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Physicochemical characteristics and thin layer chromatography of the essential oil of spearmint: *Mentha spicata* L, from western Algeria

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Abstract

The species chosen for this study is sweet mint, *Mentha spicata* L, harvested in the wilaya of El Bayadh in Algeria. The aim of this work is to highlight the plant's identification criteria, its yield in essential oil, the various physico-chemical parameters and the composition of the essential oil using different techniques (determination of several chemical indices and establishment of the list of constituents of the essential oil using chromatographic methods).

The botanical study showed the presence of two types of "Lamiaceae" type secretory hairs. These secretory hairs are the histological support for the production and storage of essential oils.

A study of the kinetics of the extraction yield shows that distillation is rapid from 0 to 120 minutes, when the yield reaches an optimum (1.08%).

The organoleptic characteristics and physico-chemical indices obtained are in line with the standards given in the bibliography.

Thin layer chromatography revealed the presence of several constituents through the appearance of several spots of different colour and Rf, three of which were identified by comparison with controls, namely menthol, cineol and carvone.

Keywords: *Mentha spicata* L; Secretory hairs; Essential oil; Yield; Thin layer chromatography

1. Introduction

The *Lamiaceae* family is characterized by the richness of its plants in essential oils [1]. The species chosen for this study is Sweet Mint, *Mentha spicata* L, harvested in the in region of El Bayadh in the west of Algeria [2]. The aim of this work is to highlight the plant's identification criteria, its essential oil yield, the various physico-chemical parameters and the composition of the essential oil using a variety of techniques (determination of several chemical indices and establishment of the list of essential oil constituents using chromatographic methods) [3].

2. Material and methods

2.1. Plant material

The plant material consists of a batch of dried leaves of spearmint: *Mentha spicata* L, harvested in region of El Bayadh (Coordinates: 33° 40′ 49″ N, 1° 01′ 13″ E, altitude: 1313 meters) [4].

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The drug consists of the aerial parts of the plant. Drying, particularly of the leafy stems, takes place after spreading them out over a fairly large surface, in a cool, dark place at a temperature of around 25ºC. Drying time: several days (24 hours according to the European Pharmacopoeia) [4].

2.2. Method

Several steps are required to study the species harvested in order to determine its various botanical and physico-chemical characteristics.

2.2.1. Botanical study

It consists of two microscopic analyses

- ***Leaf cross-section:*** After staining using the double staining technique: iodine green + carmine alum (classic technique), where the woody elements (sclerified wall) are colored green, and the rest (cellulose wall) is carmine red, with excellent contrast [5].
- ***Leaf powder:*** After air-drying, the leaves are placed in the drying oven and dried at 100°c for a further 5 minutes. The leaves are then crushed in a mortar and sieved to obtain a fine, homogeneous powder with a green color and a very pleasant odor characteristic of mints. The powder is mounted between a slide and a coverslip in Gazet du Chatelier reagent [5].

2.2.2. Physical and chemical methods

Extraction

The operating conditions for extracting the mint essence studied are summarized in the table below:

Table 1 Operating conditions for extracting the Mint essence studied [6]

	Equipment	
Parameters	Single extractor	Pilot extractor
Temperature	100°C reached in 90 min	100°C reached in 120 min
Test sample	20 grams	25 kilograms
Extraction time	180 - 240 min.	90 - 120 min.
Refrigerant	Temperature < 18°C	

Physicochemical characteristics

- Physical parameters

This involves measuring the water content of the fresh plant and the relative density of the essence:

- *The water content of the fresh plant is obtained by determining the moisture content using the Dean-Stark apparatus [7]:*

20 mg of fresh Mint are introduced into a 200-ml flask. Toluene is added until the plant is completely immersed. Heating is electric, and the operation may last from 6 to 8 hours, until the solvent supernatant in the flask becomes stable and clear. The drops of water deposited on the walls of the cooler are collected by increasing the heat. After cooling, the volume of water is noted, enabling the moisture content to be calculated using the following expression: *H % = (V/M) x 0.998 x 100; (*H: moisture content (%). V: volume of water recovered in ml. M: mass of plant matter in mg.0.998: density of water) [7].

