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Research Article

Combined Effects of Domestication and Extraction Technique on Essential Oil Yield, Chemical Profiling, and Antioxidant and Antimicrobial Activities of Rosemary (*Rosmarinus officinalis* L.)

El Hassan Sakar,[1] **Ahmed Zeroual**,[1,2] **Ayoub Kasrati**,[3] **and Said Gharby**[4]

[1]*Laboratory of Biology, Ecology, and Health, FS, Abdelmalek Essaadi University, Tetouan, Morocco*
[2]*Engineering Laboratory of Organometallic Molecular Materials and Environment (LIMOME), Department of Chemistry, Faculty of Sciences, Dhar Mahraz Fez, Sidi Mohamed Ben Abdellah University, B.P. 1796 Fez-Atlas, Fez 30003, Morocco*
[3]*Laboratory of Drug Science Biomedical and Biotechnological Research, Faculty of Medicine and Pharmacy of Casablanca, Hassan II University, Casablanca, Morocco*
[4]*Biotechnology Analytical Sciences and Quality Control Team, Polydisciplinary Faculty of Taroudant, Ibn-Zohr University, 83000 Taroudant, Morocco*

Correspondence should be addressed to El Hassan Sakar; e.sakar@uae.ac.ma

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We aimed at comparing the effects of domestication and extraction technique on the chemical profiling and antioxidant and antimicrobial activities of *Rosmarinus officinalis* essential oil (ROEO). This was isolated from wild (WR) and cultivated rosemary (CR) using microwave-assisted extraction (ME) and Clevenger hydrodistillation (CH). Domestication was the main variability source in ROEO constituents, while yield was equally determined by domestication and extraction techniques. Our results revealed important variations, owing to domestication and isolation technique, in terms of ROEO yield (1.10–2.85%), major compounds: α-pinene (14.07–42.03%), camphene (2.26–8.19%), β-pinene (0.35–3.76%), α-terpinene (0.55–2.92%), *p*-cymene (1.22–4.18%), limonene (0.64–2.79%), 1,8-cineole (31.73–40.72%), β-myrcene (2.09–3.2%), linalool (0.22–1.94%), camphor (12.12–19.66%), borneol (0.53–1.67%), and α-terpineol (1.46–7.45%) as well as minimal inhibitory concentration (MIC, 6.17–15.50 μg/mL), and antioxidant activity (IC_{50}, 2.61–8.58 mg/mL). WR performed better in terms of yield, limonene, cineole, camphor, MIC, and IC_{50}, while the remaining compounds were better expressed in CR. ME displayed high records of ROEO traits except for limonene, camphor, and verbenone (better expressed in CH). Principal component analysis confirmed the obtained findings via the separation of WR, CR, and techniques through the first two components (over 93% of data variability). In conclusion, *R. officinalis* domestication results in differentiated effects on ROEO traits, fostering a better accumulation of some compounds but reducing yield of other compounds and therefore antioxidant along with antimicrobial activity. ME could be recommended as a green method for ROEO isolation since it was more efficient in terms of the investigated ROEO traits.

1. Introduction

Rosemary is an important industrial crop belonging to medicinal and aromatic plants. It is known botanically as *Rosmarinus officinalis* L. (*R. officinalis*). Its wild populations grow primarily in the western region of the Mediterranean basin. *R. officinalis* domestication and breeding give rise to more than 20 genotypes (varieties, cultivars, etc.) [1–3]. Following these authors, *R. officinalis* has been used since ancient times for various purposes such as culinary, medicinal, and ornamental. In food science field, *R. officinalis* is known for its essential oil endowed with great antimicrobial and antioxidant properties, hence its use as food preservative. *R. officinalis* essential oil (ROEO) has also other applications including culinary, medicinal, and pharmacology, as well as a food additive [2, 4–7]. Likewise, many

pharmacological activities of *R. officinalis* have been documented in various previous works carried out on the species from different areas in the Mediterranean basin and abroad [4, 8–11]. According to these studies, *R. officinalis* possesses important antimicrobial, antioxidant, anti-inflammatory, and antitumor activities, among others.

The nutritional values of *R. officinalis* along with its bioactive compound profiling were compiled and reviewed in the study by Ribeiro-Santos et al. [2]. *R. officinalis* contains different vitamins (total ascorbic acid, thiamin, riboflavin, niacin, vitamin B6, vitamin E, folate, vitamin B12, vitamin A, and vitamin D), fatty acids (saturated, monounsaturated, and polyunsaturated), and minerals (Ag, Al, As, B, Ba, Bi, Ca, Cd, Co, Cu, Cr, Cu, Fe, K, Li, Mg, Mn, Na, Ni, P, Pb, Se, Sr, Ti, V, and Zn). Their contents depend on plant parts. Moreover, various kinds of phytochemicals are found in *R. officinalis* depending on a set of factors such as plant part, processing technique, and geographical origin, among others [2, 12]. The most important ones are borneol acetate, camphor, eucalyptol, α-pinene, β-pinene, β-caryophyllene, verbenone, myrcene, borneol, camphene, and 1,8-cineole. Their content can change considerably according to plant part, plant phenology, and extraction method [2].

