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Antibacterial and antifungal activities of polygon mint essential oils

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Abstract

In this study, we worked on a medicinal aromatic plant widely used in Morocco, namely, *Mentha pulegium* L. of the Lamiaceae family (Labiatae). This plant was characterized by the Azilal region (Middle Atlas) and another from the Gharb region, then was placed in a well-ventilated place under light and shade to dry it, followed by extraction by the method of hydrodistillation for the extraction of essential oil from pennyroyal. The spectral analyzes by (CPG) and (CPG-MS) show that the oil extracted before and after maceration is rich, especially in Pulegone for the two plants of the two regions. A study of the effect of maceration on essential oil yield showed that the defined plant essential oil yield of the Azilal region is RHE= 2.32% without maceration and RHE= 2.33% after maceration, on the other hand, the essential oil yield of the plant in the Gharb region is RHE= 2.27% before maceration and RHE= 2.30% after maceration. The antibacterial activity of the essential oil of *Mentha pulgium* from the two regions confirmed that the essential oil extracted from the plant indicated in the region of Azilal has a very significant zone of inhibition compared to that of the region of Gharb.

Keywords: *Mentha pulgium*, essential oil, maceration, aromatic plant

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1. Introduction

Medicinal plants play a significant role in drug discovery [1]. It is reported that at least 119 compounds derived from 90 plant species can be considered important drugs against most diseases [2]. Unfortunately, until today researchers have not found effective drugs [3] for several diseases namely, cancers, viral infections such as HIV, hepatitis C, infections by bacteria and fungi, diseases cardiovascular disease, inflammation, and allergy problems [4]. Nowadays traditional medicine or treatment using natural plants is still limited [5]. For this, research has been conducted on the study of natural and biologically active products [6]. These products are chemical substances of organism origin not used in the primary metabolism [7]. Indeed, the selection of the plant to be studied is a crucial factor in the ultimate success of the extraction of biologically active agents [8].

In Morocco, mint is widely used for flavoring tea and in traditional medicine. For a long time, Morocco had been trying to develop the peppermint industry and the Gharb plain, with its fertile alluvial soil, contributed to an essential oil production estimated at 5 tons in 1946. Other regions are favorable such as the south of tiflet and khemisset, with Màaziz [9][10]. Mints are plants of great perennial herbaceous vigor with a characteristic odor that leaves an impression of freshness, and they have been used for therapeutic purposes since the 16th century [11]. In therapy, mint is used against fever, weakness, cough, nausea, stomach aches, melancholy, chest diseases, hysteria, and visual disturbances [12][13], it also presents medicinal properties, for example, antioxidant [14] antifungal [15], antibacterial [16], anticancer activity and with radioactive potential [17]. Mint is also used against parasites [18]; the stems and flowers of mint are burned to drive away fleas from mattresses and domestic animals [19][20].

In this context, the objective of our work is to study the maceration and the antibacterial activity of the essential oil extracted from the *Mentha pulegium* L. plant of the Lamiaceae family (Labiaceae), harvested from two different regions, the Azilal region, and the Gharb region. The identification of the compounds presents in the essential oil to be studied was carried out using GC/MS.

2. Materials and methods

2.1. Materials

The studied plant *Mentha pulgium* L. was harvested in the region of Azilal (middle atlas), and placed in a well-ventilated place sheltered from light and shade. After drying, the plant was ready for extraction.

2.2. Methods

- ***Extraction of essential oils:***

The extraction was carried out using the hydrodistillation method. A quantity of 40% of the plant is completely immersed in 60% of distilled water, and to homogenize the boiling of the mixture some grains of pumice stone has been added. The heating is maintained at a gentle temperature for 8 hours. the vapors are condensed in the cooler and the essential oil and water are separated by difference in density.

- ***Yield variation over time.***

$$Yield = (Mxp / Mth) \times 100.$$

RHE= (mass of HE / mass of dry plant) x 100

2.3. Characterization

An analysis by gas chromatography coupled with mass spectrometry (GPC/MS) was carried out to identify and calculate the percentages of the constituents present in the essential oil extracted from the mint. The calculations were carried out according to the internal standard method.

