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Quality Control, Phytochemical Profile, and Antibacterial Effect of *Origanum compactum* Benth. Essential Oil from Morocco

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Abstract: *Origanum compactum* Benth (*O. compactum*) is widely used traditionally in Morocco to treat a broad range of illnesses, including infectious diseases. The aim of this study was to evaluate the phytochemical composition, quality control, and antibacterial activity of *O. compactum* leaf and flower essential oil. First, a quality control study on soil and irrigation water was performed to determine whether there was any risk of heavy metals endangering human health or causing stress to the plants studied. Laboratory examination of the environmental quality of the researched species revealed an almost absolute absence of metals that could endanger human health or any abiotic stressor. The essential oil was extracted by hydrodistillation. Chemical characterization was performed using gas chromatography-mass spectrometry (GC/MS). The yield of essential oil (EO) obtained by hydrodistillation of *O. compactum* leaves and flowers and moisture content were 4.27% and 12.20%, respectively. GC/MS identified 35 volatile compounds in the studied EO majorly composed of thymol (38.59%) followed by carvacrol (26.65%), o-cymene (14.33), and γ-terpinene (11.22%). The antibacterial activity of *O. compactum* leaf and flower essential oil was evaluated using the solid-state diffusion method against five Gram-negative bacterial strains and a Gram-positive strain. The results show that the essential oil of *O. compactum* leaves and flowers has a considerable inhibitory effect against *E. coli* with an MIC = 0.35 µg/mL, *E. pseudocoloides* (MIC = 0.35 µg/mL), *E. vekanda* (MIC = 0.35 µg/mL), *K. pneumoniae* (MIC = 0.7 µg/mL), *P. aeruginosa* (MIC = 0.35 µg/mL), and *S. aureus* (MIC = 0.35 µg/mL).

Keywords: *Origanum compactum* L.; essential oil; quality control; phytochemical; antibacterial activity

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1. Introduction

Medicinal herbs have received increasing attention in recent decades, as their economic and health importance has grown globally. The global demand for herbal medicines is expanding, as the cures generated from them are both effective and well tolerated by the body. Herbal treatments are now a significant element of basic health care in most nations, with approximately 80% of the world's population successfully treating themselves with herbs and plant-derived pharmaceuticals. As they contain bioactive compounds responsible for pharmacological qualities, medicinal plants represent a source of hope and a very important source for the manufacture of by-products such as essential oils and novel drugs [1].

The overuse and misuse of antibiotics in agriculture and human medicine to treat infectious diseases is to blame for the emergence of resistant strains [2,3]. Antibiotic resistance has become a major public health issue [4,5]. A British report estimated that in 2014, 700,000 deaths worldwide were linked to bacterial resistance to antibiotics [6]. Major plans to combat antibiotic resistance have been put in place, both nationally [6,7] and internationally [8,9]. Among these is the use of secondary metabolites derived from plants. Essential oils (EOs) are odiferous, highly volatile substances present in plants for defense purposes against biotic and abiotic stressors and are known for their interesting biological activities, namely antibacterial, antioxidant, antifungal, and insecticidal activity [10–12].

Oregano species are quite valuable as spices. They are also used to address health problems in traditional medicine. However, studies have shown an interest in plants of this genus because of their antimicrobial [13–16], insecticide [17], nematicide [18], antioxidant [19–21], hypoglycemic [22], and antithrombin effects [22].

Origanum compactum Benth. (*O. compactum*) belongs to the botanical family of Lamiaceae and is an endemic plant in Morocco and southern Spain [23]. It is common throughout the country's north and center. The common names are "za'tar tadlawi" and "za'tar". This dialect is also shared by other Moroccan oregano species and thymes [24]. It is a perennial plant with pubescent stems covered in long hairs, ovate-ovoid stem leaves that are hairy, inflorescences in dense and short spikes that are very purple, floral bracts that are ovate-lanceolate, rigid, coriaceous, flowers that are large, and a calyx with 5 triangular and subequal teeth with ciliated margins [25].

However, only a few oregano species' essential oils have been examined for their antibacterial properties. EOs have advantages in terms of both complex mechanisms of action and complex healing properties [26], making them potential medicines for the treatment of many illnesses. The purpose of this research was to determine the chemical composition and antibacterial activity of *O. compactum* essential oil against certain pathogenic bacteria strains.

2. Results and Discussion

2.1. Quality Control Study (Soil, Water, and Plant)

2.1.1. Irrigation Water Quality

pH and Salinity (Electrical Conductivity)

The pH is an important indicator of water quality, as the essential oil content may increase or decrease depending on water salinity and/or acidity. In the case of our study, the pH was 7.5, so it was weakly basic. We also found that the electrical conductivity (EC) was 0.71 dS/m, according to the USSLS standard, Richard 1954 (Table S1) [27]. This value indicates that salinity was average.

