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Characterization, Chemical Compounds and Biological Activities of *Marrubium vulgare* L. Essential Oil

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Abstract: As consumer trends shift towards more natural and ecological consumption patterns, industrialists are actively working towards substituting synthetic chemicals with natural and vegan products that contain bioactive properties. Thus, considering the shifts in customer demand and the growing concern around vegetable sourced productions, this work aims to contribute to the valorization of aromatic and medicinal Moroccan plants. By focusing on the *Marrubium vulgare* L. species, our objective is to carry out a physicochemical characterization to determine its chemical composition and biological activities. The volatile fraction collected by hydrodistillation (0.61%) and analyzed by GC-MS (gas chromatography coupled to mass spectrometry) contains five main compounds: 3-Thujanone, Eugenol, Topanol, Menthone and Piperitone. The antioxidant activity has been estimated by applying the DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging test and the ferric reducing antioxidant power (FRAP). The values of inhibitory concentration prove that our oil is a good antioxidant, with values of IC_{50} = 1.136 mg/mL and IC_{50} = 2.998 mg/mL, respectively, for the DPPH and FRAP tests. The results of the antifungal activity indicate a significant inhibition of mycelial growth for both tested molds, as well as a total inhibition of spore production at a concentration of 0.25 µL/mL.

Keywords: *Marrubium vulgare* L.; essential oil; GC-MS; physicochemical characterization; antifungal; antioxidant

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1. Introduction

Plant history can be traced back 60,000 years to the Neanderthals and shamans who were instrumental in collecting, learning and passing on knowledge about plants during the evolution of Homo sapiens. Then the Greeks (notably Hippocrates, Aristotle, Theophrastus, Galen, and Dioscorides) and the Romans taught the art of healing with plants and recorded more than 500 medicinal plants. Later on, it was the development of the pharmaceutical industry based on chemical components that took over the healing functions of plants. However, some civilizations (Chinese, Ayurvedic, Arabic, Tibetan, Indian, etc.) still rely on these ancestral and less expensive therapeutic systems [1,2].

Moroccan civilization is one in which plant-based therapies are still common and popular. This is due to its very rich flora, developed because of its significant mountain

ranges and Mediterranean and Atlantic climates, particularly suitable for acclimatization in all temperate regions of the world [3].

In Morocco, "Merriout" refers to the *Marrubium* genus, belonging to the Lamiaceae family, which contain 226 different genera [4] and have various species which have been the subject of phytochemical and pharmacological investigations due to their general properties which are linked to their variety of molecules and active principles. With 30 species distributed in Europe, North Africa and Asia [5], this genus presents a wide variety of biological activities, including antimicrobial [6], cardioprotective [7], antitumor [8], antioxidant [9,10], antidiabetic [11], antiviral [12], anti-inflammatory [13], antifungal [14], antiparasitic and antiprotozoal [15,16]. This led to the interest of manufacturers, resulting in the increasing development of processes using extracts and active principles of plant origin.

There are many applications of essential oils extracted from medicinal plants in the food, cosmetic, and medical industries. Researchers have used several conventional (steam distillation, hydrodistillation, hydrodiffusion, and solvent extraction) as well as non-conventional (solventless microwave extraction, subcritical liquid extraction, and supercritical fluid extraction) extraction techniques to recover these active principles [17] such as menthol, isomenthone, menthone, limonene, caryophyllene, α-terpineol, geranyl, linalool, thymol, carvacrol, etc. [18]

Our study aims to focus on the *Marrubium* genus by valorizing the biodiversity of aromatic plants in Western Morocco through the search for active bio-substances and, more precisely, the essential oil of the species *Marrubium vulgare* L. from Zaër which, to our knowledge, has not been examined before. The objective is to improve the extraction yield, to determine the chemical composition by GC-MS, to study the physico-chemical characteristics and to evaluate some biological activities of its essential oil, such as antioxidant and antifungal power.

2. Materials and Methods

2.1. Reagents

Ethanol, potassium hydroxide, phenolphthalein (PP), hydrochloric acid, chloroform, acetic acid, potassium iodide, thiosulfate, DPPH, ascorbic acid, potassium ferricyanide, and $FeCl_3$ were used for this study. The sources for these products are OX-FORD, SCIENCEMED, VWR PROLABO CHEMICALS, SIGMA-ALDRICH, and HON-EYWELL.

2.2. Plant Sample

Marrubium vulgare L. is a member of the Lamiaceae family, known as the white horehound or Merrîwa (Arabic). The genus *Marrubium* has 30 species spread in Europe, North Africa and Asia [19]. The harvest of the plant that was the subject of our study takes place at the level of the region of Zaër (Western Morocco). The plant is air-dried and preserved until use.

2.3. Essential Oil Extraction

100 g of *M. vulgare* leaves and distilled water (600 mL) were placed in a flask, and the steam distillation process was carried out using the Clevenger apparatus for 3 h [20]. The collected essential oil (volatile part) is kept in the dark at 4 °C for later use. The conservation of essential oils requires well-stoppered bottles that are protected from air and light to avoid their oxidation and polymerization. We estimated the yield of essential oil in relation to the total mass of the dry plant.

