields are given in Table 1. The mean values of the extraction yields for the related factors at each level were calculated according to the assignment of the experiment (Fig. 1). The mean values of the five levels of each factor (e.g., pressure) reveal how the extraction yield will change when the level of that factor is changed. Fig. 1 shows the variations in extraction yield as a function of change in different levels of the factors studied. The density of $CO_2$ is an important parameter that can affect the solubility of an organic molecule in $CO_2$. The density determines the number of interactions between $CO_2$ and molecules of the organic compounds. If sufficient interactions occur, the cohesive forces between individual molecules of the organic compound are broken and solubilization will occur. Therefore, the solubilization of organic molecules in $CO_2$ will be a function of the molecular weight of the organic compound and the level of interaction between $CO_2$ and organic molecules. At 10.1 MPa, the total amount of the extract is small, but as pressure increases, the amount increases (Fig. 1), due to the increase of $CO_2$ density and consequently its dissolving ability. As temperature increases, the extraction rate decreases, due to the decrease of the solvent density. The static and dynamic extraction times of the supercritical fluid were found not to be significant as the main effect.

Due to the limited solubility of polar organic compounds in supercritical carbon dioxide, quantitative extraction of these compounds with pure supercritical $CO_2$ is impossible. The addition of a polar modifier to supercritical carbon dioxide has been shown to represent a

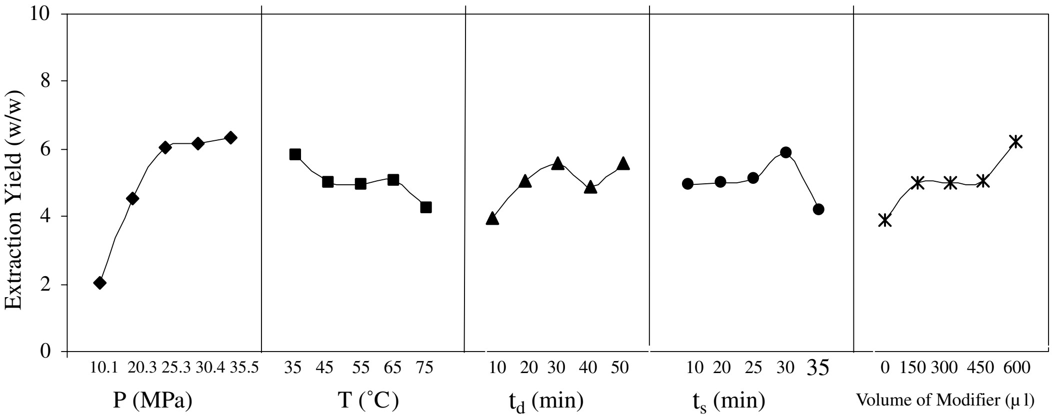

Fig. 1. Effects of temperature, pressure, dynamic and static extraction times and volume of the modifier on extraction yield.

tremendous increase in the extraction efficiency of polar organic compounds. In the present research, the modifier (methanol) enhanced the solubility of solutes in the supercritical $CO_2$ and consequently the efficiency of the extraction increased.

The SFE extracts and hydrodistillation of *S. mirzayanii* showed a relatively simple GC–MS chromatographic pattern. Detailed identification and quantization of the compounds found in *S. mirzayanii* seed oil, produced by SFE under different conditions, were performed by GC–MS, as reported in Table 2. Products obtained by hydrodistillation were also analysed by GC–MS. In addition, the results are shown in Table 2, for comparison. The major compounds were: α-thujene (0.04%), α-pinene (0.17%), myrcene (1.87%), *p*-cymene (0.35%), 1,8-cineole (8.03%), *cis*-β-ocimene (0.29%), γ-terpinene (0.09%), *trans*-linalool oxide (0.79%), linalool (9.01%), *trans*-pinocarveol (0.03%), nerol oxide (0.43%), *p*-mentha-1,5-dien-8-ol (0.57%), terpinen-4-ol (0.45%), α-terpineole (4.51%), *trans*-carveol (0.30%), nerol (0.34%), linalyl acetate (7.63%), *E*-anethole (0.51%), terpendiol (0.11%), α-terpinyl acetate (0.1%), 8-acetoxy linalool (10.97%), β-elemene (0.84%), α-gurjunene (0.38%), β-selinene (1.3%), α-muurolene (0.3%), γ-cadinene (2.0%), δ-cadinene (5.80%), cadina-1,4-diene (0.40%), α-cadinene (0.30%), α-calacorene (0.50%), spathulenol (0.20%), δ-cadinal (0.10%), farnesol (0.30%) and 14-hydroxy-α-muurolene (0.20%). Significant difference in the linalyl acetate content between the SFE and the hydrodistillation product can be notified from Table 2. However, the recovery of linalyl acetate in SFE is better than hydrodistillation. Finally, SFE shows various results in comparison with the conventional hydrodistillation procedure. Furthermore, SFE gives a better selectivity for compounds of interest; changing extraction variables is less tedious, and it requires a shorter extraction time. The major disadvantage of the oil obtained by SFE is the presence of co-extracted cuticular waxes.