- The relative density (dtt) of essential oil at a given temperature is the ratio between the mass of a given volume of the sample and the mass of the same volume of water at the same temperature dtt = Mass of a given volume of this species at a given T°/ Mass of the same volume of water at this T°[8]:

Take 4 pre-weighed test tubes. To the 1st tube, add 1 ml of distilled water and weigh. To the other three, add 1 ml of essential oil per tube and weigh. Calculate the respective masses of water and essential oil (average). The relative density d_t^t is determined [8].

- Chemical parameters

This involves determining the various indices relating to essential oils

- Acid Index (AI): This number expresses in milligrams the quantity of potassium hydroxide required to neutralize the free acids present in 1g of substance (m). It measures the state of alteration of an oil.

Dissolve 10 g of substance in 50 ml of a mixture of equal volumes of Ethanol 96c and petroleum ether. Heat to 90°C to dissolve the substance to be examined. The solvent must first be neutralized with KOH 0.1M in the presence of 50 ml of Phenolphthalein.

Titrate at approx. 90°C with 0.1 M KOH: *AI = 5.610 x (n/m);* (n: is the volume of titrant) [9].

- Saponification Index (SI): This number expresses in milligrams the quantity of potassium hydroxide required to neutralize the free acids and saponify the esters present in 1 g of substance (m). In a 250 ml borosilicate glass flask fitted with a reflux condenser, place the test sample, 25 ml 0.5 M KOH and a few glass beads. Heat under reflux for 30 minutes. Add 1ml Phenolphthalein and titrate with 0.5 M hydrochloric acid (n1).

Perform a blank test under the same conditions (n2): SI =28.05 x (n2 - n1) / m [10].

- Ester Index (EI): This number expresses in milligrams the quantity of potassium hydroxide required to saponify the esters present in 1 g of substance. It is calculated from the saponification and acid indices: EI = IS – IA; [9,10].
- Unsaponifiable: These are substances, non-volatile at 100-105° C, obtained by extraction, with an organic solvent, of a solution of the substance to be examined after saponification.

In practice, an aqueous dilution of the saponification medium is extracted with ethyl dioxide; after washing and removal of the solvent, the residue is weighed. Acidimetry, i.e. the absence of significant quantities of fatty acids in the residue, must be verified [9,10].

Table 2 Operating conditions for determining the various chemical indices [8,9,10]

	Chemical indices	
Parameters	**Acidity Index AI**	**Ester Index EI**
Temperature	Ambient temperature 20°C	Boiling / 30 min
Test sample of Extracted Essential oil	2 grams	2 grams
Reagent	KOH at 0.1 mol/l KOH at 0.5 mol/l	HCL at 0.5mole/l
Indicator	Phenolphthalein (pink)	Phenolphthalein (colorless)

Thin layer chromatography (TLC)

Essential oil constituents are separated by thin-layer chromatography in a layer of silicic acid using a solvent consisting of a mixture of benzene and isopropyl ether. The components are characterized by the various colorations they give off by revelation with sulfuric vanillin, and compared with controls [5,6].

- TLC plate: Kieselgel GEL 60 F254 (20 x20 mm) Merck.
- Migrating solvent: benzene and isopropyl ether [8 parts - 2 parts] for 100 ml.
- Sample diluted 1/10th in 96° alcohol (1 ml petrol and make up to 10 ml with alcohol).
- Controls diluted to 1/100th in 96° alcohol which are: Menthol, Cineol, Carvone.
- Deposition of prepared solutions: 5 µl of diluted essence and 2.5 µl of control solutions.

- The plate is placed in the tank and development takes approximately 1 hour at 20°C. The plate is then removed from the tank to dry.
- Revelation: first, under Wood's light to trace the solvent front, and then by spraying the entire plate with Paris & Godon vanillin sulfur [5,6].