2.4. Extraction Kinetics

The purpose of this study is to determine the time required for extracting the maximum amount of oil while avoiding losses in time and energy. The kinetics consists of determining the yield as a function of extraction time [21]. The yield is determined by taking the essential oil at intervals of 30 min over a period ranging from 0 to 180 min, taking into account that

the beginning of the extraction starts with the formation of the first drop of distillate. This stage corresponds to the increase in temperature of the boiling water.

2.5. GC-MS Characterization

The essential oil chromatographic analysis was performed at the University Center for Analysis, Expertise, Technology Transfer and Incubation CUE2TI of the University IBN Tofail Kenitra with a gas chromatograph type 456-GC coupled to a mass spectrometer type EVOQ TQ. Fragmentation was performed by electronic impact at 70 eV. The column used was a capillary column type Rxi-5Sil MS (30 m × 0.25 mm ID × 0.25 µm df). The column temperature was initially programmed at 40 °C for 8 min at a rate of 2 °C/min up to 300 °C for the remaining 20 min. The carrier gas was helium with a flow rate of 1.50 mL/min. The injection temperature was maintained at 250 °C and the injection volume was 1 µL. The range was 30–800 m/z. The apparatus was connected to a computer system managing a NIST MS Search 2.0 mass spectra library and driven by MS Data review software.

2.6. Physicochemical Indices of Essential Oils

The physicochemical properties are a means to verify and control the quality of the essential oil. For this reason, we have determined the following physicochemical indices: refractive index, ethanol miscibility, peroxide index, acid index, ester index and saponification index.

2.6.1. Index of Refraction

The ratio between the incidence sine angle and the refractive sine angle of a light ray of a specified wavelength passing from the air into the essential oil is maintained at a constant temperature. The refractometer would allow a direct reading of the refractive index.

2.6.2. Evaluation of Miscibility with Ethanol

An essential oil is considered miscible with V volumes or more of ethanol of a determined alcoholic strength at a temperature of 20 °C. To reach a limpid solution for 1 volume of essential oil, we needed to progressively add at least 20 volumes of ethanol at 20 °C.

2.6.3. An Acid Index (AI)

This indicates the number of milligrams of potassium hydroxide required to neutralize the free acids contained in 1 g of essential oil. We weighed and introduced 2 g of oil into a 100 mL flask to which we added 5 mL of 95% ethanol and 5 drops of 0.2% phenolphthalein (PP). Then we titrated the solution with the potassium hydroxide solution (0.1 mol/L) contained in the burette that we continuously added until the solution turned. The calculation of the AI is determined by the following formula:

$$AI = V \times c \times 5.61/m$$

where V is the volume in milliliters of potassium hydroxide solution used for the titration, c is the exact concentration in moles by liter of the potassium hydroxide solution, and m is the mass in grams of the test sample.

2.6.4. Ester Index (EI)

The ester index are the number of milligrams of potassium hydroxide necessary to neutralize the acids released by the hydrolysis of the esters contained in 1 g of essential oil. We weighed and introduced 2 g of essential oil into a flask. Then, using a burette, we added 25 mL of the potassium hydroxide solution as well as fragments of pumice stone or porcelain. We fitted the condenser, placed the flask on the heating mantle, and left it to heat for one hour. After cooling the flask, we removed the tube and added 20 mL of water, followed by 5 drops of phenolphthalein solution. The excess potassium hydroxide that remained was titrated with the hydrochloric acid solution. At the same time, a blank was

performed under the same conditions and with the same reagents. The calculation of the EI follows the formula:

$$EI = (28.05 \times (V_0 - V_1)/M) - I_A$$

where M is the mass in grams of the test, V_0 is the volume in mL of the HCl solution (0.5 mol/L) used for the blank, And V_1 is the volume in mL of the HCl solution (0.5 mol/L) used to determine the EI of the essential oil [22].

2.6.5. Peroxide Index (PI)

The peroxide is the number of micrograms of active peroxide contained in one gram of product, and potassium iodide is oxidized to release iodine under the conditions of the method described. We put 1 g of oil in a tube and placed it in an Erlenmeyer flask, then added 10 mL of chloroform and shook the solution. We added 15 mL of acetic acid CH_3COOH, followed by 1 mL of saturated aqueous KI, shook the flask, and left it in the dark for 5 min. We added 75 mL of distilled water and shook for 1 min. We carefully titrated in the presence of starch and released iodine with a solution of $Na_2S_2O_3$ (0.01 N) until complete discoloration of the solution was reached. The calculation of the PI is determined by the formula:

$$PI = 8000\ V/m$$

where m is the mass of the test and V is the volume of the thiosulfate solution N/100.

2.6.6. Saponification Index (SI)

The saponification index is the amount of soda expressed in grams needed to saponify 1 g of oil [23]. The saponification index is calculated according to the following formula:

$$SI = AI + EI$$

where AI is Acid Index and EI is Ester Index.