Risk Related to Nitrate Pollution

The presence of organic matter in soil can be determined by analyzing nitrates in water. Nitrates enter water through the leaching of nitrogenous products in the soil and the decomposition of natural organic matter. Its contribution to the soil, and hence to water, is thus highly dependent on the amount of organic matter present and the environmental circumstances [28]. Indeed, nitrate itself is not toxic, but its transformation into nitrites harms health [29]. In our case, the nitrate and nitrite content in the water analyzed in the Masmouda area were low. The recorded values were in the order of NO_3^- (23 ppm) and NO_2 (0.0001 ppm). These values confirmed the safety of water according to DIAEA/DRHA/SEEN 2008 standards (Table S2) [30].

Mineral Composition

The species studied are consumed by humans, can accumulate large quantities of heavy metals when they are in contact with polluted water or contaminated oil, and can induce disturbances in their metabolism [31]. Generally, heavy metals are carcinogenic depending on the type, concentration in the plant, exposure time, plant species, and the

possible presence of other elements [32]. The water in our test area was of good quality and could not cause any danger to the plants. This is noted in the results recorded in Table 1, where we noticed the total absence of heavy metals (Ag, As, Cd, Cr, Cu, Ni, and Pb). Mineral elements are divided into macro and microelements. The highest contents were illustrated for Ca, K, Mg, Na, P, Fe, and S, and the lowest were recorded for Co, Mn, Zn, Cr, Cu, Se, B, As, Cd, Mo, Ni, Pb, and V. The results are recorded in Table 1. These results indicate that calcium is the major element in this species, with a concerted effort of about 1876 mg/kg. Potassium was the second most abundant element, with a concentration of 1421 mg/kg. The other major elements have values in the order of 304 mg/kg for Mg, 170.8 mg/kg for P, 76.53 mg/kg for Fe, and 21.85 mg/kg for Na. The elements with lower content were 3.23 mg/kg for boron, 4.85 mg/kg for manganese, and 1.79 mg/kg for zinc. The other elements, Cd, Cu, Cr, Ni, As, Co, and Pb, showed only a few traces. These results are of great importance for the use of our species as a natural medicinal plant by the local population. The high content of Ca, K, Mg, and Fe, as well as the absence of Cd, Cu, Cr, Ni, As, Co, and Pb, may suggest that this studied species is a healthy and non-toxic nutrition.

Table 1. Water analysis of the Masmouda area using the ICP method.

Elements in ppm	Mean ± SD	Elements in ppm	Mean ± SD
Ag	0.0000 ± 0.0000	Ni	0.0000 ± 0.0000
Al	0.0182 ± 0.0008	Pb	0.0023 ± 0.0003
As	0.0000 ± 0.0000	Sb	0.0117 ± 0.0016
B	0.0597 ± 0.0007	Se	0.0000 ± 0.0000
Ba	0.0378 ± 0.0094	Sn	0.0262 ± 0.0011
Be	0.0000 ± 0.0000	Te	0.0282 ± 0.0001
Cd	0.0000 ± 0.0000	Ti	0.0122 ± 0.0004
Co	0.0016 ± 0.0006	Tl	0.0134 ± 0.0036
Cr	0.0000 ± 0.0000	V	0.0002 ± 0.0001
Cu	0.0000 ± 0.0000	Zn	0.0109 ± 0.0033
Fe	0.0044 ± 0.0008	k	1.0100 ± 0.0350
Li	0.0143 ± 0.0000	Na	51.260 ± 0.0800
Mn	0.0001 ± 0.0000	Ca	122.87 ± 0.0600
Mo	0.0027 ± 0.0001	Mg	8.7700 ± 0.0040

2.1.2. Soil Quality

Soil pH and Salinity

The pH is an important parameter because the degree of acidity or basicity plays a very important role in the assimilation of nutrients by the plant. The results of pH analysis, presented in Table S3, showed that the soil taken from the Masmouda Mountains had a basic pH with an alkaline tendency of about 8.50, according to the DIAEA/DRHA/SEEN 2008 standards [30].

The salinity analysis of the studied soil measured by electrical conductivity (EC in dS/m) revealed a value of 0.119 dS/m. According to DIAEA/DRHA/SEEN 2008 standards [30], according to Table S4, this value was less than 4 dS/m, which means the studied soil was not saline.

Total Phosphorus and Nitrogen

Phosphorus and nitrogen are major elements that are essential for the growth and development of plants. In particular, they play an essential role in the establishment of the root system, photosynthesis, and plant reproduction. Their variations are determined by the soil's physicochemical properties [33,34]. The results of the analysis showed that our soil was characterized by a low concentration of phosphorus and total nitrogen, assimilable to P_2O_5 (Table S5**)**, according to Delaunois (2008) standards [35]), and N_2 with values of about 16 ppm and 1.64%, respectively.

Soil Organic Matter

Soil organic matter (SOM) is an important indicator of soil quality degradation because of its contribution to soil stability, mineral fixation, and as a substrate for soil microorganisms. According to Table S6, our results showed that the analyzed soil had a very low organic matter content of 12.1 ppm (<0.7%). Generally, the organic matter of soils is influenced by several factors, namely climate, vegetation, and soil texture [36].

