Parametric and kinetic modeling, chemical composition, and comparative analyses of Algerian *Mentha pulegium* L. essential oil extracted from flowers and leaves by hydrodistillation

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Abstract

*Mentha pulegium* L. is a plant with numerous health benefits that is utilized in traditional Algerian medicine. The objective of the current study was to analyze the chemical compositions of the essential oils produced by the *M. pulegium* flower and leaf parts after identifying the best hydrodistillation operating parameters and modeling the kinetic extraction. According to parametric tests, first order extraction kinetics result in yields of 1.7±0.06% (w/w) for leaves through 60 minutes and 4.00±0.10% (w/w) for flowers through 30 minutes. Five compounds were found in flowers, whereas 16 compounds were found in leaves. For leaves, a total of 15 constituents accounting for 100% of the oil were found; for flowers, a total of 5 constituents accounting for 100% of the oil were found. Predominance of oxygenated monoterpenes, such as pulegone (53.09%), menthol (12.53%), and neoisopulegol (5.7%) was determined in leaves, respectively pulegone (83.40%), isopulegylacetate (7.98%), and menthol (3.63%) in flowers. The results indicate that the experimental conditions used provided good yields in the extraction of essential oils, particularly from mint blossoms via hydrodistillation. As a result, there are options for lowering the time and energy required for mint oil extraction while still producing a high-quality product.

**Keywords:** chemical composition; essential oil yield; kinetic modelling; *Mentha pulegium* L.

Introduction

*Mentha pulegium* L. is one of the plants used in the traditional medicine of Algeria. It stimulates gastric secretions, reduces gas and colic, and combats fermentations. It is one of the best digestive drinks, beneficial in especially for those with liver failure, and eliminates intestinal worms as described by Patricia *et al.* (2019), *M. pulegium* essential oil is of particular interest due to its usage in various fields such as cosmetics, perfumery, and pharmaceutical industries (Werka *et al.*, 2007; Shahmohamadi *et al.*, 2011; Swamy *et al.*, 2015). It can be...
extracted from different parts of the plant, such as leaves and aerial parts by using distillation-extraction (Diaz-Maroto et al., 2007; Mata et al., 2015), steam distillation (El Asbahani et al., 2015), and microwave-assisted hydro-distillation (Petrakis et al., 2008).

The species of the genus *Mentha* are easy to recognize by their very characteristic smell, as much they are difficult to distinguish one from the other, because of the intermediate shapes of hybrid origin, which connects them (Benayad et al., 2008). They are represented by 18 species and about 11 hybrids, which are subdivided into subspecies, forms, varieties, sub varieties, cultivars and selections (Sutour et al., 2010). The species *M. pulegium* is very widespread in Algeria. It is a perennial plant with its rhizomes, low, 10 to 55 cm high, common in humid environments, which exudes a lemony scent. The stems with square section are more or less erect, greenish or greyish, very branched. The leaves, opposite, small (0.8-1.3 cm × 5-6 mm), are almost whole oval or oblong and provided with a short petiole, rounded base, obtuse apex. The flowers, which appear in summer, from July to September, are lilac pink, sometimes white, and are grouped in the axils of the leaves in glomeruli widely spaced along the stem (Quezel et al., 1963).

The majority of *M. pulegium* essential oils are characterized by the predominance of pulegone (Roy et al., 2018) accompanied by menthone, piperiten oxide and isopulegol (Politeo et al., 2018). Furthermore, the essential oil of *M. pulegium* is known for its antimicrobial, antifungal, insecticidal, antiviral, antioxidative, and anti-inflammatory activities (Cherrat et al., 2014; Abdelli et al., 2016; Brahmi et al., 2016; Nickavar et al., 2018).