2.7. Antioxidant Activity

Two methods were applied to assess the antioxidant activity of our essential oil. First, the capacity of the oil to reduce the DPPH radical was tested. Then the FRAP, which refers to the ability of the oil to reduce ferric ions (Fe^{3+}), was measured.

2.7.1. DPPH (1,1-Diphenyl-2-picrylhydrazyl) Assay

To study the antioxidant activity of our essential oil, we used the DPPH (1,1-diphenyl-2-picrylhydrazyl) method. For this purpose, solutions of increasing concentrations were prepared by diluting the stock solution with ascorbic acid (0–0.2 mg/mL) used as a standard. The DPPH solution was prepared by solubilizing 3 mg of DPPH in 100 mL of ethanol. Consistent with the technique, we mixed 2 mL of the freshly prepared DPPH solution in a tube with 0.1 mL of each solution. A negative control (or blank) was prepared in parallel with ethanol. Then, the whole solution was incubated in the dark for 30 min and the absorbance measured at 517 nm using a spectrophotometer (UV-2005, Selecta, Barcelona, Spain). Three optical density measurements were determined for each solution.

Antioxidant activity evaluation is expressed as a percentage of DPPH radical inhibition according to the following formula:

$$\%\ IP = ((Abs\ control - Abs\ sample)/Abs\ control) \times 100)$$

where % IP is the percentage of inhibition and Abs is the absorbance. Using this formula, we draw the line that represents the variation of the percentage inhibition as a function of the various concentrations in each sample (y = ax + b). From this line, it is possible to deduce

the concentration that allows reducing 50% of DPPH for the sample studied and for ascorbic acid. This concentration, called IC_{50}, is calculated according to the following equation:

$$IC_{50} = (50 - b)/a$$

where IC50 is the concentration required to reduce 50% of DPPH, a represents the line slope, and b is the intercept of the line [24].

2.7.2. FRAP (Ferric Reducing Antioxidant Power) Assay

Based on the previous literature, which set the protocol of reducing power by the essential oil of *M. vulgare* on ferric ions (Fe^{3+}) [25], we combined 1 mL of extract at different concentrations (0 to 1 mg/mL) with 2.5 mL of a phosphate buffer solution (0.2 M, pH 6.6) and 2.5 mL of a 1% potassium ferricyanide $K_3Fe(CN)_6$ solution. The mixture was centrifuged at 8653.32× *g* for 10 min. We added 2.5 mL of distilled water and 0.5 mL of 0.1% $FeCl_3$ solution to 2.5 mL of supernatant. At the same time, a sample blank is prepared under similar operating conditions.

Absorbance was measured at 700 nm and the standard antioxidant solution, ascorbic acid, was used as a positive control. For presentation of the results, the concentration of the sample corresponding to an absorbance of 50 (IC_{50}) was calculated from the equation in the line, representing the variation of absorbance within the concentration.

2.8. Evaluation of the Antifungal Activity

2.8.1. Fungal Material

The studied fungal strains, *Fusarium pseudograminearum* and *Fusarium moniliforme*, belong to the mycotheque of the Laboratory of Vegetal, Animal and Agro-Industrial Production, Faculty of Science, Kenitra. They are isolated from saffron and regularly transplanted on PDA (Potato Dextrose Agar) culture medium. The identification was made based on their morphological characters and it was verified at the National Center for Scientific and Technical Research (CNRST) of Rabat.

2.8.2. Antifungal Effects of the Essential Oil

The antifungal power of the essential oil was tested in our study by the direct contact method [26] on the isolated mold species, namely *Fusarium pseudograminearum* and *Fusarium moniliforme*. The essential oil was tested with the following concentrations: 0.083; 0.250; 0.500; 2.500; 5.000; and 12.500 µL/mL of PDA medium. These concentrations were obtained by adding 5, 15, 30, 300, 150 and 750 µL of the essential oil to 60 mL of warm PDA medium in a test tube. After shaking, the medium was poured into glass Petri dishes of 9 cm in diameter. The inoculation was performed by depositing a mycelium disk of about 0.6 cm in diameter from a 3 to 7 day pre-culture (28 °C) onto the center of the dish. A Petri dish containing 20 mL of PDA without essential oil was inoculated to serve as a control. For each concentration, three tests were performed. After an incubation of 7 days at a temperature of 28 ± 4 °C and taking into account the mycelium growth of the control, the antifungal index was calculated using the following formula:

$$\text{Antifungal index} = (1 - Da/Db) \times 100$$

where Da is the diameter of the test growth area and Db is the diameter of the control growth area.

2.8.3. Effect of Essential Oil on Spore Germination

The activity of the essential oil on sporulation was evaluated with the cultures where the fungistatic effect was observed. The spores obtained after dilution in distilled water were numbered with the Malassez cell, and the results were expressed as the number

of spores/mL of the suspension. The number of spores is determined according to the following formula:

$$\text{Number of spores (spore/mL)} = \text{Average number} \times 10^5 \times \text{Dilution factor}$$

3. Results and Discussion

3.1. Extraction Yield and Organoleptic Characteristics

The essential oil of *M. vulgare* obtained by hydrodistillation is light beige in color and has a strong aromatic odor.