Given their different interests (economic, medicinal, etc.) along with increasing market demand, several medicinal and aromatic plants (MAPs) are domesticated and cultivated under higher agronomic inputs (fertilization, irrigation, etc.) for large-scale production. *R. officinalis* became an important industrial crop. Moreover, the increasing market demand for MAPs leads to overexploitation of wild PAM populations. In such a context, a number of agencies are calling for conservation and recommending the cultivation of wild MAPs [13–15]. Cultivation practices as well as environmental conditions influence, to a large extent, plant morphological traits, biomass, and chemical composition [2, 16, 17]. Domestication-induced effects on *R. officinalis* were studied in terms of organic extracts [18, 19]. According to these evidences, extracts from wild *R. officinalis* demonstrated high values of yield, antioxidant, antimicrobial, alkaloids, flavonoids, tannins, saponins, as well as some minerals. MAP domestication and its effects on essential oil yield and chemical profiling were previously investigated in other species [18, 20–22]. It was demonstrated that MAP domestication results in low essential oil yield as well as significant variations in terms of chemical profiling and therefore antioxidant as well as antimicrobial activity. For instance, Abdellaoui et al. [22], while studying the effects of domestication on fennel (*Foeniculum vulgare* Mill.), reported that wild *F. vulgare* recorded the highest yield of essential oil ($3.67 \pm 0.13\%$), whereas cultivated *F. vulgare* exhibited the lowest yield ($2.13 \pm 0.07\%$). Moreover, the wild *F. vulgare* essential oil showed the highest phenolic content (222.24 mg/mL) and antioxidant power based on β-carotene bleaching assay ($IC_{50} = 0.694$ mg/mL) and TBARS assay ($IC_{50} = 1.193$ mg/mL).

The chemical profiling and biological activities of *R. officinalis* have attracted the attention of many studies. However, little is known about its domestication and ROEO changes, as well as related biological activities. To the best of our knowledge, no detailed information exists on the effects of domestication and extraction technique on yield, chemical profiling, and related biological activities of ROEO, hence the originality of the current work. The objectives are as follows: (i) to assess antioxidant and antimicrobial activities of ROEO, (ii) to investigate the ROEO chemical profiling, and (iii) to evaluate the effects of domestication and the EO isolation technique on ROEO yield, chemical profiling, and related antioxidant and antimicrobial activities.

2. Materials and Methods

2.1. Plant Material Domestication and Sampling. The plant species was first identified at the Faculty of Science and Technology (FST, Sidi Mohamed Ben Abdellah University, Fez, Morocco), and the following code was attributed to *R. officinalis*: ref. LCOA-FST 018/2018). A total of 100 stem cuttings of *R. officinalis* were randomly collected in 2018 at the vegetative phenophase from a natural population situated in Taounate province, located in central-northern Morocco (34°31′48″N, 4°42′36″W). To preserve the natural population's genetic identity, clonal propagation was used. The cuttings were transplanted immediately into the experimental area at the National Agency for Medicinal and Aromatic Plants (ANPAM, Taounate, Morocco) with a planting distance of $0.1\,m \times 0.1\,m$. During the establishment phase (the first month), an irrigation level consisting of 80% of the soil field capacity was applied in the morning twice a week. Thereafter, the irrigation was applied once a week. In both cases (wild populations and domesticated plants), the soil was loam clay and no fertilizers were applied during the trial period.

R. officinalis sampling was done at full blooming phenophase (BBCH 67) for both cultivated (domesticated) and wild (natural population used for domestication, nearby ANPAM) with three independent samples ($n = 3$). In fact, aerial parts (leaves) were collected in April, 2020. Each sample consisted of about 2 kg of fresh leaves. The samples were subjected to drying in a ventilated, dark room for a week. All extractions and measurements were then carried out on a dry basis. From a climatic standpoint, Taounate Province is known to have a Mediterranean climate type: humid in winter but semiarid in summer, following the Köppen-Geiger classification. It receives 472 mm (on average) of annual precipitation with 14.2°C as an average temperature.

2.1.1. Microwave-Assisted Extraction. The microwave method has several advantages over traditional alternatives methods such as a shorter isolation time (about 15 min versus at least 3 h needed for hydrodistillation), an environmental impact (a lower energy cost), and a cleaner method (since there are no residues generated or solvents used). Microwave-assisted extraction also enhances biological properties (antimicrobial and antioxidant activity)

and provides more valuable essential oils (EOs, with a higher amount of oxygenated compounds) [23].

Extraction via solvent-free microwave-assisted has been carried out as described in the study by Lucchesi et al. [24] in a Milestone "DryDist" microwave-laboratory-oven type. It is a microwave reactor with a maximum power of 2.45 GHz and 10^3 W. The temperature inside the apparatus was monitored through an external infrared sensor. From each sample, 150 g of the plant material was heated under atmospheric pressure with a set power density of $1\ W{\cdot}g^{-1}$during 15 min without the use of any solvents. The release of EO contained within plant tissues is fostered by direct interaction between microwaves and biological water being present in fresh plant material. Thanks to earth gravity, the mixture containing hot "crude juice" and in situ water moves on a spiral condenser where it can be easily condensed. The oily condensate was gathered continuously into a receiving flask. At the end, EO was recovered, dried over anhydrous sodium sulphate, and kept in amber vials at 4°C for other determinations.

2.1.2. Clevenger Hydrodistillation. To isolate EO from both wild and cultivated *R. officinalis*, dried aerial parts were subjected to hydrodistillation via a Clevenger-type apparatus as described in the study by Zeroual et al. [25]. Three independent distillations involving each 100 g of plant material were made by boiling for three hours. They were run in a 1 L flask topped by a column consisting of 60 cm in length associated with a refrigerant, as described in Jennan et al. [26]. Isolation of the obtained EO from water was done via decantation. EO was then dried over anhydrous sodium sulphate and kept in amber vials at 4°C for further use.