This work was conducted firstly to study the effects of the main operating parameters such as temperature (40˚C to 80˚C), volume of water (1500 ml to 2500 ml), time after cutting (0 to 180 days) and extraction time (0 to 3h) of the hydrodistillation extraction of essential oil yield of *M. pulegium* for leaves and flowers and to evaluate the suitable of kinetic modeling study of the extraction, on the other hand this work aimed to compare the chemical composition of essential oils from leaves and flowers of the same plant *M. pulegium* and to compare the chemical composition of the essential oil of *M. pulegium* of Algeria with the chemical composition of the essential oil of *M. pulegium* of other countries. we noted that there are few studies about the yield and the chemical composition, of *M. pulegium* flowers essential oil.

**Materials and Methods**

**Plant materials**

*M. pulegium* leaves and flowers were collected in May and in August respectively. after drying the plant material (leaves, flowers), the essential oil was extracted from the flowers and leaves by hydro-distillation using a Clevenger-type apparatus (Guenther, 1972)

**Parametric study**

Based on literature many factors could affect the yield of essential oil, the factors that could affect the essential oil yield and studied in our paper are: time of extraction(min), temperature (°C), volume of water (L), time after cutting (days) and nature of organ (leaves and flowers).

The studied response is essential oil yield of *Mentha* expressed as:

\[ Y = \frac{\text{MEO}}{M} \times 100 \quad (1) \]

Where Y: the essential oil yield (%), MEO: mass of essential oil (g) and M: the vegetal matter mass (g).

**Kinetic study**

The essential oils were extracted by hydro-distillation using a Clevenger-type apparatus. 100 g of dry plant material was weighed, crushed by hand and placed in a round bottom flack with the addition water under
the temperature of 80 °C. The kinetic study was conducted to determine the time of extraction of leaves and flowers essentials oils, then to determine the yield of each oil, which were separated from water by decantation without any organic solvent. The essential oils were stored in sealed glass vials at 4 °C prior to analysis.

**Modeling kinetic study**

The mathematical model describing the kinetic of essential oil hydro-distillation is derived for a batch distillation vessel in which a plant material and water are added. For water distillation of the essential oil, the plant material is immersed in water, while for steam distillation the plant material as a porous bed is placed on a perforated plate above the water. The produced water vapor heats the plant material and carries the essential oil from the external surface of the plant particles. The mixture of water and essential oil vapors is condensed in a heat exchanger and then separates into the floral water and the essential oil. In the case of water distillation, the floral water is usually returned to the distillation vessel.

The kinetics mechanism proposed by Hervas *et al.* (2006) was used to study the extraction process under equilibrium conditions, as shown in Equation (2):

$$\frac{dc}{dt} = k (C_0 - C)$$

(2)

Where $C$ is the weight of essential oil produced, $t$ is the extraction time in hours, $C_0$ the initial essential oil present, and $k$ is the effective diffusion coefficient.

Integrating Equation (2) between the initial moment and a given point at time $t$ gives rise to Equation (3):

$$C = C_0(1 - e^{-kt})$$

(3)

For the data analysis, Equation (2) was linearized, as shown in Equation (4):

$$\ln \left(1 - \frac{C}{C_0}\right) = kt$$

(4)

**Analysis of chemical composition of essential oil**

**GC analysis**

GC analyses were performed using an Agilent 6850 GC equipped with a Flame Ionization Detector (FID) and fused non polar column DB-5 (length 30 m × 0.25 mm i.d × 0.25 µm film thickness). The oven temperature was programmed at 60 °C for 5 min, then 60 °C to 250 °C at 3 °C/min and held isothermal at 250 °C for 20 min. Injector and detector temperature were set at 250 °C and 280 °C, respectively. Helium was used as a carrier gas with a flow rate of 1 ml/min. The essential oils were diluted in hexane (1/10, v/v). The samples were injected using the split less sampling technique, and the volume of injection was 0.2 µl.