2.1.3. Quality Control of Plant Material (*O. compactum*)

Indeed, having a plant material with more reproducible active compounds in terms of quantity and quality requires quality control of the plant material, especially at the botanical level, from the harvest to the preparation of the powder or powder extract. Because of environmental conditions, harvesting and drying methods influence the quality of plant species [37].

pH and Ash Content

From the pH test (Table 2), we found that it varied between 6.27 and 6.34, so the *O. compactum* studied was very weakly basic. Green plants take water, CO_2 and minerals to produce their organic matter in the leaf cells. After calcination at a temperature of 550 °C in a muffle furnace until whitish ash was obtained, we found that the average percentage of organic matter was 9.34, while the ash content (89.39) was deduced according to the following formula: % Ash = 100-OM% (Table 2). The ash content varies depending on the species studied, the part used on the stage, and the place of harvesting. These results are very important for the use of our species as a natural aromatic and medicinal plant [38].

Table 2. pH and Ash Content. Results are expressed as mean ± SD.

Plant Species		pH	MO%	Ash
O. compactum	Test 1	6.34	8.96	91.03
	Test 2	6.27	9.73	87.76
	Mean ± SD	6.305 ± 0.049	9.345 ± 0.544	89.39 ± 2.312

2.2. Essential Oil Yield Extraction

The results indicated that the extraction yield of *O. compactum* leaves and flowers represented a percentage of 4.27 ± 0.20%. Likewise, the moisture content and the average volume had the highest percentages of 12.20% and 3.75%, respectively. It is clear from the results that the yield measurement of *O. compactum* leaves and flowers was influenced by different harvest times and vegetative stages. Moreover, it seems that the yields are related to the localities of the harvest. From these results, it can be concluded that the yield varied according to the vegetative stage. Significant differences were obtained in the yield of *O. compactum* leaves and flowers (2.10%) from the Rabat region [19]. Previously published results of other species of the genus *Origanum* reported that the lowest yields were observed for Tunisia (1.70%) and Saudi Arabia (1.30%) countries [39,40]. In addition, the EO yield of *O. compactum* that we obtained remained higher than that of *O. vulgare* collected from Iran (0.5%) [41]. In addition, *Origanum* sp. (*O. boissieri*, *O. saccatum*, *O. solymicum*, *O. ayliniae*, *O. sipyleumand*, and *O. hypericifolium*) from Turkey showed the lowest yields values: 0.51%, 1.13%, 0.65%, 0.60%, 0.73%, and 0.26%, respectively [42]. On the other hand, our results are similar to other studies reported by Boughendjioua and Seridi (2017) on the *O. vulgare* species (2.50%) [43]. The yield of EO of *O. compactum* extracted by Babili et al. (2011) and Chebli et al. (2003) was around 2.10%, and 5.4%, respectively [19,44]. The yields of EO obtained from the leaves and flowers of *O. campactum* are relatively high, which justifies its industrial exploitation. It is evident that the EO yield varied greatly during the harvest period, which had a significant effect on the oil content. Other factors that could influence

the yield of essential oils include the duration and conditions of drying, the period and medium of harvest, as well as the extraction technique.

2.3. Chemical Composition of Essential Oils

Volatile profile analysis by GC/MS identified the composition of the essential oil of *O. campactum* (Figure 1 and Table 3). A total of 30 compounds, representing 100% of the total chromatographic area, were identified. The extracted oil was strongly dominated by oxygenated monoterpenes (61.02%), and hydrocarbon monoterpenes (35.55%). On the other hand, sesquiterpene compounds were found in modest quantities (3.89%). Other major compounds that were identified as significant components were thymol (38.59%), carvacrol (26.65%), o-cymene (14.33), and γ-terpinene (11.22%). The other identified constituents were present in different amounts, such as (E)-caryophyllene (1.62%), α-terpinene (1.4%), linalool (0.81%), myrcene (0.80%), caryophyllene oxide (0.84%), α-thujene (0.22%), and α-pinene (0.47%). We also noted the presence of γ-cadinenes and δ-cadinenes in very low amounts (0.13 and 0.18%, respectively). However, the percentage yield of these elements varied from one growth stage to another. It has been noted that changes in the chemical composition of essential oils can occur over time due to geographic origin, climate and ecological conditions, harvesting season and drying process, as well as extraction procedures [45,46]. Thus, the chemical composition depends on all these factors that can promote the biosynthesis of certain molecules, and stop the synthesis of others [47,48]. Similar results were observed by Bouyahya et al. (2019) on *O. compactum* from the northwest of Morocco, where carvacrol (24.71%) and thymol (15.32%) were the major components [47]. In most cases, carvacrol was typically the main ingredient (12.6–88.7% of the oil) [49], and the total amount of the two phenolic monoterpenes carvacrol and/or thymol as well as their biosynthetic precursors, *p*-cymene and γ-terpinene, made up around 75% of each essential oil [40,50]. Other compounds have also been reported as important essential oil components, such as caryophyllene [51]. Moreover, it is known that the *Origanum* species presents great variability in its essential oil composition due to numerous parameters, mainly the origin of the plant, plant age, environmental factors such as season, climatic conditions (humidity, temperature) as well as agricultural practices [51,52]. The preponderance of these compounds, on the other hand, is known for their antibacterial and antifungal characteristics and has been investigated as a possible source of novel antimicrobial compounds, agents aiding food preservation, and treatment alternatives for infectious disorders.