3. Results

3.1. Botanical results

The botanical study is carried out by microscopic analysis of the cross-section and leaf powder of the species studied. This microscopic analysis shows (figures 1,2)

Figure 1 Leaves of *Mentha spicata* var. nana (different stages)

3.1.1. Cross-section (Fig 2- Section (a))

- Bifacial mesophyll with a single layer of palisading parenchyma and 4 to 6 layers of lacunar parenchyma.
- Libero - woody bundle in the form of an open arch surrounded by meatus parenchyma.
- Secretory hairs with unicellular foot and elliptical unicellular head (+ + +).
- Rare secretory hairs with unicellular foot and swollen oval head composed of 3 to 8 radiating cells.
- Multi-cellular tector hairs with strangulated terminal article.
- Short single- or two-celled conical tector hairs present.

3.1.2. Powder (Fig 2- Section (b-c-d))

- Epidermal fragment with sinuous walls and striated cuticle.
- Secretory hairs with unicellular foot and elliptical unicellular head (+ + +).
- Rare secretory hairs with unicellular foot and swollen oval head composed of 3 to 8 radiating cells.
- Multi-cellular tector hair debris with strangulated terminal article.
- Absence of calcium oxalate crystals

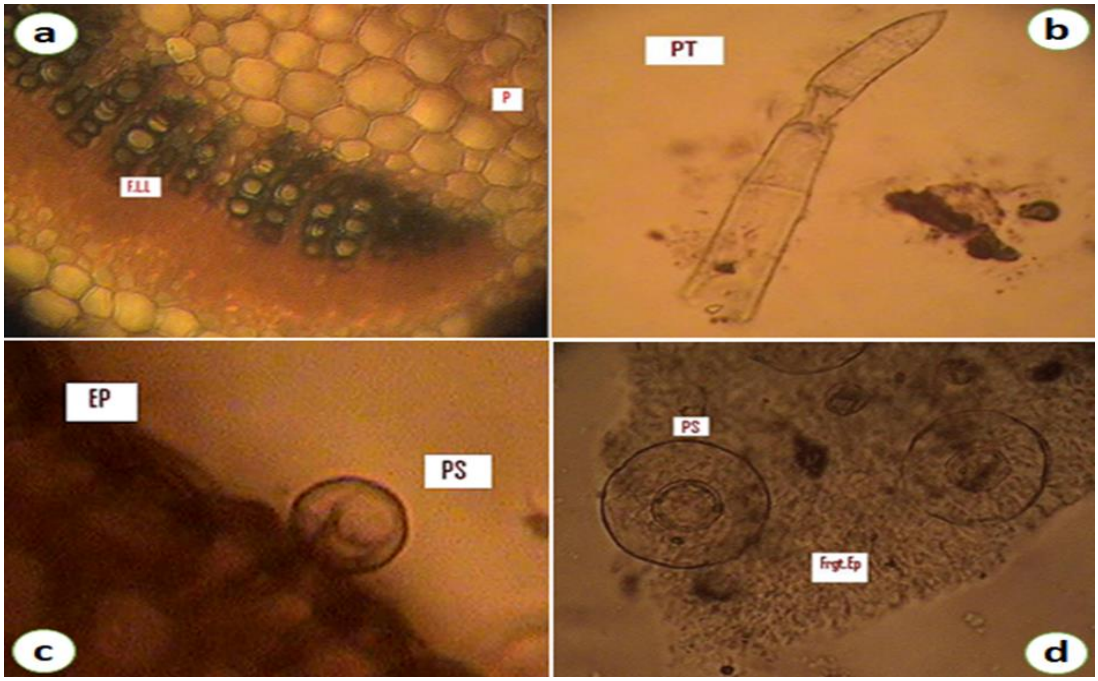

Figure 2 Microscopic elements of *Mentha spicata* leaf (Gr. x 400)

(*Mentha spicata* leaf powder: b. Tector hairs, d. Secretory hairs, front view/ Cross-section of *Mentha spicata* leaf: a. Woody bundle and parenchyma, c. Secretory hair attached to epidermis PS: secretory hair, PT: tector hair, EP: epidermis, P: parenchyma, Fgt EP: epidermal fragment, F.L.L: libero ligneous bundle (woody bundle).