**GC-MS analysis**

The volatile compounds were analyzed by gas chromatography coupled to mass spectrometry (GC–MS). Analyses were performed on a GC/MS Varian 3900 chromatograph coupled to a Saturn 2100T mass spectrometer. The ionization mode used was electronic impact at 70 ev. We used the same conditions us GC. Most constituents were identified by comparison of their GC linear retention indices (RI), determined with reference to a homologous series of $C_8$–$C_{27}$ n-alkanes. The identification was confirmed by comparison of the mass spectral with those stored in the MS database (National Institute of Standards and Technology NIST08 and Wiley libraries) and also by comparison with mass spectra from literature data of Adams (2007). The percentage composition was calculated from the summation of peak areas of the total oil.
Results

Parametric study

Effect of parameters on yield in leaves and flowers

Effect of temperature on yield extraction in leaves and flowers: The effect of temperature on oil extraction of flowers and leaves was studied in the range of 40 °C to 80 °C, using (100g of solid material, 2000ml volume water, 60 min time of extraction and the day of storage was 7 days) (Figure 1).

Figure 1. The effect of temperature on *M. pulegium* essential oil extraction operating conditions (volume water 2000 ml, extraction time 60 min and drying 7 days)

Effect of time after cutting on yield extraction for leaves and flowers

Experiments had been conducted on four samples in different time after cutting 0, 7 days, 90 days and 180 days) by keeping the other parameters constant, Figure 2 showed the effect of time after cutting on yield of *Mentha* essential oil for flowers and leaves.

Figure 2. The effect of time after cutting on *M. pulegium* essential oil extraction operating conditions (temperature 80 °C, extraction time 60 min and water: material ratio 20 ml/g)
Effect of volume water on extraction yield for leaves and flowers

The effect of water volume on oil extraction of flowers and leaves was studied in the range of 1500 ml to 2500 ml using 100 mg of *Mentha* plant with keeping the other parameters constants (T=80 °C, t=60 min after drying 7 days) (Figure 3). 

![Figure 3](image_url)

**Figure 3.** The effect of the water and material ratio on *M. pulegium* essential oil extraction operating conditions (temperature 80 °C, extraction time 60 min and drying 7 days)

Effect of extraction time on oil yield extraction for leaves and flowers

The effect of extraction time on oil yield was studied by keeping the other parameters constant, Figure 4 shows the effect of time on extraction yield for flowers and leaves.

![Figure 4](image_url)

**Figure 4.** The effect of time extraction on *M. pulegium* essential oil yield operating conditions: (temperature 80 °C, time after cutting 7 days and water: material ratio 20 ml/g)
Modeling kinetic study

To describe the mathematical model of kinetic extraction, according to the equation three (Hervas model) we draw the graph of $\ln \left(1 - \frac{C}{C_0}\right) = kt$ and calculate the properties of graph for flowers and leaves to evaluate the proposed kinetic models and extraction phase (Figure 5).

![Graph of ln(1 - C/C0) = ft](image)

**Figure 5.** Plot of $\ln \left(1 - \frac{C}{C_0}\right) = f(t)$ Extraction time for flowers and leaves

After determine the order of reaction, we compared between the experimental and data values, Table 1 show the mathematical and experimental values of yield extraction according to the time for flowers and leaves.

| T (h)   | Leaves Experimental | Mathematical model | Flowers Experimental | Mathematical model |
|---------|---------------------|--------------------|----------------------|--------------------|
| 0       | 0                   | 0                  | 0                    | 0                  |
| 0.083   | 0.544               | 0.454              | 1.125                | 1.576              |
| 0.166   | 0.953               | 0.788              | 2.62                 | 2.539              |
| 0.25    | 1.149               | 1.035              | 3.15                 | 3.13               |
| 0.333   | 1.237               | 1.213              | 4.05                 | 4.05               |
| 0.416   | 1.363               | 1.344              | 4.05                 | 4.05               |
| 0.5     | 1.465               | 1.44               | 4.05                 | 4.05               |
| 1       | 1.702               | 1.702              | 4.05                 | 4.05               |
| 1.5     | 1.702               | 1.702              | -                    | -                  |
| 2       | 1.702               | 1.702              | -                    | -                  |