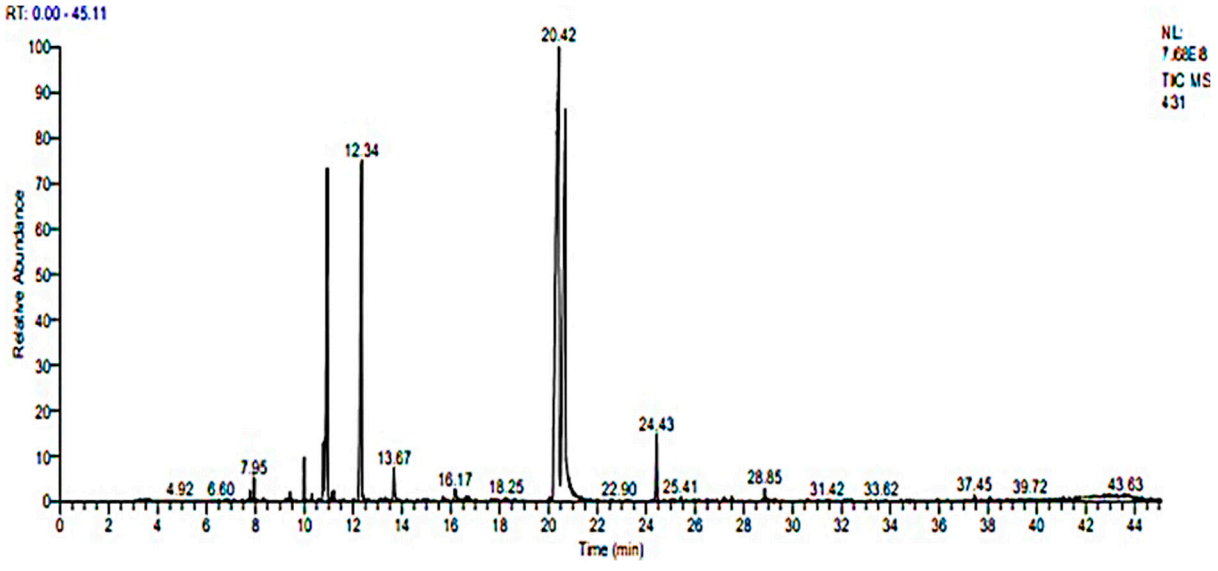

Figure 1. Chromatographic profiles of GC/MS analysis of the *O. campactum* EO sample.

Table 3. Phytoconstituents identified in the essential oil of *O. compactum* by GC-MS.

N°	KI	Chemical Compound	Relative Abundance (%)
1	930	α-Thujene	0.22
2	939	α-Pinene	0.47
3	954	Camphene	0.06
4	979	β-Pinene	0.06
5	979	1-Octen-3-ol	0.16
6	990	Myrcene	0.80
7	1002	α-Phellandrene	0.12
8	1011	δ-3-Carene	0.05
9	1017	α-Terpinene	1.41
10	1026	*o*-Cymene	14.33
11	1029	β-Phellandrene	0.13
12	1029	Limonene	0.2
13	1059	γ-Terpinene	11.22
14	1085	*m*-Cymenene	0.05
15	1088	Terpinolene	0.07
16	1096	Linalool	0.81
17	1169	Borneol	0.11
18	1177	Terpinen-4-ol	0.38
19	1188	α-Terpineol	0.08
20	1188	α-Terpineol	0.17
21	1238	Chrysanthenylacetate	0.06
22	1290	Thymol	38.59
23	1299	Carvacrol	26.65
24	1419	*(E)*-Caryophyllene	1.62
25	1454	α-Humulene	0.08
26	1513	γ-Cadinene	0.13
27	1523	δ-Cadinene	0.18
28	1583	Caryophyllene oxide	0.84
29	1959	Geranyl benzoate	0.16
30	1959	Geranyl benzoate	0.11
	Total Identification in %.		100
	Oxygenated monoterpenes in %.		61.02
	Hydrocarbon sesquiterpenes in %.		2.78
	Oxygenated sesquiterpenes in %.		1.11
	Hydrocarbon monoterpenes in %.		35.55

2.4. Antibacterial Activity

The antibacterial activity of *O. compactum* leaf and flower essential oil was evaluated by the solid-state diffusion method against five Gram-negative bacterial strains, *E. coli*, *E. pseudocoloides*, *E. vekanda*, *K. pneumoniae*, and *P. aeruginosa*, and a gram-positive strain which is *S. aureus*. The results of this antibacterial activity are presented in Table 4. The measurements of inhibition zones allowed us to classify strains according to antibiotics. Indeed, all negative gram bacterial strains tested showed resistance to amoxicillin. It is also noted that all bacterial strains tested showed sensitivity to imipenem. Compared to previous reports, the results of the antibacterial activity of *O. compactum* essential oil showed that the essential oil of this plant had excellent activity against all strains tested, with inhibition zone diameters varying between 16.2 mm and 27 mm. As indicated in Table 4, the results of the evaluation of the minimum inhibitory and bactericidal concentrations of *O. compactum* leaf and flower essential oil showed that the MIC and BMC values varied according to the microbial strains tested. Indeed, the MIC values of this oil ranged from 0.35 to 0.70 µg/mL, and the MBC values ranged from 0.35 to 2.8 µg/mL. Regarding the BMC/MIC activity ratio, our results indicate that it varies between 1 and 4. These values allow us to affirm that the essential oil tested for *O. compactum* is bactericidal.