Based on the extraction process carried out in our laboratory, the essential oil of *M. vulgare* from Morocco was obtained with a yield of 0.61%. Our yield is superior to the results found in the literature by Zawislak, G. (Lublin Poland) [27], El-Leithy, A.S. and al. (Egypt) [28], and Khanavi and al. (Iran) [29] who found, respectively, yields of 0.03%, 0.06% and 0.09%.

A number of factors can explain this difference in performance, including [30,31]: variation in botanical origin, factors of the environment and cultural practices, the degree of maturity of the plant, the part of the plant used, extrinsic factors (temperature, humidity, drought, etc.), seasonal variation, and the photoperiod.

3.2. Extraction Kinetics

In the experiments used to establish extraction kinetics (Figure 1), the process was periodically stopped for 180 min. The essential oil was collected and weighed at 30 min intervals. From these measurements of the weight of the essential oil, we determined the percent yield of each catch. This allowed us to plot the evolution of extraction yields as a function of time. This follow-up allowed us to show that a duration of 2 h is sufficient to extract the maximum amount of oil. We have thus optimized the time gained from an energy point of view. The result is illustrated in Figure 1.

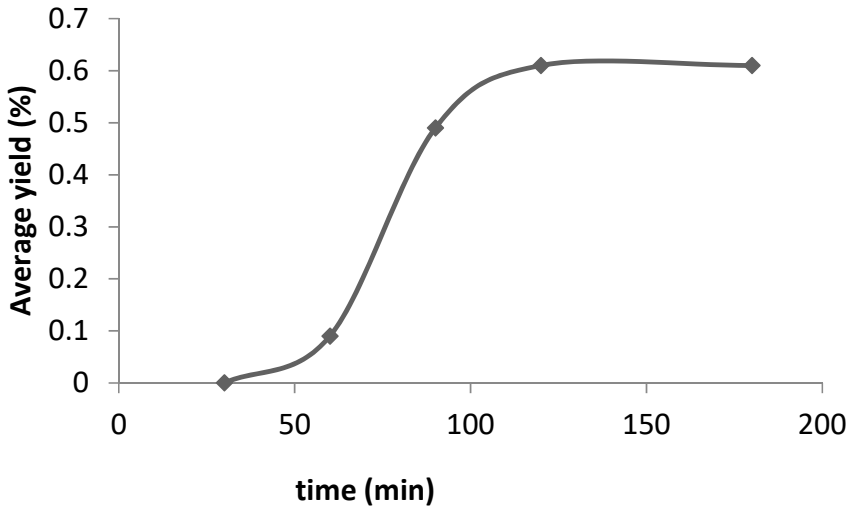

Figure 1. Extraction kinetics of the essential oil of *Marrubium vulgare*.

3.3. Physicochemical Characteristics of the Essential Oil

Physicochemical characteristics are among the factors used to determine the quality of essential oils. Due to the absence of reference values for this plant, we compared our results with those given in the literature for the following Lamiaceae: *Mentha pulegium* L. [32], *Thymus vulgaris*, and *Thymus fontanesii* [33]. The results of the physical and chemical properties are grouped in Table 1.

Table 1. Physicochemical indices for essential oil of different Lamiaceae.

Physicochemical Characteristics	Values Compared with			
	***Marrubium vulgare* L.**	***Thymus vulgaris***	***Thymus fontanesii***	***Mentha pulegium* L.**
Physical character				
Refractive index at 20 °C	1.343 ± 0.0006	1.491–1.510	1.499	1.480–1.490
Relative density	0.940 ± 0.0006	0.9–0.955	0.921	0.930–0.944
Ethanol miscibility	1V/1V ± 0.0000	2V/1V–3V/1V	0.6V/1V	3V/1V
Chemical character				
Peroxide index	8000 ± 0.0044	ND	8000	ND
Acid index	0.561 ± 0.0011	8.4	1.458	ND
Ester index	14.020 ± 0.0053	18.2	16.83	ND
Saponification index (mg KOH/g of essential oil)	14.581 ± 0.0042	ND	ND	ND

ND: Not Determined.

3.3.1. Physical Properties

- Refractive index at 20°: The refractive index of the *M. vulgare* essential oil is equal to 1.343. It is lower than those of *T. vulgaris*, *T. fontanesii* and *M. pulegium*, which have refractive indices of 1.491 to 1.510; 1.499; and 1.480 to 1.490, respectively. In general, for essential oils, the refractive index is high. It is superior to water at 20 °C, which is 1.335.
- Relative density: The value density of our oil is 0.940. It is higher than that obtained from *T. fontanesii* (0.921) and is in the range given for *T. vulgaris* (0.9–0.955) and *M. pulegium* (0.930 to 0.944).
- Ethanol miscibility: Our essential oil is miscible with 1 volume of ethanol, which corresponds to a much lower result than those obtained in previous works for *T. vulgaris* and *M. pulegium*, but it is higher than that given for *T. fontanesii*.