The comparison between the experimental data and the mathematical model is shown in Figure 6. After kinetic study (Figure 4), the separation time was 60 minutes for the leaves with a yield of $1.7 \pm 0.06\%$ (w/w) and 30 minutes for the flowers with a yield of $4.00 \pm 0.10\%$ (w/w).
Chemical composition of leaves and flowers of essential oils M. pulegium

Essential oils of M. pulegium were subjected to detailed analyzes by CG/MS. The constituents identified, according to their order of elution on the non-polar DB-5 column, their retention indices and their relative percentages are listed in Table 2.

Table 2. Chemical composition of leaves and flowers of essential oils of M. pulegium identified by GC and GC–MS

| Components                | RI    | % Leaves | % Flowers |
|---------------------------|-------|----------|-----------|
| 1. Ethyl-amyl carbinol    | 997   | 0.8389   | -         |
| 2. Limonene               | 1024  | 0.8137   | -         |
| 3. Neoisopulegol          | 1144  | 5.7848   | 2.4889    |
| 4. Menthone               | 1160  | 12.5386  | 3.6365    |
| 5. Neomenthol             | 1162  | -        | 2.4936    |
| 6. Pulegone               | 1226  | 53.0979  | 83.4008   |
| 7. Piperitone             | 1259  | 5.6349   | -         |
| 8. Isopulegylacetate      | 1278  | 3.9639   | 7.9803    |
| 9. Caryophyllene oxide    | 1574  | 2.194    | -         |
| 10. Humulene epoxide II   | 1604  | 1.9947   | -         |
| 11. Oleic acid            | 2142  | 0.7714   | -         |
| 12. Octadecanol acetate   | 2208  | 3.3686   | -         |
| 13. Manool oxide          | 2216  | 5.0515   | -         |
| 14. Methyl eperuate       | 2220  | 1.4374   | -         |
| 15. Methyl sandaracopimarate | 2254 | 1.4784   | -         |
| 16. Methyl communate      | 2259  | 1.0314   | -         |

RI: Retention time, OM: Oxygenated monoterpenes, SO: Oxygenated sesquiterpenes, MH: Mono hydrocarbons.
CG/MS analysis revealed a determined number of constituents for essential oils containing oxygenated monoterpenes (64.51%), oxygenated sesquiterpenes (4.18%) and mono hydrocarbons (0.81%) in leaves; oxygenated monoterpenes (85.88%), and oxygenated sesquiterpenes (7.98%) in flowers. This analysis showed that pulegone (53.09%), menthol (12.53%), neoisopulegol (5.7%) and Piperitone (5.69 %) were the most abundant components in the essential oil of leaves. Flowers essential oil was dominated by pulegone (83.40%), isopulegylacetate (7.98%) and menthol (3.63%).

Discussion

Parametric study

The results indicate that the extraction yield increases with an increase in extraction temperature, the improvements recorded were from 0.46% to 1.70% with 40 to 80 °C, respectively, for leaves and from 1.48% to 4.05% for flowers (Figure 1), this effect due to the reduction of the oil kinematic viscosity with an increasing mobility of biopolymers in cellular walls by the increasing of temperature. These results were in accordance with those reported by Tchiegang (2004) Based on these findings the temperature 80 °C was taken as optimum temperature for better yield.

The yield of essential oil had increased from 0.36% to 1.70% for leaves and from 3.91 to 4.05% for flowers after cutting and storage the materials 7 days, this means that the drying materials give better yield than fresh materials, after 7 days the extraction yield reduced. As a result, it is suggested that the material should be distilled and the storage time should not be longer than 7 days storage. Because essential oils contain many volatile compounds, materials which were stored in long times can cause the diffusion of essential oil into the air by the effect from the surrounding environmental factors such as temperature and humidity.