ROEOs were isolated using two different methods: microwave-assisted extraction and Clevenger hydrodistillation are described below. ROEO yield was calculated and expressed as g/100 g per dry matter (DM) according to the following equation:

$$\text{ROEO Yield}\,(\%, \text{DM}) = \frac{\text{ROEO}\,(g)}{\text{Test sample}\,(g)} \times 100. \quad (1)$$

2.2. Phytochemical Profiling of ROEO Using GC/MS. Analysis of ROEO, isolated by both techniques, was carried out as described in the study by Talbaoui et al. [27]. It was run on a TRACE GC ULTRA equipped with nonpolar VB5 (95% methyl polysiloxane and 5% phenyl), a capillary column (30 m × 0.25 mm i.d. and 0.25 μm for film thickness). It is coupled directly to a mass spectrometer (Polaris Q) and operates in electron impact EI (70 eV) mode. Both temperatures (of injector and detector) were set at 250 and 300°C, respectively. The oven temperature was programmed to raise at 4°C/min for 40–180°C and at 20°C/min for 180–300°C. The gas carrier was helium (flow rate: 1 mL/min); samples consisting of each 1 μL were injected following a splitless mode.

2.3. DPPH Free Radical Scavenging Activity. The power to trap the radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was determined by the standard method described by Brand-Williams et al. [28] with slight modifications. Briefly, 0.2 mL of various concentrations of ROEO was added to 1.8 mL of a DPPH methanolic solution (0.11 mM). After incubation for 30 min in darkness at room temperature (23 ± 2°C), absorbances were read at 517 nm against a blank solution of methanolic DPPH. DPPH radical scavenging activity (also known as antioxidant activity, AA) has been computed according to the following equation:

$$\%\,(\text{AA}) = \left[\frac{\left(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}\right)}{\text{Abs}_{\text{control}}}\right] \times 100, \quad (2)$$

where $\text{Abs}_{\text{control}}$ is the absorbance of the control (containing all reagents except the ROEO sample) and $\text{Abs}_{\text{sample}}$ sample is the absorbance of the ROEO at $\lambda = 517$ nm. Ascorbic acid was used as a positive control, and the concentration of ROEOs that inhibits 50% of DPPH (IC_{50}) was deducted.

2.4. Determination of Antimicrobial Activity. The microbial strains used in this investigation are of significant concern for human health problems as well as food spoilage. ROEOs were tested, for antimicrobial activity against the 4 strains, including Gram-positive, Gram-negative, and a fungus: *B. subtilis* (*Bacillus subtilis* ATCC 3366), *C. albicans* (*Candida albicans* ATCC 10231), *E. coli* (*Escherichia coli* ATCC 25922), and *S. aureus* (*Staphylococcus aureus* ATCC29213).

To determine the minimum inhibitory concentration (known as MIC), the agar dilution method was used following the Natural Committee for Clinical Laboratory Standard [29]. All determinations were performed in nutrient broth for microbial strains. Increasing concentrations (7.5–20 μg) from ROEOs were added separately to 1 mL nutrient broth tubes containing 10^5 CFU/mL of live microbial strains. To evenly spread ROEOs throughout the broth, the tubes (10 mL of broth) were immediately transferred into an incubator shaker. The highest dilution, which fits the lowest concentration, for which there is no visible bacterial growth, corresponds to the MIC. Thereafter, tubes displaying no growth were cultured on nutrient agar plates to verify whether the inhibition was reversible or not.

2.5. Statistical Analyses. All determinations as well as measurements were performed in triplicate. Quantitative differences were assessed through the general linear model followed by Duncan's test. Results were expressed as mean ± standard deviations (SD, $n = 3$). Differences were considered significant at a 5% probability level. Population normality was checked using the Shapiro test. Principal component analysis (PCA) and Pearson correlation matrices were carried out on mean values by means of the Statgraphics package (StatPoint Technologies, Inc., Virginia, USA) version XVII.

Table 1: The mean squares from combined analyses of variance for ROEO yield and its major compounds.

Source of variation	Df	Yield ($\times 10^{-3}$)	α-Pinene	Camphene ($\times 10^{-3}$)	β-Pinene	α-Terpinene ($\times 10^{-2}$)	p-Cymene ($\times 10^{-2}$)	Limonene ($\times 10^{-2}$)	1,8-Cineole	β-Myrcene ($\times 10^{-2}$)	Linalool ($\times 10^{-2}$)
Domestication: D	1	2017.2***	1162.69***	96840.0***	23.27***	1463.0***	2048.9***	1073.5***	34.7***	199.26***	609.19***
Technique: T	1	2613.3***	205.34***	180.1*	0.97**	8.5	35.4*	20.0**	51.9***	27.30**	24.37
Replicate (R)	2	7.3	0.17	6.9	0.01	0.8	0.2	2.2	0.2	0.04	4.22
D × T	1	0.1	171.31***	0.4	1.18*	2.9	20.3*	4.7*	70.1***	6.90*	26.11
Residual	4	5.9	0.20	8.8	0.02	1.3	1.2	0.4	0.2	0.79	6.47
Total (corrected)	9										

*, **, and *** indicate significance at 0.05, 0.01, and 0.001 levels of probability, respectively.

Table 2: Mean values of yield (%, db), chemical composition (%), minimal inhibitory concentration (MIC, (μg/mL)), and IC_{50} (mg/mL) for essential oils isolated from wild and cultivated *R. officinalis* using microwave-assisted extraction and the Clevenger hydrodistillation method. Results are mean values followed by SD ($n = 3$). For each line, values followed by the same letter are not significantly different at the 5% probability level.