In order to study the antibacterial and antifungal activity of the essential oil, tests by the agar diffusion method on 25 strains, namely Enterobacteriaceae (Enterococcus focalize. Escherichia coli, Enterobacter Freundii; Serratia marcesens, etc.), Non-Enterobacteriaceae (Pseudomonas aerogenosa, acenitobacterbaumanii); Gram-positive Cocci (staphylococcal and streptococcal), Gram-positive Bacillus (Listeria spp) and for yeasts we tested *Candida albicans*.

3. Results and Discussions

3.1. Study of maceration on yield

The essential oil yield for the Azilal region (average atlas) is **RHE= 2.32%** without maceration is 2.33% after maceration, on the other hand for the Gharb region the essential oil yield is **RHE= 2.27%** without maceration is **2.30%** after maceration.

3.2. Analysis by GC and GC/MS

Qualitative and quantitative analyses of essential oils were made using gas chromatography, and gas chromatography coupled with mass spectrophotometry for the two plants, respectively.

3.3. *Chemical composition of the essential oil before maceration*

Figure 1 gives us the percentage of the main majority products with their respective retention time. Indeed, the retention times can give information on the nature of the molecules and the areas of the peaks provide a relative quantification.

From the chromatogram, it can be seen that the majority of the product corresponds to the peak which appears for a retention time equal to 19.78 min. Analysis by GC and GC-MS allows us to confirm that our oil is rich in Polygon.

3.4. *Chemical composition of the essential oil after maceration*

This chromatogram gives us the main majority compounds of the extracted gasoline. The results give us the main majority compounds with their respective retention times. :2-Octyne, 1,1-diethoxy-. The two methods of analysis enabled us to identify the main compounds of the essential oil extracted after maceration. It was noted that the majority of products appear for the following peaks: 19.80; 17.4; we also note the formation of new products. This allows us to say that the maceration of the plant influences the chemical composition.

3.5. Antibacterial and antifungal activity

According to figure 3 and table 1, it can be seen that the essential oil of Middle Atlas mint (HE (1)) has a very significant activity because this oil inhibits almost the majority of bacteria (80%). This is remarkable for *Enterobacteriaceae*, *Staphylocossus*, and *Listeria spp*. On the other hand, it remains ineffective on *Streptococcus pnomoniae* and *Panteo*.

Table 1: Results of antibacterial and antifungal activities

Type of germ	Bacterial strains	Inhibition diameters (mm)					
		Essential Oil N°1			Essential Oil N°2		
		Brute	Dilution 1/2	Dilution 1/4	Brute	Dilution 1/2	Dilution 1/4
Enterobacteries	*Enterococcus foecalis*	16	11	12	14	12	14
	Yersina ATCC	23	19	9	-	-	-
	Serratia marcesens	16	12	9	-	-	-
	Salmonella Entritidis	19	11	15	12	-	-
	Esherichiacoli beta *lactamas à spectre* *Élargie (E.C BLSE)*	20	19	21	14	11	-
	Escherichia coli *O157*	24	18	16	12	-	-
	Shigella spp	22	22	19	12	-	11
	ENTEROBACTERIE *COLOACAE*	11	11	-	-	-	
	PROTEUS spp	20	16	14	-	-	
	Klebsiella spp	24	18	11	14	-	-
	Marganellaspp	28	26	20	13	8	11
	SALMONELLE spp	18	15	10	-	-	-
	Klebsiella BLSE	15	12	-	-	-	-
	Panteospp	-	-	-	-	-	-
	Salmonella Groupe *C*	19	20	15	11	-	-
COCCI GRAM+	*Staphylococcus* *Aureus ATCC*	28	22	14	12	8	10
	Streptococcus *pneumoniae*	-	-	-	-	-	-
	Staphylococcus *epedermis*	22	20	15	-	-	-
	Streptococcus groupe	16	11	-	-	-	-