The results show on (Figure 3) indicated that the yield of essential oil increased by the increasing of volume water, the optimal yield obtained on 2000 ml, because when heating the mixture of water and material, the water vapor permeates the epidermis, which contains essential oils, breaks down the essential oils, and attracts the oil by steam. If the amount of water is insufficient to dissolve the colloids and salt wrapping the pouch of the essential oil, the oil is unable to escape. Using more water for extraction will cause greater diffusion of oil into the water, leading to enhanced solubility and increased the yield of soluble components, after this value the yield reduces from (4.05% to 3.95% for flowers and from 1.70% to 1.65% for leaves) this changes due that the excess water could dissolve or emulsify the oil, reducing the amount of oil yield and the economic efficiency of the distillation due to increased energy consumption and extraction duration.

The results indicated that the extraction yield increased by increasing of time to take the optimal value after 60 min for leaves and flowers (Figure 4).

The first-order kinetic model is shown through the graph in Figure 5 that shows the parameters of the model, such as k, Co and the determination coefficient were calculated from the slop and chart intercept. The results show that the determination coefficient of the model has a very high determination coefficient (R²= 0.997) with k=6.171 for flowers and (R²= 0.935) with k = 3.7534 h⁻¹ for leaves, and from these results we conclude that Harvest model is the best model to describe the hydro-distillation of M. pulegium essential oil.

The dry flowers extraction time were half the dry leaves extraction time. This is due to where the essential oil is located; it is closer to the surface of the flowers, while in the leaves they are further apart.

Analyzing the Figure 6 we noted that diffusional model based on mass trans-fer fitted very well the experimental data. The diffusional model is based on material balance across internal surface of particle assuming that the components to be extracted are uniformly distributed (First order) inside the particle and the surface resistance is negligible.

The amount of essential oils obtained from M. pulegium and the extraction time was variant compared to previous works. In Algeria, the essential oil of M. pulegium extracted from the aerial part gave a yield of 0.8% (w/w) for 4 hours (Benomari et al., 2017). The dried leaves of M. pulegium from Morocco, yield 1.66% (w/w)
of oil for 3 hours as reported by Derwich et al. (2010) and a yield of 1.9% (w/w) during the same time was found by Cherrat et al. (2014). In other studies, the yield of essential oil from Iran was 3.5% (v/w) at the time of 3 hours (Shahmohamadi et al., 2011) and the yield of essential oil from Brazil gave 0.1-0.2% (v/w) through 2 hours (Oliveira et al., 2011). In Portugal a yield of about 1.54% (w/w) through 2 hours was obtained by Stoyanova et al. (2005). Reported flowers and leaves yields of plants collected from India were 2.4 and 2.6% for 3 hours (Agnihotri et al., 2005). In Uruguay Lorenzo et al. (2002) found the yield of 1.93% (v/w), and in Czech Republic the yield ranged from 0.9-1.9% (v/w) for 2 hours (Pavela et al., 2014).

**Chemical compositions**

One of the most valuable natural organic compounds obtained from the essential oils of this plant is Pulegone (Roy et al., 2018). Pulegone was found to be one of the main constituents of *M. pulegium* oils followed by menthone in many studies (Díaz-Maroto et al., 2007; Mata et al., 2007; Shahmohamadi et al., 2011; Cherrat et al., 2014; Nickavar et al., 2018).

Determination of the chemotype and composition is fundamental. The different chemotypes of *M. pulegium* are characterized by the dominant secondary metabolite (Kimbaris et al., 2017). The pulegone chemotype is most characteristic of *M. pulegium*, but the least common is piperitone which is an isomeric compound of Pulegone. In addition, menthol, menthone, and piperitone oxide (E) chemotypes also exist as mentioned by Benomari et al. (2017) and Kimbaris et al. (2017).

