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ORIGINAL RESEARCH PAPER

CHEMICAL CHARACTERIZATION AND INSECTICIDAL EVALUATION OF THE ESSENTIAL OIL OF *Mentha suaveolens* L. AND *Mentha pulegium* L. GROWING IN MOROCCO

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Abstract: In this study the chemical composition and insecticidal evaluation of the essential oil of *Mentha suaveolens* L. and *Mentha pulegium* L. which are 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 gas chromatography - mass spectroscopy (GC-MS). The major compounds which are characterized in the essential oil of *Mentha suaveolens* L. were piperitenone (**1**) (33.03%), pulegone (**2**) (17.61%), piperitone (**3**) (9.18%); and of *Mentha pulegium* L. were pulegone (**2**) (73.33%), menthone (**4**) (8.63%). The study of the insecticidal activity of the essential oils on two species of devastating insects of the stored foodstuffs: *Sitophilus oryzae* and *Rizopertha dominica* was investigated. The mortality percentage of the species of insects was recorded in the first hours of treatment by the essential oils in the amount of 3, 12 and 50 µL.

Keywords: *chemical composition, essential oils, GC/MS, insecticidal activity, Mentha pulegium, Mentha suaveolens*

INTRODUCTION

Many plants 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, and 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 insecticidal activity of the essential oil of *Mentha suaveolens* L. and. *Mentha pulegium* L.

MATERIALS AND METHODS

Plant material

The plant samples used in this study were collected manually in Rabat, the capital of Morocco, during their flowering stage in the end of June. The plant was identified by Professor M. Fenane, Scientific Institute, "Mohammed V Agdal" University Rabat, Morocco, based upon the morphology of their leaves and stems. Voucher specimens have been deposited in the "Laboratoire des Substances Naturelles et Thermolyse Éclair", Faculty of Sciences, University "Mohammed V Agdal", Rabat, Morocco.

Essential oil extraction

The collected plants 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 using ethyl acetate (two times distilled), 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 oils was performed using a Hewlett-Packard 6890 GC in mode SCAN. The column is capillary HP 19091S-433, length 30 m, diameter 250 µm and its temperature maximum 325ºC. The detector is a mass spectrometer in EI mode at 70 eV at a temperature 150ºC, the temperature of the source was 230ºC. Helium was used as the carrier gas with a flow rate of 1 mL/min. Initially, the furnace temperature was 40°C and then rose with 10ºC/min. The final temperature was 250ºC; the total time of the operation was 32 min. Injector: manual injection, with constant pressure: 2.15 psi, volume injected: 2 µL with a syringe of 10 µL, the solvent used is the *n*-hexane. Maximum mass is 300 g and minimal mass is 80 g. The identification of the components was based on comparison of their mass spectra with those of Wiley and NBS Libraries [17] and those described by Adams [18]. Furthermore, the components relative concentrations were calculated based on GC peak areas without using correction factors.

Insecticidal activity

The study is related to two devastating insects of the stored foodstuffs. They are two Coleopters: *Rhysopertha dominica* and *Sitophilus oryzae*. The toxic effect was evaluated in Petri dishes (9 cm diameter), fitted latticework on closely connected to obtain a perfect ventilation, containing each one 5 g of corn on which we deposited a volume *C* (n) of essential oil using the microsyringes. Ten adults of each species of insect were then placed separately in Petri dishes at a rate of 5 repetitions. The witnesses do not have sudden treatment. The tests were carried out under conditions of ambient temperature (18°C) and of relative humidity 70%. The percentage of mortality was determined by counting the number of dead insects every 24 h during four days.
C (n) corresponds to: *C* (1) = 3 µL; *C* (2) = 12 µL; *C* (3) = 50 µL.

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 [19 – 21].
For the investigation of the essential oil of *Mentha suaveolens* L. and *Mentha pulegium* L., the oil was obtained by hydro-distillation. It had a light yellow color, pungent odor at room temperature and its output to the dry plant material was found to be 4.33% for *Mentha suaveolens* L. and 2.33% for *Mentha pulegium* L.. The composition of the essential oil was determined by gas chromatography-mass spectrometry on the basis of the GC retention times as summarized in Tables 1 and 2. The structures of the major compounds are presented in Figure 1.