	Wild rosemary		*Cultivated rosemary*	
	Microwave	Clevenger	Microwave	Clevenger
ROEO yield (%, db)	02.85 ± 0.08^{a}	01.93 ± 0.07^{c}	02.04 ± 0.07^{b}	01.10 ± 0.09^{d}
ROEO composition (%)				
α-Pinene	42.03 ± 0.15^{a}	26.20 ± 0.07^{b}	14.78 ± 0.28^{c}	14.07 ± 0.80^{d}
Camphene	02.52 ± 0.09^{c}	02.26 ± 0.04^{d}	08.19 ± 0.07^{a}	07.96 ± 0.13^{b}
β-Pinene	00.35 ± 0.04^{c}	00.41 ± 0.02^{c}	03.76 ± 0.08^{a}	02.57 ± 0.24^{b}
α-Terpinene	00.62 ± 0.05^{c}	00.55 ± 0.02^{d}	02.92 ± 0.09^{a}	02.66 ± 0.18^{b}
p-Cymene	01.83 ± 0.06^{c}	01.22 ± 0.08^{d}	04.18 ± 0.03^{a}	04.10 ± 0.14^{b}
Limonene	02.40 ± 0.07^{b}	02.79 ± 0.08^{a}	00.64 ± 0.04^{d}	00.77 ± 0.20^{c}
1,8-Cineole	31.73 ± 0.48^{d}	40.72 ± 0.11^{a}	33.16 ± 0.08^{b}	32.48 ± 0.56^{c}
β-Myrcene	02.54 ± 0.09^{c}	02.09 ± 0.02^{d}	03.21 ± 0.08^{a}	03.06 ± 0.03^{b}
Linalool	00.22 ± 0.07^{c}	00.23 ± 0.04^{c}	01.94 ± 0.04^{a}	01.36 ± 0.45^{b}
Camphor	12.12 ± 0.08^{d}	19.66 ± 0.12^{a}	16.51 ± 0.17^{c}	18.79 ± 0.42^{b}
Borneol	00.96 ± 0.11^{c}	00.53 ± 0.04^{d}	01.67 ± 0.28^{a}	01.57 ± 0.05^{b}
α-Terpineol	01.77 ± 0.07^{c}	01.46 ± 0.24^{d}	07.45 ± 0.21^{a}	07.05 ± 0.20^{b}
Verbenone	00.18 ± 0.03^{d}	00.77 ± 0.04^{b}	00.36 ± 0.04^{c}	00.86 ± 0.09^{a}
Bornyl acetate	00.06 ± 0.02^{b}	00.04 ± 0.00^{b}	00.85 ± 0.03^{a}	00.83 ± 0.04^{a}
β-Caryophyllene	00.00 ± 0.00^{c}	00.10 ± 0.05^{b}	00.19 ± 0.02^{a}	00.12 ± 0.00^{b}
α-Caryophyllene	00.00 ± 0.00^{c}	00.15 ± 0.06^{b}	00.26 ± 0.03^{a}	00.15 ± 0.01^{b}
Monoterpene hydrocarbons	49.75	33.43	34.47	32.13
Oxygenated monoterpenes	49.52	65.46	64.30	65.17
Sesquiterpene hydrocarbons	00.00	00.25	00.45	00.27
Other oxygenated compounds	00.06	00.04	00.85	00.83
Total	99.32 ± 0.25^{c}	99.01 ± 0.17^{d}	99.61 ± 0.37^{a}	98.37 ± 0.31^{b}
MIC (μg/mL)				
E. coli	09.83 ± 1.26^{d}	11.50 ± 1.32^{c}	13.87 ± 1.31^{b}	15.50 ± 1.32^{a}
S. aureus	06.83 ± 0.29^{d}	08.83 ± 0.76^{c}	11.17 ± 0.76^{b}	12.83 ± 0.76^{a}
B. subtilis	06.17 ± 0.29^{d}	07.83 ± 0.29^{c}	09.67 ± 0.76^{b}	11.50 ± 0.50^{a}
C. albicans	07.50 ± 0.50^{d}	09.83 ± 1.06^{c}	12.67 ± 0.58^{b}	13.83 ± 0.76^{a}
IC_{50} (mg/mL)	02.61 ± 6.18^{d}	06.48 ± 5.72^{b}	04.39 ± 5.87^{c}	08.58 ± 5.85^{a}

*db = dry biomass and ROEO = *R. officinalis* essential oil.

3. Results and Discussion

3.1. Data Variability Analysis. The combined analyses of variance for ROEC yield and its chemical compounds are summarized in Table 1. Based on these outcomes, both factors (domestication and extraction technique) as well as their interaction impacted significantly (at least at $p < 0.05$, Table 1). However, domestication was the main source of variability since it explained around 92% of the variance in the investigated variables except for yield and cineole. These were mainly under the dependency of extraction technique and the interaction of domestication with the extraction technique.

3.2. Essential Oils' Yield, Profiling, and Their Activities. Results of yield, ROEO chemical profiling, antioxidant, and antimicrobial activities are presented in Table 2. From these results, it appears that the extraction technique and rosemary's origin (wild or cultivated) impacted ($p < 0.05$) ROEO yield, composition, as well as antioxidant and antimicrobial activities. Microwave-assisted extraction had the best records for ROEO yield for both rosemary samples. Furthermore, ROEO isolated from wild rosemary demonstrated its superiority in terms of yield ($02.85 \pm 0.08\%$) obtained via microwave-assisted extraction. Taking together all the results of ROEO chemical profiling, 16 compounds were revealed with significant differences ($p < 0.05$) between extraction techniques and rosemary origins. Nature of the major compounds (whose % ≥1) depended mainly on rosemary domestication (Table 2). Representative chromatograms for ROEO isolated by both techniques are shown in Figure 1. In ROEO from wild rosemary, 8 compounds were detected, in a decreasing order (in the case of microwave extraction), α-pinene, 1,8-cineole, camphor, camphene, β-myrcene, limonene, α-terpineol, and p-cymene. ROEO isolated from cultivated rosemary (obtained by both techniques, Table 2) was found to have 11 major compounds following microwave extraction: cineole, camphor, α-pinene, camphene, α-terpineol, p-cymene, β-pinene, β-myrcene, α-terpinene, linalool, and borneol. A similar trend was observed for ROEO from Clevenger hydrodistillation; however, significant differences ($p < 0.05$) were seen regarding the amount of major compounds between the two techniques. These outcomes are comparable with previously published literature on ROEO profiling from