Table 2 shows the components, obtained by hydrodistillation and different conditions of the SFE. The major components of oil based on hydrodistillation were linalyl acetate (7.6%), linalool (9.0%), 1,8-cineol (8.0%), 8-acetoxy linalool (11.0%), α-terpineole (4.5%), *E*-anethole (4.5%) and δ-cadinene (5.8%). In all of the oils obtained by the SFE, linalyl acetate was the major constituent. As it can be concluded from Table 2, the yield of linalyl acetate in the best extraction conditions was 41.5% in comparison with 7.6% of hydrodistillation. The recovery of linalyl acetate by the SFE is better than that of the hydrodistillation. The composition of the SFE products and hydrodistilled *S. mirzayanii* essential oils was different. Table 2 indicates that the number of the essential oil components extracted by the SFE (maximum 20 for Run 1) is lower than those obtained by the hydrodistillation method (34 components). Therefore, the SFE procedure is more selective than hydrodistillation. The hydrodistillation results achieved from the research was similar to the results obtained by Javidnia et al. (2002).

Table 3 shows analysis of variance (ANOVA) results for the calculated models. It is worthy to note that the obtained data are only valid for the studied sample. The ANOVA results of this experiment indicated that the pressure of SFE plays an important role in the SFE of *S. mirzayanii* and appears to be significant for all of analytes. This means that extraction recovery is enhanced as the pressure increases. The pressure increase causes an increase of the fluid density and thus it could have an important effect. Increase of the solvating power of the supercritical fluid is responsible for the quantitative recoveries. The effect of other parameters (i.e. temperature, static and dynamic times and modifier volume) on the studied range, on the extraction efficiency of the plant was not significant. Thus in Fig. 1 the variation of extraction efficiency due to the variation of the parameters is located in the range of standard deviation of the method.

## 4. Conclusion

In this work, the effect of the process parameters was studied in the supercritical fluid extraction of *S. mirzayanii.* The SFE method was compared with the hydrodistillation method in the extraction of the essential oil of *S. mirzaya-*

Table 2
Composition (%) of *Salvia mirzayanii* oils obtained by SFE and hydrodistillation (the compounds were listed in order of elution time from a DB-5 column)