3.3.2. Chemical Properties

- Peroxide index: The index is a quality indicator showing the freshness and age of the oil. A lower index means that the oil is fresh. The peroxide value of our essential oil is 8000, similar to that of *T. fontanesii*.
- Acid Index: The AI value for *M. vulgare* essential oil is 0.561. This result is inferior to those determined for *T. vulgaris* (8.4) and *T. fontanesii* (1.458). This is due to the fact that the oil is placed in a tinted glass bottle, because light has been shown to alter the structure of the oil and cause acids to proliferate. In fact, the oil oxidizes, degrades rapidly, and causes an increase in the acid index. If the index is less than two, the oil is well-preserved, which is the case for our essential oil.
- Ester index: The ester index of our essential oil is 14.020; it is lower than those recorded for *T. vulgaris* and *T. fontanesii*. The greater the amount of ester in the oil, the higher its quality.
- Saponification index: The saponification number indicates the fatty acid content (esterified and free) of the oil. In our case, *Marrubium vulgare* L. oil has a saponification number of 14.581, which means that to saponify one gram of oil, 14.581 g of soda must be used.

3.4. GC/MS Analysis

Through analysis by gas chromatography coupled with MS, we can identify 30 compounds in this essential oil. The chromatogram and chemical composition of *M. vulgare* obtained are presented in Figure 2 and Table 2, respectively. If we compare these results

with those of the same species (*M. vulgare*) cultivated in different countries, we notice that the constituent compounds of the essential oil are very different. This is related to the environmental effects established by its botanical origin. Compared with the literature, the main compounds identified by Bayir, B. et al. for *Marrubium vulgare* L. originating from Turkey are α-Pinene (28.85%), β-Pinene (18.31%), β-Phellandrene (17.40%), and 2-hexenal (14.80%) [34]. The essential oil of *M. vulgare* from Tunisia has a β-bisabolene chemotype [35], while the main compounds of the Moroccan Marrubium essential oil are the following: Monoterpenols (14.57%): (+)-β-Linalool, α-Terpineol, Eugenol; Monoterpenes (2.64%): (-)-Camphene, p-Menth-4-en-3-one, Menthone; Monoterpene ketones (41.76%): 3-Thujanone, Piperitone, Geranylacetone, β-Ionone; and Sesquiterpenes (1.12%): Isocaryophyllene, β-Bisabolene, Humulene, and β-Selinene.

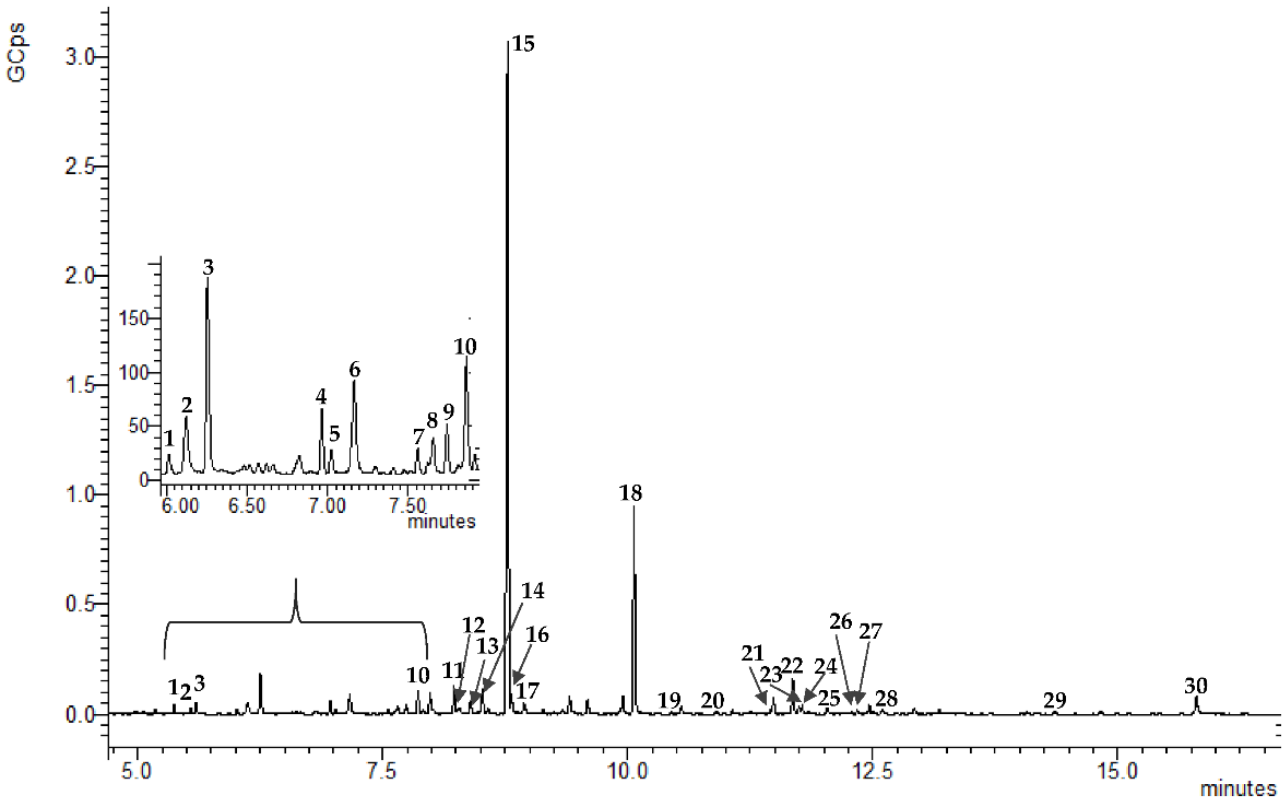

Figure 2. Chromatogram of *Marrubium vulgare* L. essential oil.