Morocco. El Kharraf et al. [30] studied ROEO from a wild *R. officinalis* population from Figuig province in eastern Morocco (under an arid Mediterranean climate). According to this study, ROEO is dominated by 1,8-cineole (148 mg/mL), camphor (40 mg/mL), α-pinene (28 mg/mL), α-terpineol (10.6 mg/mL), borneol (8.40 mg/mL), camphene (8.14 mg/mL), and limonene (6.64 mg/mL). In a recent work by Elyemni et al. [31], ROEO yield was found to be $1.35 \pm 0.04\%$ (cultivated *R. officinalis* from Fez region, Morocco) and $2.24 \pm 0.05\%$ (wild *R. officinalis* from Figuig province, Morocco). In a study performed across 7 Iranian populations, Bajalan et al. [32] reported a ROEO yield range of 0.6–2.35 mL/100 g, and the major compounds were 1,8-cineole (5.63–26.89%), camphor (1.66–24.82%), and α-pinene (14.69–20.81%). Bajalan et al. [33] studied quali- and quantitative variations of EO in 21 Iranian *R. officinalis* accessions collected in contrasting environments. They outlined important variations in terms of ROEO yield and its composition. In fact, they found that ROEO yield ranged from 0.53 to 2.6 mL/100 g with significant variations among the studied accessions. According to the same work, major compounds were 1,8-cineole (5.32–28.29%), camphor (1.58–25.32%), and α-pinene (14.19–21.43%). Our ROEO profiling was consistent with that reported by other authors for *R. officinalis* grown in various agro-climatic regions in the Mediterranean basin and aboard [30, 32–36]. As discussed in the study by Bajalan et al. [32], ROEOs from Italy, Morocco, Tunisia, and Turkey were reported to have 1,8-cineole as the main component, accounting for over 40% [34]. ROEO from French, Greek, and Spanish has 1,8-cineole along with α-pinene and camphor in similar contents (in the range 20–30%) according to the study by Ojeda-Sana et al. [37].

More recently, Rathore et al. [38], while investigating ROEO from some *R. officinalis* accessions grown in the western Himalaya, found similar chemical profiling with important season-to-season variations. In fact, ROEO major compounds were dominated by 1,8-cineole, ranging from 32.50 to 51.79% depending upon harvest season and from 38.70 to 42.20% as a range value in the studied accessions. These authors demonstrated a dynamic variation of ROEO yield and profiled it according to year and season. In fact, ROEO yield reached its peak in the autumn (0.87%), decreased to 0.68% in the summer, and reached its lowest value in the rainy season (0.48%). According to the same authors, similar trends were outlined in the case of ROEO profiling. In autumn, Indian ROEO was dominated equally by 1,8-cineole (32.5%) and camphor (31.5%). Moreover, 1,8-cineole content increased over camphor to reach 37.35% in the summer and 51.79% in the rainy season.

Domestication seemed to affect ROEO yield and chemical profiling and thus modify related antioxidants along with antimicrobial activities. Wild *R. officinalis* showed higher yield values regardless of the ROEO isolation technique. Monoterpene hydrocarbons (mainly α-pinene, camphene, and p-cymene) were higher in wild *R. officinalis* as compared to the cultivated one but the picture was reflected for oxygenated monoterpenes (consisted mostly in 1,8-cineole, β-myrcene, camphor, and α-terpineol), sesquiterpene hydrocarbons (mostly α-caryophyllene), and other oxygenated compounds (represented by bornyl acetate). These outcomes are consistent with those reported by Elyemni et al. [31] for wild and cultivated *R. officinalis* collected from two contrasting localities. Such differences could be attributed to genetic factors and environmental conditions. As explained by Elyemni et al. [31], wild *R. officinalis* receives neither fertilization nor irrigation. As discussed in the study by Serralutzu et al. [39], α-pinene along with camphor showed statistically significant correlations with temperature, but it was not the case for borneol. According to other authors [40], changes in light intensity as well as water availability result in opposite effects on the relative abundance of EO compounds formed via the activity of two kinds of enzymes. These are pinene synthase (responsible for the biosynthesis of α-pinene, β-pinene, camphene, and myrcene) and bornyl diphosphate synthase (involved in the biosynthesis of borneol, camphor, and bornyl acetate). This is in line with our results: α-pinene (the main monoterpene hydrocarbon in our ROEO) was reduced to half in cultivated *R. officinalis* (in the case of Clevenger hydrodistillation) conducted under drip irrigation and to one-third in the case of microwave-assisted extraction.