Table 4. Results of antibacterial activity of *O. compactum* essential oil.

Bacteria	*O. compactum*				Amoxicillin 25 µg/disc	Imipenem 10 µg/disc
	IZ (mm)	MIC (µL/mL)	MBC (µL/mL)	MBC/MIC	IZ (mm)	IZ (mm)
E. coli	20.3	0.35	0.7	2	6	26
E. pseudocoloides	19.65	0.35	0.35	1	7	28
E. vekanda	20	0.35	0.7	2	7	25
K. pneumoniae	18.5	0.7	2.8	4	6	28
P. aeruginosa	16.2	0.35	0.35	1	6	25
S. aureus	27	0.35	0.7	2	19.5	61

MIC: Minimum Inhibitory Concentration, **MBC:** Minimum Bactericide Concentration, **IZ:** Zone of Inhibition, ***E. coli***, *Escherichia coli* (susceptible); ***E. pseudocoloides***, *Escherichia pseudocoloides*; ***E. vekanda***, *Escherichia vekanda*; ***K. pneumoniae***, *Klebsiella pneumoniae*; ***P. aeruginosa***, *Pseudomonas aeruginosa*; ***S. aureus***, *Staphylococcus aureus*.

These results correspond to the work of [52], who proved during their studies that the essential oil of the species *O. vulgare* is very active against tested bacterial strains, such as *E. coli*, *P. aeroginosa*, and *S. aureus*. Additionally, antibacterial tests on *O. vulgare* oil from Brazil showed significant antibacterial potential against *S. aureus*, *E. coli*, and *P. aeruginosa* [53]. These findings justify that the genus *Origanum* has powerful antibacterial properties. It has been well reported that the phenolic compounds present in plant extracts, especially essential oils, are more effective, with a wide spectrum of antibacterial activity [54,55]. In this context, the presence of high levels of thymol (38.59%), carvacrol (26.65%), *p*-cymene (14.33%), and γ-terpinene (11.22%) as dominant components in this essential oil and the potential synergistic phenomenon between them could be involved in the high antimicrobial activity demonstrated in this study. Boughendjioua et al. (2017) demonstrated in a previous study that *O. vulgare* essential oil, which is rich in thymol (23.49%) and carvacrol (21.31%), has very high antibacterial activity [43]. Indeed, it has also been shown that *K. pneumoniae* is affected by oxygenated monoterpenes, such as carvacrol (71%) [56]. In other studies, the essential oil of *O. vulgare* native to Saudi Arabia and Jordan, with a chemical profile characterized by the majority compounds carvacrol (79.5%) and thymol (68.73%), respectively, assesses the MIC against *E. coli*, *P. aeruginosa*, and *S. aureus* of 55, 196, and 63 µg/mL for Saudi Arabia and 107 and 325.83 µg/mL for Jordani [40]. Thus, the antibacterial activity of the essential oil of *O. vulgare* in Iran, which is rich in thymol (27.4%), is in agreement with our results. It has significant antibacterial activity against *S. aureus* (MIC and MBC, 0.15 and 0.3 mg/mL, respectively) [57]. *O. compactum* essential oil's propensity to stick to microorganisms' cellular membrane lipids may be one explanation for its antibacterial activity, increasing the porosity of the membranes and causing damage [58]. Additionally, this essential oil of *O. compactum* antibacterial activity may be related to the lipophilic qualities of its constituents, which enable it to permeate cellular membranes and inhibit several targets at once [59]. All of these indications point to the bioactive chemicals found in *O. compactum* essential oil, such as thymol, carvacrol, *p*-cymene, and γ-terpinene, as the source of the oil's antibacterial properties.

3. Materials and Methods

3.1. Study Area

The province of Ouazzane has a rich and varied ecological heritage, with forests, mountains, and rivers. It shelters diversified fauna, as well as dense vegetation and other forest species. This regional flora, which is rich in aromatic and medicinal plants, has the potential to be valorized as a source of high-value products for local inhabitants (Figure 2).

