CHEMICAL CHARACTERIZATION AND TOXICOLOGICAL EVALUATION OF THE ESSENTIAL OIL OF *MENTHA PIPERITA* L. GROWING IN MOROCCO

A. Debbab[1*], B. Mosaddak[1], A.H. Aly[2], A. Hakiki[1], M. Mosaddak[1]

[1] Université Mohammed V Agdal, Faculté des Sciences, Département de Chimie, Laboratoire des Substances Naturelles et Thermolyse Eclair, Avenue Ibn Battouta BP 1014, Rabat, Morocco.
[2] Alexandria University, Faculty of Pharmacy, Department of Pharmacognosy, Alexandria, Egypt

* Corresponding author: abdessamad.debbab@yahoo.fr

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Abstract: In this study the chemical composition and oral acute toxicity of the essential oil of *Mentha piperita* L. growing in Morocco were investigated. The volatile extract was isolated using hydro-distillation technique followed by continuous liquid-liquid fractionation (Water / Ethyl acetate). The essential oil was then analyzed by gas chromatography (GC) and chromatography-mass spectroscopy (GC-MS). The major compounds which characterized the essential oil of this plant were linalool **(1)** (60.72 %) and its acetate **(2)** (20.79 %), as well as geraniol **(3)** (3.26 %), 1,8-cineol **(4)** (2.33 %) and limonene **(5)** (1.54%). The acute toxicity of *Mentha piperita* L. oil was investigated in mice. The total essential oil in form of suspension in water with Tween 80 was tested by gavage. Acute toxicity evaluation of this essential oil showed a mortality percentage of 0, 10, 30, 50, 70, 100 for the doses 250, 500, 1000, 2000, 3000, 4000 mg/kg body weight of mice,

respectively. Moreover, the lethal amount 50 (LD_{50}) of the essential oil of *Mentha piperita* L. was found to be 1612.45 mg/kg with confidence limits 1461.41 mg/kg and 1779.11 mg/kg.

Keywords: *Mentha piperita* L., *essential oils, chemical composition, GC/MS, Lethal dose (LD_{50}), Mice.*

INTRODUCTION

Many plant species used in traditional medicine are common sources of drugs and insecticides. They were found to produce a vast and diverse assortment of organic compounds having medicinal and pharmaceutical importance [1 – 3]. Furthermore, essential oils and crude extracts from roots, leaves, twigs and flowers are widely used in food, tea, cosmetic, pharmaceutical and perfumery industries [4, 5].

Family *Lamiaceae* consists of about 250 genus and 6700 species. This family comprises aromatic, annual or perennial herbs or undershrubs and is long recognized because of the medicinal and culinary value of its members [6, 7], which are in many cases used as flavoring agents and cents. The *Lamiaceae* family is well represented in North Africa and plant species of this family are widely used in the Moroccan folk medicine.

Furthermore, a review of the literature reveals that the aerial materials from the genus *Mentha* of some members are used for herbal teas and condiments [8] and antioxidant activity [9, 10]. Additionally, it is known for its antifungal [11, 12], antibacterial [12], antimutagenic and chemopreventive [13], anticancer and radioprotective potential [14, 15] activities.

The purpose of the present study was to extract, explore and characterize the chemical composition and evaluate the toxicological activity on mice of the essential oil of *Mentha piperita* L.

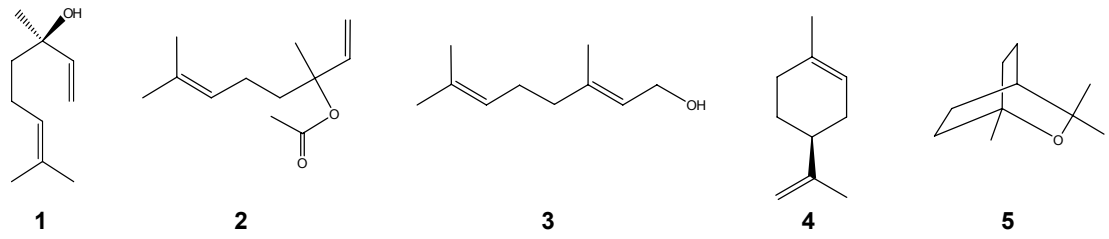

Figure 1. *Chemical structures of linalool (**1**), its acetate (**2**), geraniol (**3**), 1,8-cineol (**4**) and limonene (**5**)*

MATERIALS AND METHODS

Plant material

The plant samples used in this study were collected manually in Meknes, a city located in the middle of North Morocco (33°54′14.17″ N, 5°33′26.96″ W, elev. 1686 ft), during their flowering stage in the end of June 2004. The plant was identified by Prof. A. Ouyahya, Institut Scientifique, Université Mohammed V Agdal, Rabat, Morocco, based upon the morphology of its leaves and stems. Voucher specimens have been deposited in the "Laboratoire des Substances Naturelles et Thermolyse Éclair", Faculty of Sciences, University Mohammed V, Rabat, Morocco. The morphological characters of *Mentha piperita* L. used in this study are shown in table 1.