3.5. Antioxidant Activity

Oxidative stress is the inability of the body to defend itself against reactive oxygen species (ROS) as a result of the disruption of the endogenous balance between ROS and oxidizing agents (OA). This imbalance can potentially lead to structural and functional damage [36]. Reactive oxygen species are toxic to cells, but also essential because they are involved in the regulation of a large number of physiological processes, including cell signaling [37]. These stress-related conditions have been linked to serious diseases such as cancer, cardiovascular disease, degenerative diseases and premature aging [38,39]. In practice, multiple experimental methods are utilized to examine antioxidant activity. The free radical reduction method DPPH (1,1- diphenyl-2-picrylhydrazyl) and the ferric reducing antioxidant power (FRAP) assay have been performed in this study.

Table 2. Chemical composition of essential oil of *M. vulgare*.

No.	Compounds	Retention Time (min)	*m/z* by MS (%)	Peak Area %	Formula
1	3-Octanol	5.596	59 (100), 55 (70), 83 (50), 57 (14), 101 (12)	0.526	$C_8H_{18}O$
2	Benzenemethanol	6.122	79 (100), 77 (75), 108 (58), 107 (38), 51 (29)	0.976	C_7H_8O
3	Benzeneacetaldehyde	6.254	91 (100), 65 (31),	2.509	C_8H_8O
4	β-linalol	6.966	71 (100), 93 (80), 55 (78), 69 (48), 80 (33)	0.629	$C_{10}H_{18}O$
5	Nonanal	7.023	57 (100), 59 (70), 55 (69), 56 (60), 74 (50)	0.320	$C_9H_{18}O$
6	Benzeneethanol	7.165	91 (100), 92 (57), 65 (27), 122 (17), 71 (10)	1.564	$C_8H_{10}O$
7	Ketoisophorone	7.559	68 (100), 96 (100), 59 (20), 74 (15), 152 (13)	0.278	$C_9H_{12}O_2$
8	Lilac aldehyde	7.621	55 (100), 59 (95), 67 (62), 74 (55), 71 (44)	1.867	$C_{11}H_{18}O_2$
9	Menthone	7.740	69 (100), 55 (89), 112 (83), 70 (51), 97 (40)	2.02	$C_{10}H_{18}O$
10	α-Pinene oxide	7.982	67 (100), 93 (65), 109 (63), 123 (44), 68 (43)	1.274	$C_{10}H_{16}O$
11	α- Terpineol	8.228	59 (100), 93 (56), 81 (35), 121 (29), 67 (27)	1.718	$C_{10}H_{18}O$
12	Camphene	8.287	93 (100), 121 (82), 55 (75), 59 (60), 79 (50)	0.433	$C_{10}H_{16}$
13	4-Vinyphenol	8.470	120 (100), 91 (98), 59 (86), 74 (52), 65 (47)	0.173	C_8H_8O
14	Cyclohexanone, 2-isopropyl-2,5-dimethyl-	8.517	69 (100), 97 (62), 55 (52), 83 (38), 81 (30)	1.484	$C_{11}H_{20}O$
15	3-Thujanone	8.773	81 (100), 67 (98), 152 (48), 109 (42), 82 (34)	39.922	$C_{10}H_{16}O$
16	p-Menth-4-en-3-one	8.872	67 (100), 109 (95), 59 (87), 81 (85), 55 (76)	0.192	$C_{10}H_{16}O$
17	Piperitone	9.951	69 (100), 59 (86), 70 (75), 55 (72), 112 (70)	1.401	$C_{10}H_{16}O$
18	Eugenol	10.064	164 (100), 77 (80), 91 (64), 103 (62), 55 (52)	12.225	$C_{10}H_{12}O_2$
19	Isocaryophyllene	10.903	59 (100), 91 (90), 69 (82), 79 (78), 93 (70)	0.165	$C_{15}H_{24}$
20	Geranylacetone	11.065	69 (100), 59 (32), 67 (34), 74 (26), 107 (26)	0.195	$C_{13}H_{22}O$
21	β-Ionone	11.457	177 (100), 91 (42), 59 (33), 77 (32), 79 (32)	0.251	$C_{13}H_{20}O$
22	Topanol	11.684	205 (100), 57 (50), 220 (25), 91 (18), 105 (16)	2.017	$C_{15}H_{24}O$
23	β-Bisabolène	11.746	69 (100), 93 (75), 67 (53), 91 (47), 79 (44)	0.445	$C_{15}H_{24}$
24	Ethyl 2-Cyclohexyl-2-oxoacetate	11.779	83 (100), 55 (56), 111 (31), 111 (22), 53 (11)	0.485	$C_{10}H_{16}O_3$
25	Dihydroactinidiolide	12.037	111 (100), 67 (75), 109 (58), 137 (42), 79 (27),	0.369	$C_{11}H_{16}O_2$
26	Tabanone	12.281	59 (100), 91 (75), 74 (58), 69 (53), 55 (51)	0.658	$C_{13}H_{18}O$
27	Humulene	12.357	93 (100), 91 (42), 79 (36), 59 (33), 77 (30)	0.325	$C_{15}H_{24}$
28	Methyl stearidonate	12.599	79 (100), 91 (80), 55 (75), 59 (70), 93 (64)	0.250	$C_{19}H_{30}O_2$
29	β-selinene	14.355	91 (100), 55 (96), 59 (96), 79 (96), 74 (83)	0.188	$C_{15}H_{24}$
30	Hexadecanoic acid	15.803	73 (100), 55 (84), 60 (80), 57 (77), 69 (41)	1.448	$C_{16}H_{32}O_2$