Microwave-assisted extraction was reported to perform better as compared to Clevenger hydrodistillation with respect to ROEO yield as well as monoterpene hydrocarbons but not for oxygenated monoterpenes, sesquiterpene hydrocarbons, and other oxygenated compounds. Our results were in line with previously published literature. For instance, Elyemni et al. [41] reported that microwave-assisted extraction results in higher total oxygenated compounds and lower total nonoxygenated compounds when compared to Clevenger hydrodistillation. A higher proportion of oxygenated compounds is likely in microwave-assisted extraction due to the low water content of the isolation system as well as the speed of the heating process as compared to conventional Clevenger hydrodistillation. In this context, the thermal and hydrolytic degradation of oxygenated compounds is considerably reduced [24,42]. Oxygenated compounds are known to have a high dipole moment and therefore interact more easily with microwaves and can be easily isolated, as highlighted in the study by Kosar et al. [43]. Oxygenated compounds are more valuable compared to hydrocarbons with regard to their contribution to fragrance as well as the therapeutic characteristics of EOs. They can therefore be used as an EO quality indicator.

With respect to IC_{50}, the lowest value was reported in ROEO obtained from wild *R. officinalis* via microwave extraction (2.61 ± 6.18), while cultivated *R. officinalis* according to Clevenger extraction exhibited the highest value (8.58 ± 5.85 mg/mL) against ascorbic acid ($IC_{50} = 0.03 \pm 0.01$ mg/mL) as a positive control. Wild *R. officinalis* showed the lowest IC_{50} and therefore the most effective DPPH scavenging capacity, especially when ROEO was obtained by microwave-assisted extraction. These results are comparable with those reported by El Kharraf et al. [30], who found an IC_{50} of 6.88 ± 0.00 mg/mL.

The lowest MIC values in ROEO in both kinds of *R. officinalis* and extraction techniques were found in both

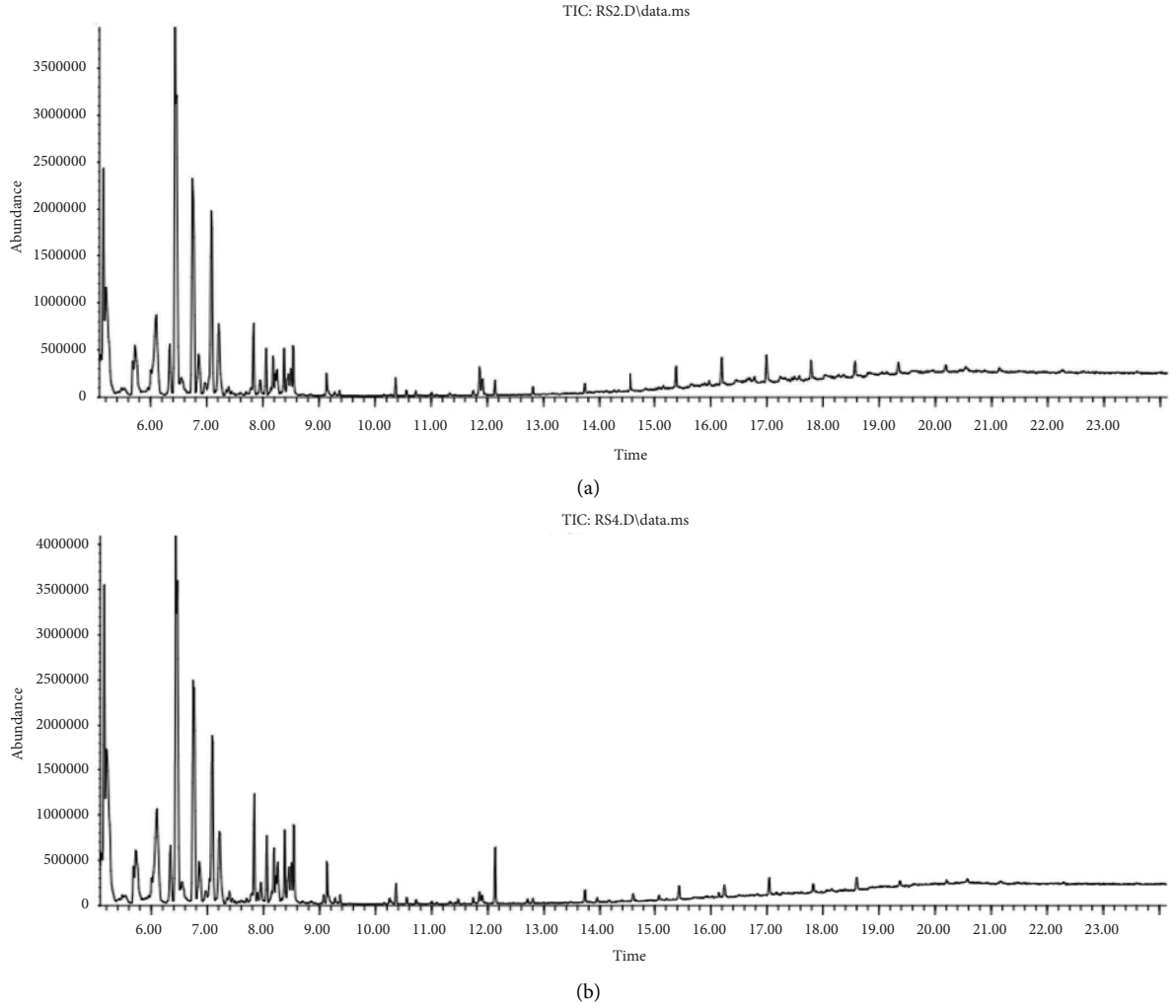

Figure 1: Chromatograms for ROEO isolated by microwave-assisted extraction (a) and Clevenger hydrodistillation (b).

Gram-negative bacteria, *B. subtilis* (06.17 ± 0.29) and *S. aureus* ($06.83 \pm 0.29\,\mu$g/mL), while the greatest level of MIC was reported in a Gram-positive bacterium (*E. coli*, $15.50 \pm 1.32\,\mu$g/mL). These values were slightly higher than those reported by El Kharraf et al. [30] for wild *R. officinalis* (0.63–2.5 μL/mL). Elyemni et al. [31] reported small values of MIC (0.315–2.5 mg/L) in the case of cultivated *R. officinalis* and 0.625–5 mg/L for the wild one. This could be ascribed to the domestication effect but also to genotypic differences among both ROEO samples and both ROEO isolation techniques, and microbial strain sensitivity, among others.