Table 1. *Morphology of Mentha piperita* L.

Average height	0.80 ± 0.05 cm	
General aspect	robust	
Color of the stem	green with violet tinge	
Number of internodes	5 ± 1	
Diameter of the stem	0.3 ± 0.05 cm	
Leaf	Color	green
	Form	oval lanceolate
	Nervation	corrugated
	Width / length	1 / 2
	Margin	dentate
	Tip	acute
Inflorescence	Flowering	May - August
	Form	verticil
	Average length	7.5 ± 0.5 cm
	Color of the corolla	pink
	Pilosity - lower - upper	 glabrous glabrous

Essential oil extraction

The collected plants, without roots, were dried at room temperature for three weeks. The essential oil was extracted from the dry material of plant by hydro-distillation. The principle consisted of immersing the dry plant material (60 g) in distilled water contained in a round glass flask (boiling flask 5 L). This mixture was heated until boiling for 3 hours and the produced vapor carrying the volatile substances of the essential oil was then passed through a cooling system (condenser) where condensation occurred. The essential oil was extracted by liquid-liquid fractionation by ethyl acetate (which was distilled two times), and the obtained organic phase was evaporated under reduced pressure. The essential oil was stored at a temperature of +4°C in well-filled, tightly closed glass vials wrapped in aluminum foil to avoid exposure to light and oxygen [16].

GC-MS analysis

The gas chromatography-mass spectrometry analysis of the obtained essential oil was performed using a Hewlett-Packard 6890 GC equipped with DB-Wax (30 m, 0.25 mm i.d., 0.25 μm film thickness) and a JEOL GC-mate as detector in EI mode at 70 eV. Helium was used as the carrier gas with a flow rate of 1 mL/min. Initially, the oven temperature was 50°C (isothermal, 5 min), and then increased to 250 °C at 2.5 °C/min. The injector temperature was 250 °C. Retention indices for components were determined according to Van de Dool [17], using *n*-alkanes as standards. The identification of the components was based on comparison of their mass spectra with those of Wiley and NBS Libraries [18] and those described by Adams [19]. The identity of some of the oil components was confirmed by GC analysis by injection with authentic substances.
Furthermore, the components relative concentrations were calculated based on GC peak areas without using correction factors.

Toxicological bioassay

A total number of 60 mice (Les Oncins, France, supplied by Department of Pharmacology, Faculty of Medicine and Pharmacy, Rabat, Morocco), 2 to 2.5 months old, weighing 20 to 30 g each, were randomly divided into six groups each of 10 animals, 5 males and 5 females, (see table 2). The animals were bred in the animal room, housed under the optimum temperature, of 24 °C, and nutrition.
Mentha oil was volumetrically diluted with water/Tween 80 (purchased from Somaprol, Casablanca, Morocco) and five different doses of 250, 500, 1000, 2000, 3000, 4000 mg/kg body weight of mice were prepared. We initially carried out an approximative determination of 5 groups each 3 animals with the same number of males and females, to determine the DL_{100} and the DL_0. The doses were administered by gavage (enforced oral administration) using a syringe supplied with a catheter [20]. For the determination of lethal dose 50 (LD_{50}), which is the amount of a substance that, when administered by a defined route of entry (e.g. oral or dermal) over a specified period of time, is expected to cause the death of 50 per cent of a defined animal population, each group was injected with a particular dose of oil, while the control group was injected with water/Tween 80 (see Table 3). The animals were observed and weighed daily for a period of two weeks.

Table 2. *Criteria of the animals*

Criteria	Mouse
Stock	OF1 (ONCINS, France)
Sex	as many males as females
Age	2 to 2.5 months
Physiological State	nonpregnant healthy mice
Weight	20 to 30 g

Table 3. *Doses administered to the mice*

Dose in mg/kg body weight of mice	Number of mice	Sex		Administered volume
		males	females	
250	10	5	5	0.5 mL/20 g body weight of mice
500	10	5	5	
1000	10	5	5	
2000	10	5	5	
3000	10	5	5	
4000	10	5	5	

RESULTS AND DISCUSSION

The taxonomy of the genus *Mentha* is particularly complex because of multiple possibilities of hybridization between various species. This makes botanical identification an insufficient tool. Accordingly, it is necessary to look at the chemical composition of these plants. Thus, the recent concept of chemotype comes to join the botanical characters and thereby presenting a reliable way to confirm plant identity and eventually improve the commercial value of cultivated plants [21 – 23]. *Mentha piperita* L. is a hybrid mint which originated probably due to accidental hybridization between *M. aquatica* and *M. spicata*. It is adapted to almost all areas and can be found in different altitudes. Being a sterile species, this species is propagated mostly by cuttings of its vegetative parts, such as internodes and underground stolon fragments. However, hybridization experiments were carried out between *M. piperita* and other species of mints using techniques such as protoplast fusions [24, 25].