Regarding the method of trapping the DPPH radical, it is reported that this radical involves a hydrogen atom transfer process [40,41]. Antioxidants reduce diphenyl picryl-hydrazyl with a purple color to a yellow compound, diphenyl picrylhydrazine, whose color intensity is inversely proportional to the concentration of antioxidants present in the

medium [42]. Due to its simplicity, stability and reproducibility, this test is widely used. The second is a colorimetric assay based on the reduction of ferric ions (Fe^{3+}) in $K_3Fe(CN)_6$ to ferrous ions (F^{2+}) contained in $K_4Fe(CN)_6$ [43]. The results of this study are presented in the Table 3.

Table 3. The antioxidant power of *M. vulgare* essential oil represented by the inhibitory concentrations of the two antioxidant tests.

Extract	DPPH			FRAP		
	IC_{50} (mg/mL)	Equation	R^2	IC_{50} (mg/mL)	Equation	R^2
M. vulgare EO	1.136 ± 0.0008	y = 58.683x − 5.823	0.989	2.998 ± 0.0004	y = 0.1659x + 0.002	0.997
Ascorbic acid	0.086 ± 0.0002	y = 521.66x + 4.323	0.993	0.086 ± 0.0002	y = 5.2173x + 0.047	0.997

Table 3 presents the values of IC_{50} which represent the concentration of the oil necessary to inhibit 50% of the DPPH free radical. Analysis of values achieved allows us to conclude that ascorbic acid has very potent antiradical activity with an IC_{50} equal to 0.086 mg/mL and a linear regression (R^2 = 0.993). This value is similar to that obtained by Bentabet N. et al. (0.08 mg/mL) [44]. Moreover, our essential oil has a very interesting DPPH free radical scavenging capacity with an IC_{50} of 1.136 mg/mL, which can be explained by the nature and type of chemical constituents present in our oil.

In addition to this work, and to evaluate the reducing power of ferric ions (Fe^{3+}) using *M. vulgare* essential oil, another test was performed. The FRAP method was used. Results, presented in Table 3, indicate that this test also demonstrated the significant effect of our oil (2.99 mg/mL, R^2 = 0.997) in comparison with ascorbic acid (0.086 mg/mL, R^2 = 0.997). The work carried out underlines the relevance of the species *Marrubium vulgare* for applications in pharmacology and phytotherapy. Based on these results, it can be deduced that this plant is one of the natural sources of antioxidant high value compounds.

3.6. Evaluation of the Antifungal Activity

The antifungal activity of *M. vulgare* essential oil on the tested molds was evaluated by the direct contact method. The results obtained are reported in Figure 3.

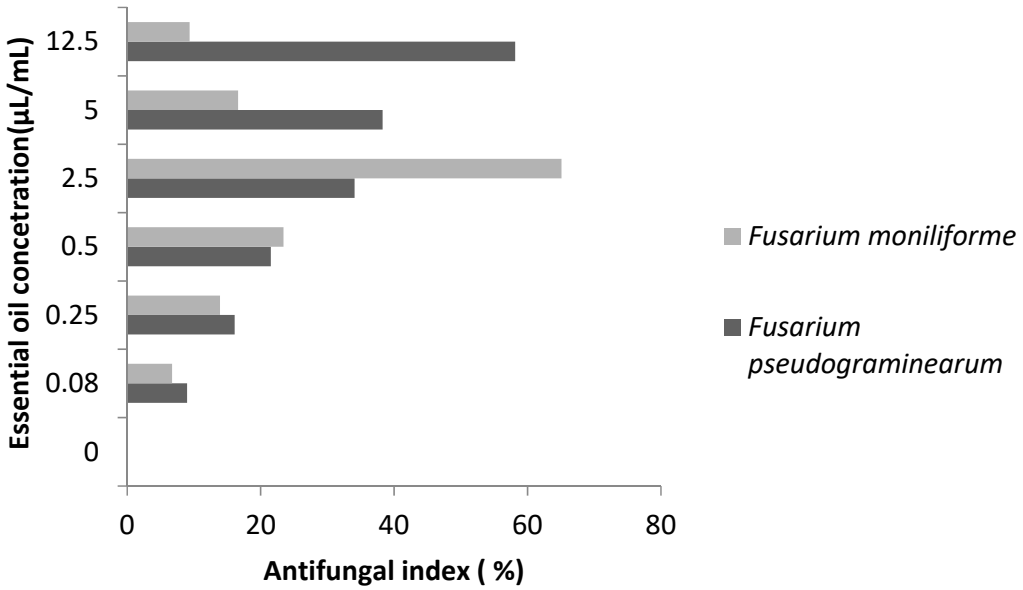

Figure 3. Antifungal index of *M. vulgare* essential oil.