	D						
No Enterobacteriaceae	*Psudomonas aeruginosa ATCC*	-	-	-	-	-	-
	Acinetobactere bounanii	28	20	18	21	16	17
BACILLE G+	*Listeria spp*	11	-	-	-	-	-

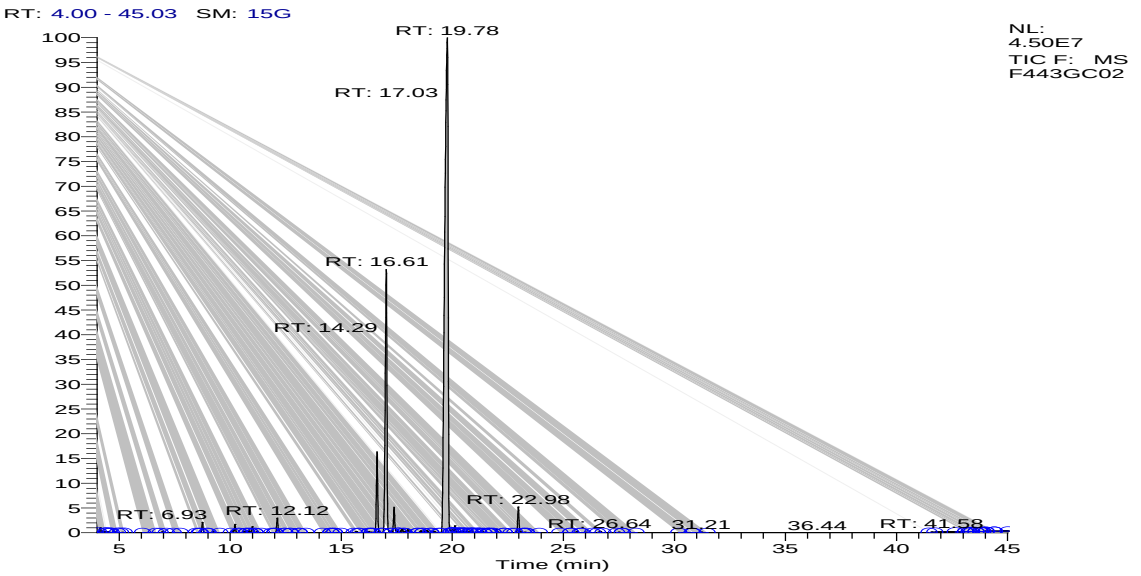


Figure 1: Chromatogram of the relative abundance as a function of the time of the essential oil before maceration.