For the investigation of the essential oil of *M. piperita* L., the oil was obtained by hydro-distillation. It had a clearly light yellow color (almost colorless) and its output to the dry plant material was found to be 1.72 %. The composition of the essential oil was determined by gas chromatography-mass spectrometry (Figure 1) on the basis of the GC retention times as summarized in Table 4.

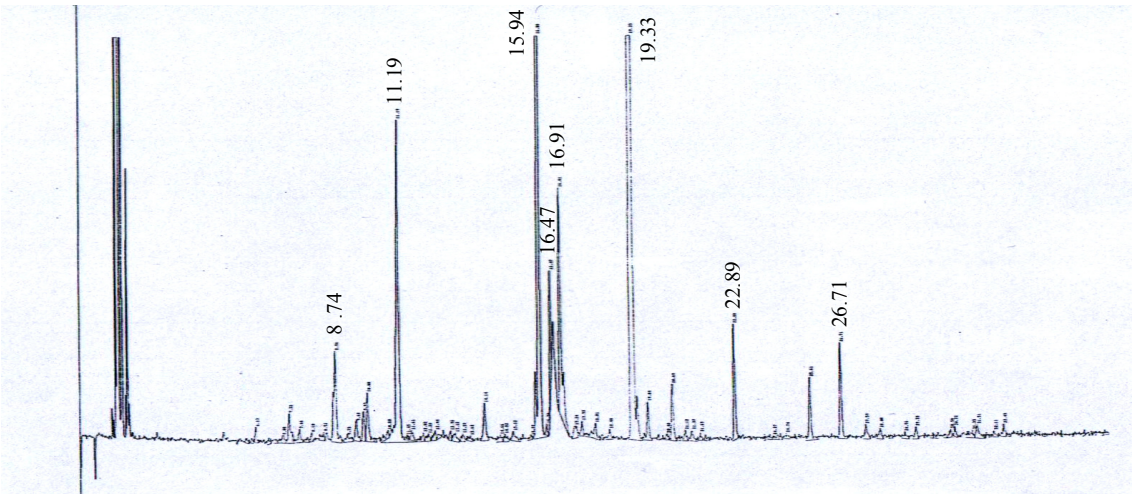

Figure 1. *GC profile of the essential oil*

Table 4. *Chemical composition of Mentha piperita* L. *essential oil*

Compound	Amount (%)	Rt (min)
Linalool	60.72	19.33
Linalyl acetate	20.79	15.94
Geraniol	3.26	11.19
1,8-cineol	2.33	16.91
Not identified	2.10	22.89 8.74 23.61 26.71 14.14
Limonene	1.54	16.47
Neomenthol	Trace	13.62
Menthol	Trace	13.37
Pulegone	Trace	11.65
Isomenthone	Trace	10.20
Menthone	Trace	9.33
Menthyl acetate	Trace	17.32
Piperitone	Trace	12.02

The treatment of many conditions using essential oils has become more and more popular over the last few decades. Essential oil/herbal therapies hold great promise for the treatment of medical illness and have been the basis of many pharmacological drugs [26]. However, most plants produce chemical compounds which disturb the human metabolism, exerting directly or indirectly a toxic action. In some cases ingestion of tiny quantities of these compounds may cause serious poising [27 – 30]. Therefore, the increasing concern with quality of life issues and thus the side effects of commonly used essential oil/herbal therapies implies a growing need to evaluate the toxicological potential of such therapies. This evoked our interest to evaluate the toxicity of the essential oil of *Mentha piperita* L., a plant frequently used as an aromatic in food, tea or cosmetic and perfumery products. Results of the toxicological bioassay showed that all the doses of the essential oil administered to the animals caused an immediate sedation effect, as well as closed eyes and accelerated breathing. Generally, diarrhea was also observed. The mortality percentage observed is demonstrated in table 5.

Table 5. *Mortality percentage*

Dose of substance (mg/kg)	Mortality (%)
250	0
500	10
1000	30
2000	50
3000	70
4000	100

The variation of the body weight of the animals was noted each day at the same hour, and a fall of the body weight was observed which could be explained by a reduced food consumption following the ingestion of the oil. Furthermore, a follow-up of the

ponderal evolution of the mice tested, during a period of 14 days, showed that the body weight of a group of mice underwent a regression as of the first days of observation, then when the signs of toxicity started to disappear, the weight increased gradually and sometimes exceeded the initial weight. However, the remaining animals showed a continuous body weight regression leading to their final death. The LD_{50} value, calculated using the MPD program [31], was found to be 1612.45 mg/kg with confidence limits 1461.41 mg/kg and 1779.11 mg/kg.

CONCLUSION

From the previous results it could be concluded that the toxicity of *Mentha piperita* L. essential oil is low, evidenced by high LD_{50} value. This finding suggests that using the essential oil of *Mentha piperita* L. as an aromatic in food, tea or cosmetic and perfumery products as well as alternative therapy is safe and presents no negative effects.

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