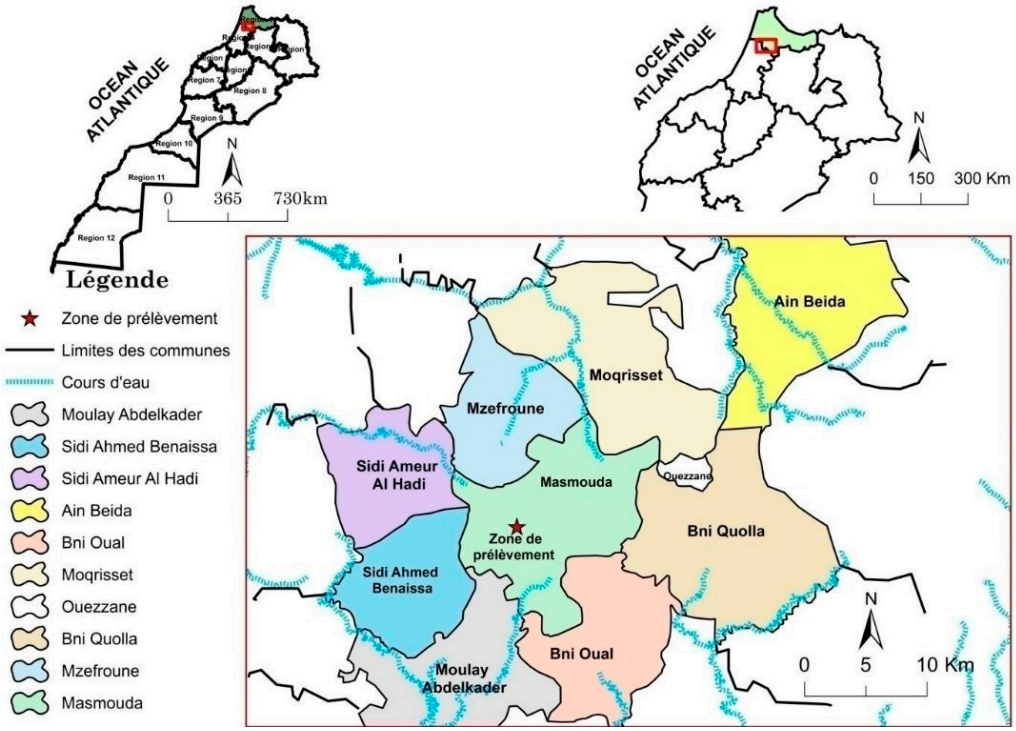

Figure 2. Geographic map of the sampling area.

3.2. Plant Material

The leaves and flowers of *O. compactum* were collected in the Ouazzane area of Morocco (34°48′36″ North 5°43′48″ West at 614 m altitude) during four different periods: in 2017 in full flowering, in 2018 (before and in full flowering), and in 2020 in full flowering. The samples were dried in the shade and protected from humidity at room temperature for about 10 days. Their botanical identification was accomplished at Rabat's Scientific Institute (Figure 3).

Figure 3. Morphological appearance of *O. compactum*.

3.3. Quality Control Study (Soil, Water, and Plant)

Within the framework of the valorization of the studied plant, a quantitative study of water and soil was carried out in the zone of the harvest to conduct a global study of the environment of the target plant. This research enabled us to assess the quality of a plant that is often used by the local populace in its natural or spontaneous state.

3.3.1. Irrigation Water Physicochemical Quality

The estimation of the physicochemical quality of water in the Masmouda area was made by measuring a set of parameters constituting the water. Abnormal results make it possible to highlight and evaluate pollution levels. Chemical pollution is probably the most frequent, much felt, and very diverse. It contains inorganic compounds, such as sodium, chloride, nitrate, phosphate, and heavy metals (lead, mercury, cadmium, etc.). The nitrate ions are reduced to nitrite ions by passing through a column of copper-plated cadmium in the presence of ammonium chloride [60]. The nitrite ions are then determined by diazotization with sulfanilamide and coupling with Naphthyl-ethylene diamine to form a strongly red-colored compound, the content of which is determined by colorimetric determination. In Table 2, we present the distribution of nitrate classes in water according to the standards DIAEA/DRHA/SEEN [30]. The pH of the water was determined using a pH meter according to the USSLS standard. Electrical conductivity (EC) was also determined to assess the salinity of the used water. The following table (Table 2) represents the distribution of water classes in terms of the EC value. Trace elements, heavy metals, and metallic trace elements were determined by inductively coupled plasma spectrometry (ICP) [61]. This method aims to estimate the quantity of accessible mineral elements that a plant can successfully extract. Inductively coupled plasma spectrometry was utilized to determine minor and major mineral content quickly and accurately. 1 g of plant powder was mineralized by heating it at 110 °C in a combination of 5 mL concentrated nitric acid (HNO_3) and 15 mL chloridric acid (HCl). After complete solubilization, the mixed sample was chilled to room temperature and diluted with ultrapure water to a final volume of 100 mL. The solution was then examined in duplicate, and trace metal concentrations were directly quantified using an ICP-AES (Agilent 5110 ICP-OES Spectrometer). The optimal instrumental conditions were maintained at 15 mL/min for stable plasma gas flow, and the auxiliary gas and nebulizer flow were maintained at 0.2 and 0.8 mL/min, respectively. The sample flow rate was 1.5 mL/min and the power was 1500 W [61].

3.3.2. Soil Quality

The soil is, above all, the source of ions that are essential for plants. The presence of excess ions, whether useful or not for plants, can then be at the origin of toxicity phenomena [62]. In this regard, four indicators of soil quality were chosen: pH, organic matter, total nitrogen, and assimilable phosphorous.

Soil pH

Soil pH was determined by the potentiometric method using a pH meter. This method consists of mixing a 10 g sample of soil with 20 mL of distilled water. The resulting mixture was stirred for 20 min, and then the reading was taken using a previously calibrated pH meter [63].