| No. | Compound | R.I.[a] | Run 1 | Run 2 | Run 3 | Run 4 | Run 5 | Run 6 | Run 7 | Run 8 | Run 9 | Run 10 | Run 11 | Run 12 | Run 13 | Run 14 | Run 15 | Run 16 | Run 17 | Run 18 | Run 19 | Run 20 | Run 21 | Run 22 | Run 23 | Run 24 | Run 25 | H.D[b] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | α-Thujene | 931 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0.04 |
| 2 | α-Pinene | 939 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0.17 |
| 3 | β-Pinene | 980 | – | 0.03 | 0.26 | 0.56 | 0.1 | 0.09 | 0.33 | 0.1 | 0.11 | 0.4 | 0.03 | 0.05 | 0.07 | 0.24 | 1 | 0.23 | – | 0.54 | 0.11 | 0.54 | 0.6 | 1.04 | 0.07 | 0.56 | 0.27 | – |
| 4 | Myrcene | 990 | – | – | 0.08 | – | 2.1 | 2.8 | 1.9 | 1.6 | 0.7 | 1.6 | 0.92 | 0.4 | 1 | 1.5 | 2.5 | – | 0.43 | 1.2 | 2.3 | 1.3 | 0.81 | 1.64 | 0.16 | 1.1 | 0.38 | 1.87 |
| 5 | *p*-Cymene | 1026 | – | 1.6 | 0.17 | 0.33 | – | 0.1 | 0.05 | 0.05 | 0.12 | – | 0.06 | – | – | 0.12 | 0.05 | 0.16 | – | 0.06 | 0.11 | 0.1 | 0.3 | 0.4 | – | 0.26 | 0.02 | 0.35 |
| 6 | 1,8-Cineole | 1033 | 1.79[c] | 8.1 | 5.8 | 7.7 | 7.7 | 11.69 | 7.2 | 9.3 | 6.2 | 8.1 | 4.1 | 4.8 | 6.4 | 7.5 | 13.8 | 4.15 | 6.7 | 12.5 | 6.7 | 11.9 | 7.3 | 13.3 | 10.45 | 9.02 | 6.4 | 8.03 |
| 7 | *cis*-β-Ocimene | 1040 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0.29 |
| 8 | γ-Terpinene | 1062 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0.09 |
| 9 | *trans*-Linalool oxide | 1074 | – | 0.12 | 0.42 | 0.58 | 0.5 | 1.13 | 0.81 | 0.42 | 0.34 | 0.3 | 0.31 | 0.4 | 0.1 | 0.32 | 0.9 | 0.47 | 0.35 | 0.73 | 0.08 | 1.1 | 0.6 | 1.2 | 0.17 | 0.82 | 0.6 | 0.79 |
| 10 | Linalool | 1098 | – | 1.5 | 1.39 | 0.42 | 0.6 | 1.9 | 1.5 | 0.87 | 1.1 | 1.6 | 0.82 | 1.1 | 1.2 | 1.53 | 1.9 | 0.52 | 1.3 | 1.8 | 1.4 | 2.01 | 1.3 | 1.97 | 1.4 | 1.63 | 1.2 | 9.01 |
| 11 | *trans*-Pinocarveol | 1139 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0.03 |
| 12 | Neroloxide | 1153 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0.43 |
| 13 | *p*-Mentha-1,5-dien-8-ol | 1166 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0.57 |
| 14 | Terpinen-4-ol | 1177 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0.45 |
| 15 | α-Terpineole | 1189 | – | 0.05 | 0.12 | – | 0.2 | 0.07 | 0.4 | – | 0.1 | 0.08 | 0.11 | 0.34 | 0.1 | 0.26 | 0.02 | 0.3 | 0.34 | 0.53 | 0.02 | 0.44 | 0.31 | 0.52 | 0.14 | 0.34 | 0.48 | 4.51 |
| 16 | *trans*-Carveol | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0.3 |
| 17 | Nerol | 1228 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0.34 |
| 18 | Linalyl acetate | 1257 | 25.1 | 24.6 | 19.9 | 22.4 | 26.3 | 32.3 | 19.7 | 29.5 | 20.9 | 21.6 | 18.4 | 19.3 | 23.3 | 34.5 | 41.5 | 13.43 | 23.06 | 29.5 | 16.6 | 30.1 | 19.1 | 27.74 | 26.5 | 23.74 | 21.3 | 7.63 |
| 19 | *E*-Anethole | 1284 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0.51 |
| 20 | Terpendiol | 1316 | 17.11 | 7.6 | 8.7 | 9.3 | 11.03 | 8.6 | 8.7 | 10.9 | 7.4 | 9.2 | 10.1 | 8.6 | 8.8 | 6.9 | 1.2 | 5.84 | 9.3 | 9.04 | 7.6 | 7.6 | 6.9 | 7.79 | 7.3 | 6.8 | 6.2 | 0.11 |
| 21 | α-Terpinyl acetate | 1350 | 15.67 | 9.9 | 8.5 | 10.1 | 11.4 | 14.05 | 9.02 | 12.2 | 8.3 | 9.2 | 9.3 | 8.5 | 9.8 | 13.7 | 12.2 | 6.6 | 10.9 | 13 | 7.6 | 12.2 | 9.1 | 12.3 | 10.2 | 8.7 | 7.9 | 0.1 |