Cryptogamic diseases caused by Fusarium cause significant damage to many plant species around the world. This results in reduced yields and food quality, which has a significant economic impact. The increasing resistance of fungi to conventional treatments and the fact that most antifungal substances have only fungistatic activity justify the search for new strategies to irradicate fungal infections and determine antioxidant powers. The antifungal power of substances of plant origin has been the subject of numerous in vitro studies [45,46]. Several studies have focused on the potential use of essential oils in the biological control of microscopic fungi [47]. In this context, the results of our research show the inhibitory effect of the essential oil of *M. vulgare* on the growth of the fungal strains tested, and can be applied to inhibit the growth of these fungi. The analysis of the results relating to mycelial growth in molds subjected to the action of the volatile fraction of different concentrations of essential oil tested allows us to note that our results are positive and that the degree of inhibition is a function of the concentration of essential oil used, as well as the nature of the mold tested. Numerically, the values of inhibition vary between 6.74% and 65.09%. The *Fusarium pseudograminearum* mycelia are inhibited by 58.15% at a concentration of 12.5 µL/mL, while those of *Fusarium moniliforme* are inhibited by 65.09% at a concentration of 2.5 µL/mL. Thus, *Fusarium moniliforme* is more sensitive than *Fusarium pseudograminearum* to *M. vulgare* essential oil.

Effect of Essential Oil on Spore Production

In order to know whether the effect exerted also influenced the sporulation of our fungi or just the mycelial growth, we adapted to the study of the sporulation. The results shown in Table 4 reveal a decreasing rate of sporulation proportional to the concentration of the medium in essential oil. The number of spores is often higher in *Fusarium moniliforme*, which is more resistant to the antifungal effect of the essential oil. In the case of *Fusarium pseudograminearum*, the sporulation is also less dense and smaller than the control (3.6×10^6 spore/mL). At lower concentrations of essential oil, sporulation is affected in both strains compared to that observed in the control. However, it is totally inhibited by the essential oil in *Fusarium moniliforme* and *Fusarium pseudograminearum* at a concentration of 0.5 µL/mL. Therefore the essential oil of *M. vulgare* showed satisfactory results and an excellent effect on the sporulation of both tested strains, and can be used as an antifungal agent. The results obtained in this study reveal a significant inhibition by the essential oil on spore production as well as on mycelial growth of the two tested Fusarium genera. Nevertheless, their action on sporulation is rather favorable. This effectiveness is probably related to its active ingredient, 3-Thujanone.

Table 4. Effect of *M. vulgare* essential oil on sporulation of tested molds.

Mold Tested	Number of Spores (spore/mL) Essential Oil Concentration (µL/mL)						
	Control Sample	**0.08**	**0.25**	**0.5**	**2.5**	**5**	**12.5**
Fusarium moniliforme	$1.59 \times 10^6 \pm 0.0001$	$9 \times 10^4 \pm 0.0002$	$3 \times 10^4 \pm 0.0042$	0	0	0	0
Fusarium pseudograminearum	$3.6 \times 10^6 \pm 0.001$	$3 \times 10^4 \pm 0.0053$	0	0	0	0	0

4. Conclusions

Marrubium vulgare L. is integrated into traditional medicine systems in Morocco and is generally recognized as safe. However, well-designed clinical trials are needed to move from its traditional use to a well-defined use for the prevention and treatment of various ailments. This work is part of the valorization of aromatic and medicinal plants from Morocco and aims to determine the chemical composition and the physicochemical characteristics of the *Marrubium*, as well as evaluate the biological activities of the essential oil produced by this plant.

The GC/MS analysis revealed that *M. vulgare* is very rich in bioactive compounds such as 3-Thujanone, Eugenol, Topanol, Menthone, etc., which can explain the interesting results

of antioxidant activity that were found. The control of our essential oil through the physicochemical characteristics has contributed to highlighting the high quality of our oil, which presents acidity and a low refractive index, evidencing that we have perfectly respected the modalities of storage in order to protect it from the oxidation and polymerization that can be caused by the air and light. The results obtained in this study reveal a significant inhibition by the essential oil on spore production as well as on mycelial growth of the two tested Fusarium genera. Nevertheless, their action on sporulation is rather favorable. Building on these encouraging results, *M. vulgare* from the Zaër region (Morocco) can be used as an alternative to other chemicals as a natural antioxidant and antifungal agent.

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