Organic Matter

Soil organic matter ensures the cohesion of soil constituents with each other. It contributes to better stability of the soil structure thanks to the numerous electrostatic bonds, and especially to the weak bonds that organic matter can ensure [64–67]. The Walkley and Black method [68], is the most extensively used for assessing the organic matter richness of the soils investigated, and it is based on the idea that potassium dichromate oxidizes the carbon in the soil. The color of potassium dichromate changes depending on the quantity of reduced products, and this color variation can be compared to the amount of organic carbon in the soil.

Total Nitrogen

The measurement of total nitrogen is carried out using the Kjeldahl (1954) method, which consists of two steps [69]. Digestion of the sample in concentrated sulfuric acid (H_2SO_4) at elevated temperatures to convert the organic nitrogen into inorganic nitrogen in

ammoniacal form and determination of the ammonium in the extract by titration of NH_3, released by steam distillation with sulfuric acid.

Assimilable Phosphorus

Assimilable phosphorus is found in the form of P_2O_5, HPO_4, and H_2PO_4, which are free in the solution and provide phosphorus feed to crop plants. The method used to determine the amount of available phosphorus in the soil is that of Olsen et al. [70] This method consists of extracting the phosphorus contained in the soil with a 0.5 M sodium bicarbonate solution at a constant pH of 8.5 and then measuring the phosphorus thus recovered in the solution by the colorimetric method. During the staining reaction, a complex of orthophosphoric acid and molybdic acid is formed and reduced. This is accompanied by a light blue color, the intensity of which varies according to the quantity of phosphorus retained in the solution. The phosphorus content was measured using a UV Visible spectrophotometer at a wavelength of 825 nm. The obtained results are expressed in ppm.

3.3.3. Quality Control of Plant Material

pH

The pH of a product determines its acidity. The method involved adding 10 mL of hot distilled water to 2 g of the tested samples. The mixture was agitated, filtered, and cooled. To record the pH value, the electrode was immersed in a considerable amount of this filtrate.

Ash Content

The principle is based on calcining the sample in a muffle furnace at 550 °C until a pale ash of consistent weight is obtained. To do this, 4 g of sample were weighed into porcelain capsules and placed in the muffle furnace set at a temperature of 550 ± 15 °C for 5 h until a light gray or whitish color was obtained; the capsules were then removed from the furnace and allowed to cool in the desiccator and weighed. The organic matter content was determined according to the following formula:

$$\boldsymbol{OM}\ (\%) = \frac{\boldsymbol{W_1} - \boldsymbol{W_2}}{\mathbf{PE}} \times \mathbf{100} \quad (1)$$

With ***OM:*** Organic matter; $\boldsymbol{W_1}$: Weight of capsule and sample before calcination; $\boldsymbol{W_2}$: Weight of the capsule and sample after calcination; **PE**: Test plug

The ash content was calculated as follows:

$$\mathbf{Ash}\ (\%) = \mathbf{100} - \boldsymbol{OM}(\%) \quad (2)$$

3.4. Phytochemical Analysis

3.4.1. Determination of Moisture Content

The oven drying process measured the water content of the gathered samples by measuring the mass of the fresh plant and its bulk after drying. Three replicates were performed. The moisture content was calculated using the following relationship:

$$\mathbf{MC}\% = (\mathbf{W0} - \mathbf{W1}) \times \mathbf{100} \quad (3)$$

With: **W0**: initial mass of the plant, **W1**: mass after drying

3.4.2. Essential Oil Extraction

The extraction of essential oil (EO) from *O. compactum* was performed by the hydrodistillation method. 100 g of the biomass consisting of the leaves and flowers of the dry plant material of *O. compactum* were immersed in a 2 L flask containing 1 L of distilled water and topped with a Clevenger apparatus and a ball cooler. The water-plant mixture was boiled

for three hours using a flask heater. The essential oil was measured in ml per 100 g of plant material, dehydrated with anhydrous sodium sulfate (Na_2SO_4), and stored under cover until use. This operation was repeated three times. The extraction yield was calculated from 100 g of the plant material using the formula:

$$\mathbf{EY}\ (\%) = \frac{\mathbf{V(EO)}}{(\mathbf{100} \times \mathbf{MC}\%)} \times \mathbf{100} \quad (4)$$

V (**EO**): Volume of EO recovered (ml); **MC**%: Moisture Content.

3.4.3. Phytochemical Analysis of the EO

The analysis of the chemical composition of the EO of *O. compactum* samples was carried out using a Thermo Electron gas chromatograph (Trace GC Ultra) coupled to a Thermo Electron Trace MS system (Thermo Electron: Trace GC Ultra; Polaris Q MS). The fragmentation is performed by the electronic impact of 70 eV intensity, and the chromatograph is equipped with a DB-5 type column (5% phenyl-methyl-siloxane) (30 m × 0.25 mm × 0.25 µm film thickness), a flame ionization detector (FID) fed by an H_2/Air-gas mixture, the column temperature was programmed at a rate of 4 °C/min rise from 50 to 200 °C for 5 min. The injection mode is split (leakage ratio: 1/70, flow rate ml/min), the carrier gas used is nitrogen with a flow rate of 1ml/min. The identification of the chemical composition of the EOs was carried out based on a comparison of their Kovats indices (KI) with those of known reference products in the literature [71,72]. It was completed by a comparison of the indices and mass spectra obtained by gas chromatography coupled with mass spectrometry (GC/MS), with different references.

$$\boldsymbol{KI} = \left[\frac{\boldsymbol{TRx} - \boldsymbol{RTn}}{\boldsymbol{RT(n+1)} - \boldsymbol{RTn}} + \boldsymbol{n}\right] \times \mathbf{100} \quad (5)$$

TRx: retention time of solute x, **TRn and TR*n* + 1**: the retention times of the linear alkanes with n and *n* + 1 carbon atoms that frame the solute peak.