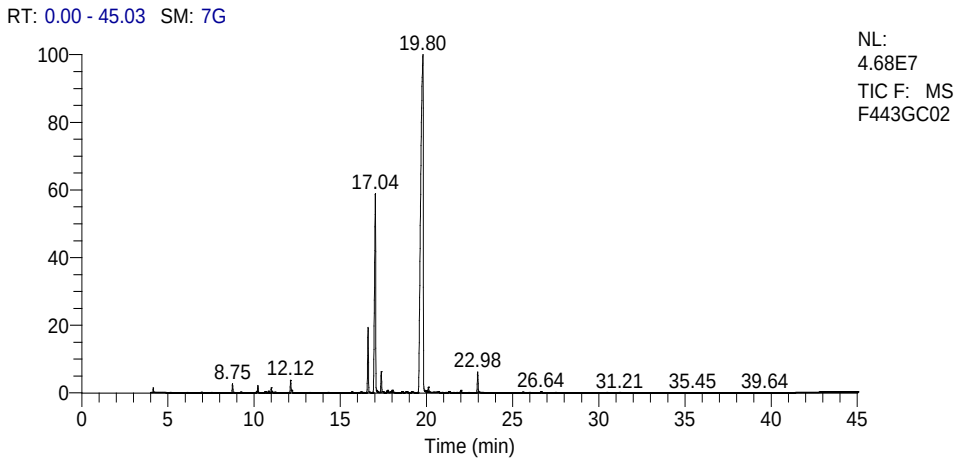
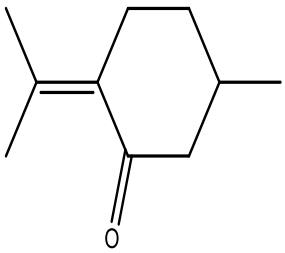
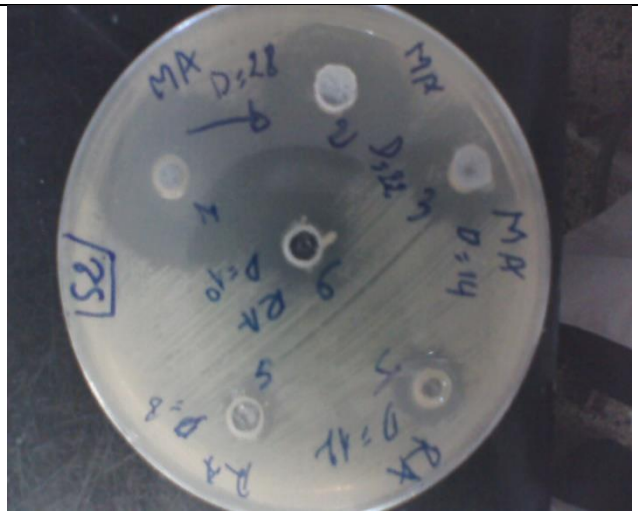
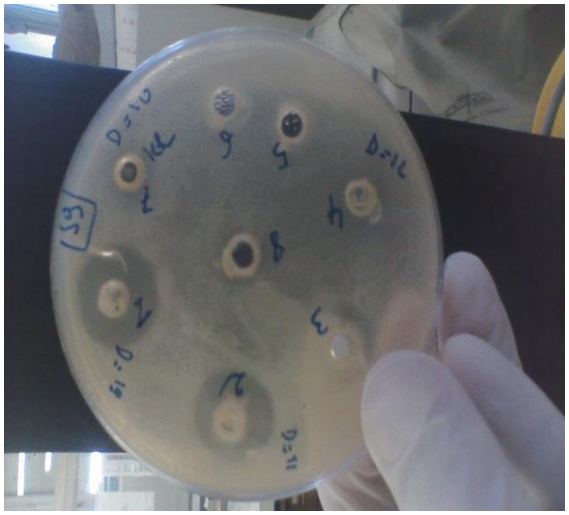


Figure 2: Chromatogram of the relative abundance as a function of time of the essential oil extracted after maceration