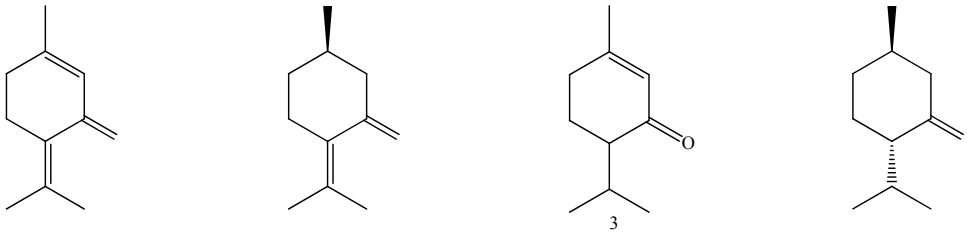

Figure 1**: Chemical structures of piperitenone **(1)**, pulegone **(2)**, piperitone **(3)**, menthone **(4)

***Table 1**. Chemical composition of **Mentha suaveolens L.** essential oil*

Compound	Amount (%)	Rt (min)
α-pinene	Trace	5.60
β-pinene	Trace	6.35
terpinolene	Trace	7.01
eucalyptol	Trace	7.29
γ-terpinene	Trace	7.74
α-fenchene	Trace	7.94
pulegone	17.61	9.52
piperitone	9.18	9.52
piperitenone	33.03	11.08
menthone	3.34	11.17
caryophyllene	traces	13.45
5-methyl-9-methylen-2-isopropylbicyclo(4.4.0)dec-1-ene	traces	13.74
7-methyl-10-isopropylbicyclo(4.4.0)dec-1-ene	traces	14.29
ledol	traces	15.67
α-cadinol	traces	16.41

***Table 2.** Chemical composition of **Mentha pulegium L.** essential oil*

Compounds	Amount (%)	Rt (min)
α-pinene	1.70	5.59
β-pinene	trace	6.36
D-limonene	0.44	7.26
eucalyptol	traces	7.29
menthone	8.63	9.34
pulegone	73.33	11.48
piperitone	traces	11.52
carvone	traces	12.43
caryophyllene	traces	13.45
α-caryophyllene	traces	13.91

The result of chemical study revealed piperitenone as the major component of *M. suaveolens* essential oil (33.03%), and pulegone as the major component of *M. pulegium* essential oil (73.33%).

The essential oil of *Mentha pulegium* is proven to be very toxic to the two species of coleopters in the first 24 h (Table 3).

The essential oil of *Mentha suaveolens* revealed toxic effect for the two species of coleopters, the percentage of mortality is 100% for the amounts of 50 µL and 12 µL.

For the amount of 3 µL we observed an acute toxicity causing the mortality of 85% in the first day of treatment then it reached 100% the second day (Table 4).

Table 3. *Percentage of mortality from species insect after treatment by essential oil of* ***Mentha pulegium***

	Sitophilus oryzae		***Rhysopertha dominica***	
	24 h	**48 h**	**24 h**	**48 h**
C (1) = 3 µL	100	-	100	-
C (2) = 12 µL	100	-	100	-
C (3) = 50 µL	100	-	100	-

Table 4. *Percentage of mortality from species insect after treatment by essential oil of* ***Mentha suaveolens***

	Sitophilus oryzae		***Rhysopertha dominica***	
	24 h	**48 h**	**24 h**	**48 h**
C (1) = 3 µL	85	100	85	100
C (2) = 12 µL	100	-	100	-
C (3) = 50 µL	100	-	100	-

CONCLUSION

From the presented results it could be concluded that the essential oil of *Mentha suaveolens* has the piperitenone as a major compound, followed by the pulegone, while the essential oil of *Mentha pulegium* was found to contain pulegone as a major compound. Moreover, the essential oils of the investigated plants could be used as effective insecticides.

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