With respect to antimicrobial activity, significant variations ($p < 0.05$) in terms of MICs were found among the investigated strains but also among ROEOs. MIC Mean values found in our study were in line with peer reviewed literature *R. officinalis* from Morocco and outwards [17, 41]. As outlined in the Results' section, the highest MIC records were found in the yeast strain (*C. albicans*), which was followed by the investigated Gram-positive bacteria (*B. subtilis* along with *S. aureus*), and finally the Gram-negative bacterium (*E. coli*). Such outcomes were consistent with previously published studies [18, 25, 30]. They reported that the yeast strains showing their superiority regarding MIC of EOs in comparison with Gram-positive as well as Gram-negative bacteria.

It is largely evidenced by studies carried out on the antimicrobial activity of EOs demonstrating that bacteria with Gram-positive are more sensitive than Gram-negative ones. This sensitivity difference could be explained by the presence of its characteristic outer membrane having hydrophilic lipopolysaccharides. These encompass the bacterial peptidoglycan layer in the case of Gram-negative. This membrane plays the role of a barrier against macromolecules (among which are hydrophobic compounds), and therefore it can limit the diffusion of such hydrophobic compounds into bacterium cytoplasm [44, 45].

Our results of MICs had proven the effectiveness of ROEO against the investigated strains. Extraction techniques seemed to induce changes in EO chemical profiling, and related biological activities have been studied in other industrial crops, including medicinal and aromatic plants. They demonstrate that these extraction techniques and solvents can significantly modify EO composition as well as

Table 3: Pearson correlation coefficients were found among the main investigated dependent variables, including essential oil yield, major compounds, minimal inhibitory concentration (MIC) in various microbial strains, and antioxidant activity (IC_{50}).

	ROEO yield	α-Pinene	Camphene	β-Pinene	α -terpinene	p-Cymene	Limonene	1,8-Cineole	β-myrcene	Linalool	Camphor	Borneol	α-Terpineol	Verbenone	Bornyl acetate	β-Caryophyllene	α-Caryophyllene	*E. coli*	*S. aureus*	*B. subtilis*	*C. albicans*	IC_{50}
ROEO yield		0.846*	−0.628	−0.485	−0.601	−0.555	0.552	0.115	−0.358	−0.489	−0.826*	−0.410	−0.613	−0.884*	−0.641	−0.578	−0.533	−0.897**	−0.906**	−0.923**	−0.878*	−0.962**
α-Pinene			−0.852*	−0.832*	−0.852*	−0.777	0.787	−0.024	−0.623	−0.831*	−0.719	−0.654	−0.847*	−0.588	−0.859*	−0.916**	−0.882*	−0.944**	−0.954**	−0.934	−0.975**	−0.694
Camphene				0.964*	0.998***	0.991***	−0.994***	−0.497	0.939**	0.968**	0.264	0.953**	0.999***	0.198	0.999***	0.766	0.697	0.908*	0.897**	0.879*	0.912	0.395
β-Pinene					0.977*	0.948*	−0.958*	−0.419	0.915**	0.999***	0.221	0.917**	0.969**	0.058	0.962**	0.855*	0.806*	0.812*	0.805*	0.770	0.844*	0.231
α-Terpinene						0.988**	−0.992**	−0.484	0.940*	0.981**	0.255	0.952**	0.999***	0.168	0.997***	0.789*	0.724	0.893**	0.882**	0.861*	0.902**	0.361
p-Cymene							−0.999***	−0.606	0.974**	0.953**	0.143	0.984**	0.992**	0.103	0.991***	0.687	0.612	0.863*	0.847*	0.832*	0.858**	0.314
Limonene								0.588	−0.972**	−0.963**	−0.154	−0.981**	−0.995**	−0.1	−0.992**	−0.709	−0.636	−0.863*	−0.847*	−0.831*	−0.861*	−0.309
1,8-Cineole									−0.745	−0.430	0.643	−0.729	−0.501	0.497	−0.485	0.112	0.195	−0.214	−0.175	−0.186	−0.155	0.29
β-Myrcene										0.920**	−0.084	0.998***	0.943**	−0.121	0.933**	0.575	0.498	0.726	0.704	0.687	0.718	0.101
Linalool											0.217	0.923**	0.973**	0.060	0.966**	0.849*	0.797	0.817*	0.809*	0.776	0.847*	0.237
Camphor												−0.038	0.251	0.922	0.279	0.597	0.611	0.600	0.630	0.630	0.631	0.872*
Borneol													0.956**	−0.065	0.948**	0.586	0.507	0.762	0.740	0.725	0.751	0.158
α-Terpineol														0.179	0.999***	0.770	0.702	0.899**	0.888**	0.869*	0.905**	0.376
Verbenone															0.213	0.329	0.320	0.590	0.611	0.637	0.574	0.972**
Bornyl acetate																0.771	0.702	0.914**	0.904**	0.887*	0.918**	0.409
β-Caryophyllene																	0.995***	0.754	0.769	0.723	0.825*	0.400
α-Caryophyllene																		0.691	0.709	0.661	0.771	0.368
E. coli																			0.999***	0.998**	0.993***	0.743
S. aureus																				0.998***	0.995***	0.757
B. subtilis																					0.986**	0.785
C. albicans																						0.716
IC50																						

*, **, and *** indicate significance at 0.05, 0.01, and 0.001 probability levels, respectively.