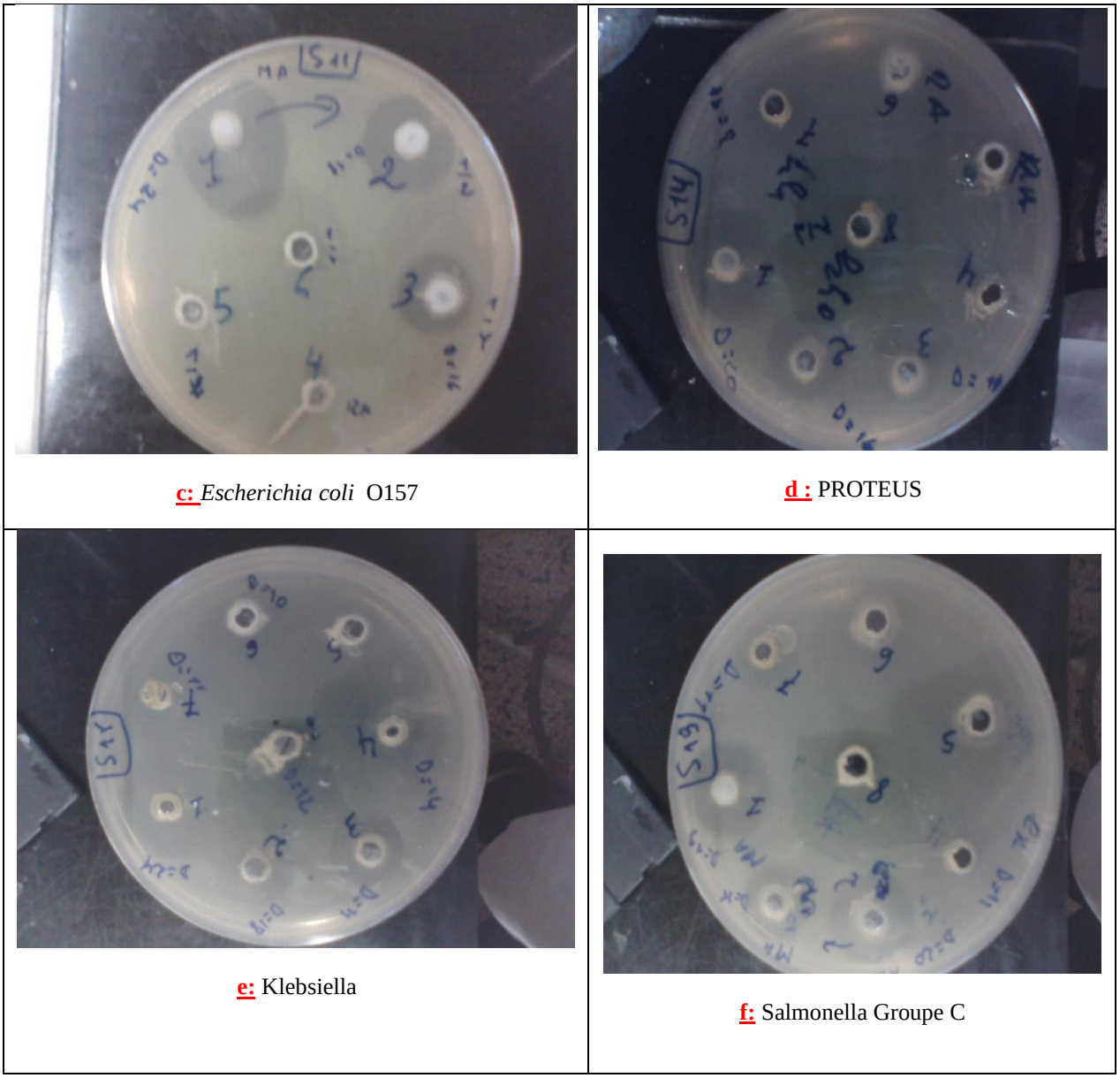




a: *Shaphylococcus aureus* ATCC



b: *Salmonelle entritidis*



c: *Escherichia coli* O157

d : PROTEUS

e: Klebsiella

f: Salmonella Groupe C

Figure 3: Action of essential oils extracted on different bacteria

The essential oil of pennyroyal from the Gharb region of Morocco (HE (2)) significantly inhibited Enterobacteriaceae, especially the strains: Enterococcus, Escherichia coli, Klebsella spp. For Cocci Gram+; HE (2) inhibited only Shaphylococcus aureus and remains ineffective for Streptococcus pneumoniae, Staphylococcus epedermis, Streptococcus group D. For Non-Enterobacteriaceae, HE (2) strongly inhibited Acinetobacter baumanii, and remains ineffective we go to the 1/4 dilution. It did not inhibit Psudomonas aeruginosa ATCC and Listeria.

4. Conclusions

The results of this study show a very significant antimicrobial efficacy for essential oils from the harvest region. Antibacterial tests show us that the essential oil from the Middle Atlas region has a remarkable zone of inhibition in its raw state and after dilution. The essential oil extracted from the plant of the Middle Atlas region is effective on the germs responsible for toxin food infections such as (*Shaphyloccus aureus*, *Lesteria*, *Echerchiacolis*, *Shgella*, *Salmonella*, *Yersina ATCC*) as well as the germs responsible for urinary tract infections and nosocomial infections (ESBL *Klebsiella*, *Escherichia coli O157*, etc.). The differences in inhibition can be explained by the chemical variability of the two extracts, which depends on the nature of the soil, the degree of pollution, and the nature of the climate.

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