3.2. Physico-chemical results

3.2.1. Extraction yield kinetics

Sequential sampling of the distillate at different time intervals enabled us to plot the evolution of extraction as a function of time, which in turn enabled us to estimate the optimum extraction time, as shown in the following table (Tab.3) and Figure (Fig.3):

Table 3 Variation in essential oil extraction yield as a function of time

Time (min)	Empty tube mass (g)	Full tube mass (g)	Essential oil mass (g)	Cumulative essential oil mass (g)	Yield (%)
0	0	0	0	0	0
5	21.07	21.38	0.31	0.31	0.38
10	20.54	20.78	0.24	0.55	0.67
15	21.22	21.34	0.12	0.67	0.82
30	21.05	21.16	0.11	0.78	0.96
45	20.76	20.79	0.03	0.81	1.00
60	21.46	21.48	0.02	0.83	1.02
90	19.66	19.68	0.02	0.85	1.05
120	21.03	21.05	0.02	0.87	1.07
150	20.74	20.75	0.01	0.88	1.08
180	20.63	20.63	0.00	0.88	1.08
240	21.01	21.01	0.00	0.88	1.08

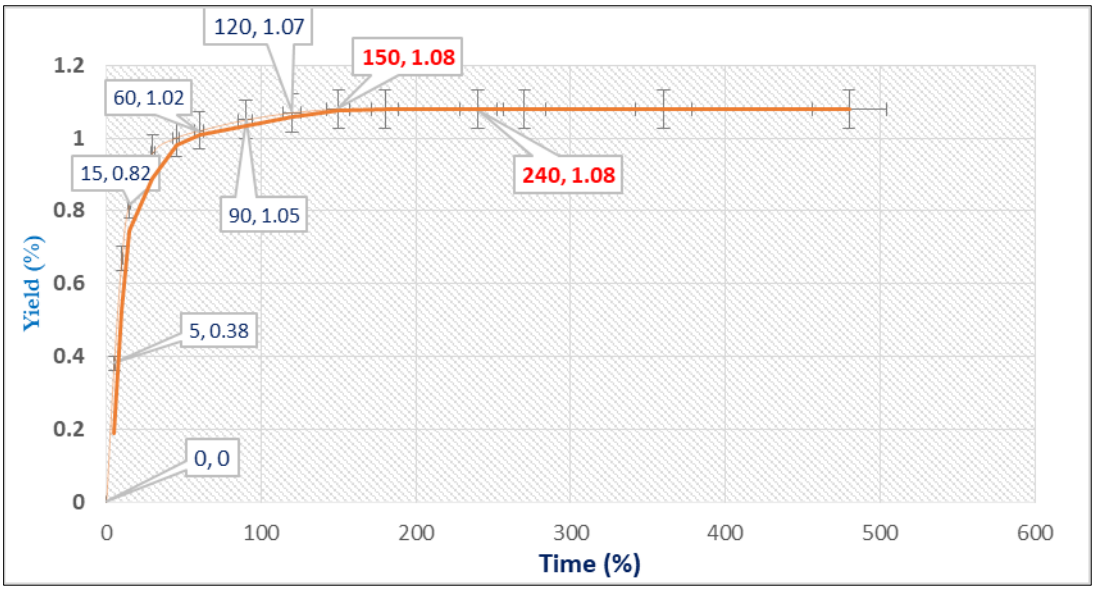

Figure 3 Kinetics of essential oil extraction yield as a function of time

The various organoleptic characteristics and physicochemical parameters are summarized in the following tables:

Table 4 Organoleptic characteristics and physicochemical parameters of MS essential oil

	Parameter	**Result**
Organoleptic characteristics	Appearance	Clear, mobile liquid
	Color	Light yellow
	Odor	Strong, pleasant (Chewing gum)
	Taste	Pungent
Physical Parameters	Moisture content	H = 19 % i.e 3,9 ml per 20 g of PE
	Essential oil content	R = 1,08 %
	Relative density at 20°C	$D_{20°c}$ = 0,934
Chemical Indices	Acidity Index	AI = 3086
	Ester Index	EI = 34,360
	Saponification Index	SI = 37,446

3.3. Results of the chromatographic study (*Thin-layer chromatography)*

After developing the TLC (migration lasting 1 hour 20 min), the plate is removed from the tank and observed under Wood's light (UV lamp), with the solvent front traced. After air-drying, the entire plate is sprayed with vanillin sulfur and placed in the oven at 100°C for 5 minutes.

The frontal ratio is calculated using the following expression: ***Rf = d/D***; (d: distance travelled by the separated substance to be identified. D: distance travelled by the migrating solvent).

Several spots appear, whose colors and Rf (front ratio) are shown in the following tables:

Table 5 Colors and Rf of essential oil thin-layer chromatography of the control and sample spots

The control			
Control spot	**Color**	**d (cm)**	**Rf**
Menthol	Blue	3.0	0.30
Cineol	Blue	6.9	0.69
Carvone	Blue green	8.1	0.81
Solvent front	D = 10 cm		
The sample			
N° of spot	Color	d (cm)	Rf
1	Blue gray	1.2	0.12
2	Blue gray	1.9	0.19
3	Blue	2.9	0.29
4	Purple	4.4	0.44
5	Purple blue	5.5	0.55
6	Blue	6.8	0.68
7	Blue green	7.9	0.79
8	Pink	9.1	0.91
Solvent front	D = 10 cm		

4. Discussion

The botanical study shows the presence of two types of secretory hairs: a short unicellular foot with an elliptical unicellular head, and a unicellular foot with a bulging multi-cell "Lamiaceae" type head (less abundant than the first type). These secretory hairs form the histological support for the production and storage of essential oils [11].

A study of the kinetics of extraction yield, as shown in the graph, shows that distillation is rapid from 0 -120 minutes, when yield reaches an optimum (1.08%). After this time (120 min), the curve shows a plateau, indicating the end of extraction [12]. The rapid increase in yield is explained by the entrainment of essential oils from the exogenously-located secretory hairs, thus facilitating extraction; the slowdown in extraction corresponds to the exhaustion of the remaining endogenously-located essence (already very low content) [13].

The organoleptic characteristics and physicochemical indices obtained comply with the standards given in the bibliography [14]. The thin-layer chromatography revealed the presence of several constituents through the appearance of several spots of different color and Rf, three of which were identified by comparison with controls, namely spots (3), (6) and (7) corresponding to menthol, cineol and carvone respectively. The carvone spot is large, reflecting the high content of this constituent in the essential oil [15,16].

5. Conclusion

The work carried out on the essential oil of spearmint *Mentha spicata* L , has enabled us to draw the following conclusions:

- Firstly, the histological and biochemical support for the elaboration and storage of these essences are the secretory hairs of two types located in the aerial parts, particularly the leaves.
- The yield of extracted essential oil is high: 1.08% (25 kg yielded approx. 100 ml), with an optimum extraction time of 90 to 120 minutes, reflecting a good cost/extraction ratio, i.e. time and energy savings with a good extraction yield.

- Organoleptic characteristics, physicochemical indices and chromatographic analysis showed that the extracted essential oil complies with the standards described in the literature, particularly pharmacopoeias, and that the main component of this essence is carvone (62.95%).

Compliance with ethical standards

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Disclosure of conflict of interest

The author and co-authors declare that they have no conflicts of interest in connection with this document.

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