| 22 | 8-Acetoxy linalool | 1346 | 5.35 | 4.5 | 4.8 | 5.01 | 6.1 | 4.8 | 4.5 | 5.01 | 3.7 | 4.2 | 5.1 | 4.3 | 4.3 | 3.9 | 0.6 | 3.07 | 4.6 | 4.3 | 3.7 | 4.1 | 3.6 | 4.02 | 3.92 | 3.3 | 3.6 | 10.97 |
| 23 | β-Elemene | 1391 | 1.12 | 0.2 | 0.14 | 0.16 | 0.18 | 0.27 | 0.07 | 0.2 | 0.25 | 0.66 | 0.1 | 0.02 | 0.12 | 0.6 | 0.7 | 0.68 | 0.07 | 0.1 | 0.9 | 0.04 | 0.75 | 0.08 | 1.34 | 0.65 | 0.81 | 0.84 |
| 24 | α-Gurjunene | 1409 | 2.73 | 5.9 | 4.2 | 0.86 | 0.5 | 7.5 | 5.2 | 0.7 | 5 | 3.8 | 0.45 | 4.9 | 5.3 | 8.4 | 9.3 | 7.51 | 5.4 | 7.3 | 5 | 7.1 | 4.4 | 6.2 | 5.4 | 5.4 | 4 | 0.38 |
| 25 | α-Guaiene | 1441 | 0.67 | 1.6 | 1.1 | 5.9 | 0.07 | 0.04 | 0.5 | 0.11 | 0.3 | 0.46 | 0.54 | 0.02 | 0.92 | 2.04 | 2.5 | 0.91 | 1.2 | 0.4 | 0.3 | 0.38 | 4 | 0.16 | 1.15 | 1.4 | 1.2 | – |
| 26 | β-Selinene | 1485 | 1 | 0.4 | 0.4 | 0.39 | 0.05 | 0.21 | 1 | 0.05 | 0.8 | 0.24 | 0.5 | 0.08 | 0.2 | 0.06 | 0.07 | 0.6 | 0.21 | 0.23 | 1.2 | 0.09 | 0.73 | 0.8 | 0.11 | 0.22 | 1.1 | 1.3 |
| 27 | Germacrene D | 1488 | 1.29 | 0.04 | 0.2 | 0.14 | 0.16 | 0.15 | 1.4 | 0.19 | 0.03 | 0.43 | 0.23 | 0.6 | 0.12 | 0.12 | 1 | 0.31 | 0.2 | 0.1 | 0.5 |  | 0.72 | 1.01 | 0.26 | 1.1 | 1.8 | – |
| 28 | Germacrene A | 1493 | 0.68 | 0.16 | 0.2 | 0.2 | 0.04 | 0.55 | 0.06 | 0.11 | 0.2 | 0.25 | 0.24 | 0.11 | 0.24 | 2.1 | 1.9 | 0.76 | 0.17 | 0.45 | 0.5 | 0.46 | 0.21 | 0.35 | 0.08 | 1.53 | 1.03 | – |
| 29 | α-Muurolene | 1499 | 3.97 | 5.5 | 5.2 | 4.5 | 5.7 | 0.35 | 5.2 | 6.6 | 4.2 | 4.5 | 5.2 | 3.4 | 3.1 | 1.6 | 0.1 | 1.59 | 2.2 | 0.9 | 3.8 | 1.8 | 2.26 | 1.27 | 2.5 | 2.8 | 3.8 | 0.3 |
| 30 | γ-Cadinene | 1513 | 1.66 | 3.4 | 2.6 | 0.8 | 0.5 | 0.27 | 2.9 | 1.6 | 2.3 | 1.8 | 2.9 | 1.8 | 0.86 | 0.82 | 0.1 | 0.83 | 0.82 | 0.2 | 2.2 | 0.16 | 1 | 0.43 | 0.93 | 1.7 | 1.9 | 2 |
| 31 | δ-Cadinene | 1524 | 0.31 | 1.9 | 2.2 | 1.7 | 0.04 | 0.05 | 0.03 | 0.07 | 0.32 | 0.03 | 0.1 | 0.03 | – | – | 1.2 | 1.37 | 0.2 | 0.1 | 1.1 | 0.03 | 0.04 | 0.08 | 0.01 | 0.02 | 0.08 | 5.8 |
| 32 | Cadina-1,4-diene | 1532 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0.4 |
| 33 | α-Cadinene | 1538 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0.3 |
| 34 | α-Calacorene | 1542 | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | – | 0.5 |
| 35 | Spathulenol | 1576 | 3 | 7.3 | 6.95 | 6.5 | 7.8 | 6.3 | 7.5 | 10.1 | 5.8 | 5.8 | 8 | 6.6 | 6.5 | 5 | 0.06 | 4.95 | 6.6 | 4.2 | 7.2 | 4.2 | 3.97 | 3.27 | 5.7 | 4.68 | 5.6 | 0.2 |
| 36 | δ-Cadinal | 1636 | 1.01 | 2.4 | 4.3 | 1.5 | 0.54 | 0.3 | 0.11 | 0.23 | 1.6 | 1.8 | 2.8 | 1.8 | 2.05 | 1.04 | – | 0.53 | 1.9 | 0.83 | 2.4 | 1.2 | 3.1 | 0.92 | 3.05 | 1.24 | 3.79 | 0.1 |
| 37 | β-Eudesmol | 1649 | 0.23 | 7.2 | 7.3 | 4.8 | 8.4 | 1.5 | 5.8 | 0.07 | 4.5 | 4.7 | 7.4 | 4.8 | 4.3 | 3.2 | 0.02 | 4.25 | 2.4 | 1.13 | 0.16 | 1.9 | 3.1 | 1.55 | 2.4 | 3.8 | 0.71 | – |
| 38 | α-Eudesmol | 1653 | 0.86 | – | – | – | – | – | – | 0.05 | – | – | – | – | – | – | – | 0.57 | – | – | – | – | – | – | – | – | – | – |
| 39 | Farnesol | 1693 | 2.82 | 3.8 | 4.5 | 4.4 | 5.3 | 0.3 | 4.3 | 4.9 | 4.4 | 4 | 4.6 | 5.3 | 4.3 | 0.3 | 0.06 | 5.91 | 4 | 0.16 | 4.4 | 1.5 | 2.8 | 0.93 | 2.2 | 2.7 | 2.84 | 0.3 |
| 40 | 14-Hydroxy-α-muurolene | 1775 | 1.88 | 0.35 | 2.2 | 1.8 | 0.06 | 0.09 | 0.2 | 0.06 | 2.7 | 0.3 | 1.8 | 2.3 | 1.8 | 0.03 | 0.12 | 2.76 | 1.2 | 0.77 | 2.8 | 0.59 | 1.3 | 0.13 | 3.6 | 1.4 | 2.5 | 0.2 |