These differences in the chemical composition of the *M. pulegium* essential oil can be explained by the various parameters belonging to two categories. Intrinsic ones that correspond to the species, organs and maturity of plants, as well as cultural methods, harvesting, temporal and environmental interactions (climate, soil, etc.) and, extrinsic ones including extraction, storage and packaging. According to (Vekiari et al., 2002), harvest time, plant organ, drying time, storage, fragmentation and conservation of the raw plant material before the extraction of its essential oil and on the other hand in the extraction method. These parameters are all factors frequently responsible for the variability of the chemical composition of an essential oil within a plant of the same genus and species (Hornok, 1983). In some Lamiaceae, storage for more than a day is sufficient to induce noticeable changes in their chemical composition (Bruneton et al., 2009).

The comparative studies for the essential oil of *M. pulegium* are summarized in Table 3.

**Table 3.** Comparative different studies about *M. pulegium* essential oil extraction

| Extraction procedure | Yield | Chemical composition | Proportion of chemical composition | Country | Reference |
|----------------------|-------|----------------------|-------------------------------------|---------|-----------|
| *M. pulegium* powder with 300 ml of methanol as a solvent was placed in a Soxhlet extractor for 8 h | Not mentioned | Pulegone Pepiritone Pepiritinone | 51% 13% 21% | Iran | (Jafari-sales et al., 2019) |
| Hydro-distillation using a Clevenger apparatus (3h) | Not mentioned | Pulegone carvone | 71.5% 5.6% | Morocco | (Chraibi et al., 2018) |
| Hydro-distillation using a Clevenger apparatus (90 min) | 1.56% | Cineole Pepiritinone Menthone | 14.6% 11.4% 14.9% | Tunisia | (Ben Chaaban et al., 2019) |
| Hydro-distillation using a Clevenger apparatus (3 h) | 2.2% | Pulegone Isomenthone Piperitenone | 62.3% 13.4% 4.4% | Greece | (Giatropoulos et al., 2018) |
| Hydro-distillation using a Clevenger apparatus (1 h) | Leaves 1.7% flowers 4.0% | Pulegone Isopulegylacetate Pepiritone | Leaves 53% flowers 43% Leaves 8% flowers 4% Leaves 5.6% flowers 0% | Algeria | Present work 2022 |
Conclusions

In this study, parameters involved in the hydro-distillation processing such as extraction time, temperature, solvent to solid ratio and time after cutting that have been taken for obtaining optimal operating conditions. In Algerian *M. pulegium* essential oil, the maximum yield was 1.7% for leaves and 4.05% for flowers at distillation conditions (the water:material- ratio of 20, 80 °C, 7 days after cutting and time extraction of 60 min for leaves and 30 min for lowers). The results were tested by using linear regression equation on Hervas kinetic model. This model was established to assess the correlation of experimental data of the extraction process. As compared to recent studies, the results showed that Hervas model (first order) is most suitable to describe the process of hydroditilation *Mentha* oil extraction with R² coefficient of (R²= 0.997) for flowers and (R²= 0.935) for leaves. The dry flowers extraction time were half the dry leaves extraction time. This is due to where the essential oil is located; it is closer to the surface of the flowers, while in the leaves they are further apart. CG/MS analysis revealed a determined number of constituents for essential oils containing oxygenated monoterpenes (64.51%), oxygenated sesquiterpenes (4.18%) and mono hydrocarbons (0.81%) in leaves; oxygenated monoterpenes (85.88%), and oxygenated sesquiterpenes (7.98%) in flowers. This analysis showed that pulegone (53.09%), menthol (12.53%), neoisopulegol (5.7%) and piperitone (5.69%) were the most abundant components in the essential o, especially il of leaves. Flowers essential oil was dominated by pulegone (83.40%), isopulegylacetate (7.98%) and menthol (3.63%). Through previous research, the dry leaves of a plant were used, as well as the aerial part, whether with or without flowers, and through our good results that we obtained, which allowed us to obtain good returns in less time with regard to flowers, which enabled us to flow in energy and time and high quality.

Authors' Contributions

Data curation: FB, DG and YBM. Methodology: FB and DG. Supervision: AH and BBZ. Writing - original draft: FB and DG. Writing - review and editing: FB and DG.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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