3.5. Antimicrobial Testing of O. Compactum Essential Oils

3.5.1. Microbial Strains

The germs tested for the antimicrobial activity of *O. compactum* essential oils are composed of six microbial strains frequent in human pathologies, belonging to two different categories (Gram (+) positive and Gram (−) negative) as in the following Table 5. These bacterial species are responsible for skin infections (*S. aureus*), urinary tract infections (*E. coli*), and nosocomial infections (*K. pneumoniae* and *P. aeruginosa*), which are the cause of major public health problems. They were maintained by subculturing on a nutrient agar medium favorable to their growth for 24 h in the dark at 37 °C. They were all clinically isolated in a hospital environment in the neonatology department of the University Hospital Centre (CHU) of Fez.

Table 5. Bacterial strains used in this study and their categories.

Bacterial Strain	Categories (Gram +/Gram −)
E. coli	Gram −
E. pseudocoloides	Gram −
E. vekanda	Gram −
K. pneumoniae	Gram −
P. aeruginosa	Gram −
S. aureus	Gram +

3.5.2. Aromatogram in Solid Medium

The essential oils extracted from *O. compactum* were tested on the different strains of microorganisms using the solid-state diffusion method, which is based on the migratory

power of volatile extracts in a solid nutrient medium [73], by measuring the diameter of the inhibition zones. A bacterial suspension with a concentration of 108 CFU/mL in sterile physiological saline was spread by flooding the surface of a 90 mm diameter Petri dish containing Mueller Hinton agar. Then, sterile Wattman paper discs of 6 mm diameter were deposited and impregnated with 2 µL of the HE, and three replicates were done. Control experiments were conducted in parallel, serving as a positive control with an amoxicillin antibiotic of 25 µg/disc and Imipenem of 10 µg/disc. Inhibition diameters were read after 24 h of incubation at 37 °C.

3.5.3. Macro Dilution in Liquid Medium (MIC and MBC)

This process consists of preparing a bacterial inoculum in a sterile saline solution standardized from a 24 h bacterial culture. An emulsion was made to test the essential oil with a 30% DMSO (dimethylsulfoxide) solution. To the test tubes containing Mueller Hinton broth and 40 µL of the bacterial suspension (107 UFC/mL), the different volumes of EO were aseptically added to reach an increasing number of final EO concentrations from C1 to C10 (0.18; 0.35; 0.7; 1.4; 2.8; 5.6; 11.2; 22.4; 44.8; and 89.6 µL/mL), three replicates were made. After an incubation of 18 to 24 h at 37 °C, the MIC was determined and corresponded to the concentration of the first tube in which there was no visible growth of the tested germ. In addition, the BMC was assessed by subculturing 100 µL of the tubes with a concentration greater than or equal to the MIC to determine the BMC. The latter were the lowest concentrations that completely inhibited the growth of the germs in the starting suspension. The Petri dishes were incubated at 37 °C for 24 h. In addition, the BMC/MIC ratio was calculated to assess the antibacterial power.

4. Conclusions

The evaluation of the environmental quality of the studied species at the laboratory level showed the almost total absence of metals that can cause risks to human health. The GC/MS study of the essential oils of this plant revealed the presence of four significant chemical components: thymol, carvacrol, *o*-cymene, and γ-terpinene. This richness in diversified chemical compounds confers on the plant multiple therapeutic benefits. From a biological point of view, the essential oils of the studied plants showed very interesting antibacterial activity on the bacterial strains, namely: *E. coli*, *E. pseudocoloides*, *E. vekanda*, *S. aureus*, *K. pneumonia,* and *P. aeruginosa*. The current findings suggest that the antibacterial properties of the essential oils of this plant's parts make them suitable for use in the food industry as natural additives, colorants, and preservatives. Thus, this essential oil could be used to prepare phyto-drugs or dietary supplements for use by patients suffering from infectious diseases caused by these pathogenic bacteria.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/ijpb13040044/s1, Table S1. Distribution of water salinity classes according to the USSLS standard, Richard, 1954 [27]; Table S2. Distribution of water nitrate classes according to standards DIAEA/DRHA/SEEN 2008 [30]; Table S3. Soil pH rating along with the studied soil pH; Table S4. Soil salinity ranking; Table S5. P_2O_5 assimilable phosphorus classes; Table S6. Distribution of soil OM classes according to DIAEA/DRHA/SEEN (2008) [30].

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