[a] Kovats retention indices on DB-5 column.

[b] Hydrodistillation.

[c] Percent of component based on the area normalization.

Table 3
ANOVA table for the experiments (at 95% confidence)

| Source of variance | Sum of square | Degree of freedom | Mean square | $F^a$ value |
|---|---|---|---|---|
| Pressure | 66.79 | 4 | 16.70 | 6.38 |
| Temperature | 6.32 | 4 | 1.58 | |
| Static extraction time | 7.02 | 4 | 1.76 | |
| Dynamic extraction time | 9.82 | 4 | 2.46 | |
| Methanol percentage | 14.40 | 4 | 3.60 | |
| Pooled error | 52.34 | 20 | 2.62 | |
| Total | 156.69 | 40 | | |

[a] $F_{\text{critical}} = 2.8661$.

*nii.* SFE showed different results in comparison with the conventional hydrodistillation procedure. In addition, SFE gives a better selectivity for the compounds of interest; changing extraction variables is less tedious and it requires a shorter extraction time. It is worthy to note that in the present study only one plant sample was used to compare the efficiency of hydrodistillation and SFE to obtain essential oils. To obtain more reliable results comparison should be done using results based on experiments on the plant samples collected independently.

The flexibility in the management of the variables involved in the SFE process permitted us to optimize the experimental conditions, considering the selectivity of a substance or classes of substances of interest. The selectivity of supercritical $CO_2$ was allowed to maximization the concentration of selected compounds. Therefore, SFE is more advantageous than the hydrodistillation for the extraction of oils from *S. mirzayanii.*

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