MICs against various pathogen strains [46, 47]. The outcomes from these studies demonstrated that solvents with higher polarities are more effective in recovering bioactive molecules and therefore, have the ability to inhibited the growth of different microbes at lower concentrations (thus, having low MICs). Similar trends of variation were observed for IC_{50}, our ROEOs were able to reduce DPPH to 50% with lesser concentrations. Following Roby et al. [48], IC_{50} is defined as number of the phenolic compound moles divided by the number of DPPH moles required in order to reduce the absorbance of DPPH to 50%. Based upon this definition, the lower the IC_{50} is, the higher the antioxidant activity is.

3.3. Correlations Study. Table 3 shows a correlation matrix among the studied dependent variables. As evidenced in these results, important positive and negative associations were revealed. For instance, ROEO yield was positively and strongly correlated to α-pinene but negatively linked to verbenone and camphor. Likewise, it was negatively and significantly correlated with MIC in all studied microbial strains. This means that higher essential oil yield results in a great proportion of α-pinene but low levels of verbenone, camphor, MIC, and $IC_{50,}$ and therefore strong antimicrobial and antioxidant capacity. Most of the ROEO constituents were linked positively to MIC for the studied microbial strains as well as IC_{50}. Regarding correlations among constituents, there are strong positive and negative correlations. Apart from limonene, α-pinene was negatively correlated with the remaining compounds.

Camphene was positively correlated with ROEO constituents except for α-pinene, limonene, and 1,8-cineole. α-Pinene was negatively correlated with limonene and 1,8-Cineole but positively correlated with α-terpinene, p-cymene, β-myrcene, linalool, camphor, borneol, α-terpineol, verbenone, bornyl acetate, β-caryophyllene, and α-caryophyllene. α-Terpinene was negatively associated to limonene, and 1,8-cineole but positively linked to p-cymene, limonene, 1,8-cineole, β-myrcene, linalool, camphor, borneol, α-terpineol, verbenone, bornyl acetate, β-caryophyllene, and α-caryophyllene. p-Cymene also was negatively linked with limonene and 1,8-cineole but positively correlated with β-myrcene, linalool, camphor, borneol, α-terpineol, verbenone, bornyl acetate, β-caryophyllene, and α-caryophyllene. Limonene was found to be positively correlated with 1,8-cineole but negatively associated with β-myrcene, linalool, camphor, borneol, α-terpineol, verbenone, bornyl acetate, β-caryophyllene, and α-caryophyllene. 1,8-Cineole was negatively correlated with β-myrcene, linalool, borneol, α-terpineol, and bornyl acetate but positively associated with camphor, verbenone, β-caryophyllene, and α-caryophyllene. β-Myrcene was negatively linked to camphor and verbenone but positively associated with linalool, borneol, α-terpineol, bornyl acetate, β-caryophyllene, and α-caryophyllene. Linalol was positively correlated with camphor, borneol, α-terpineol, verbenone, bornyl acetate, β-caryophyllene, and α-caryophyllene. Camphor had a negative association with borneol but positive correlations with α-terpineol, verbenone, acetate bornyl, β-caryophyllene, and α-caryophyllene. Borneol presented a negative correlation with verbenone but positive correlations with α-terpineol, verbenone, bornyl acetate, β-caryophyllene, and α-caryophyllene. Terpineol had positive correlations with verbenone, bornyl acetate, β-caryophyllene, and α-caryophyllene. Bornyl acetate, β-caryophyllene, and α-caryophyllene were positively correlated to each other.

Similar correlations were found by other authors [32, 33, 39]. These authors also found important correlations among ROEO constituents and plant agro-morphological characteristics on the one hand and with pedoclimatic conditions on the other hand. A strong positive correlation among such compounds could be assigned to the biosynthesis pathways shared among these compounds, as discussed in the study by Wang et al. [49]. Probably, these compounds produced from the same biosynthetic pathway or are double-bond isomers. These correlations, along with those highlighted by other authors [33, 39], seem to be very important and should be taken into account when analyzing essential oil yield, profiling, as well as related biological activities.

3.4. Principal Component Analysis. Principle component analysis (PCA) is known as one of the most popular multivariate statistical methods. It was used with the aim to reduce our data dimensionality as well as to project data sets on a reduced space. For such purposes, the PCA approach is widely in use in various fields such as agronomy and food science, among others [19, 50–62]. In our work, the first three principal components (PCs) were retained since they allow explaining about 93% of the total data variability, as indicated in Figure 2.

The 4 points plotted on Figure 2(a) are related to *R. officinalis* domestication (wild and cultivated *R. officinalis*). These appear to be separated via the first component (PC1 = 73.01%).

On the positive direction of the first component PC1, points related to wild *R. officinalis* were plotted, which was associated with great levels of ROEO yield, α-pinene, limonene, and cineole. On the opposite side of the same component (PC1), the points were distributed associated with cultivated *R. officinalis* with high values of IC_{50}, MICs in all microbial strains as well as the remaining compounds (verbenone, camphor, α-caryophyllene, β-caryophyllene, camphene, α-terpinene, bornyl acetate, α-terpineol, linalool, β-pinene, p-cymene, borneol, and β-myrcene). Similarly, Figure 1(b) presents the distribution of points linked to EO isolation techniques (Clevenger hydrodistillation and microwave-assisted extraction). These seem to be separated through the second principal component PC2, with a variability contribution of 20.72%. Points related to Clevenger hydrodistillation were plotted on the positive side of PC2. This technique, as shown in Figure 2(b), was associated with great values of all MICs belonging to all microbial strains, IC_{50}, verbenone, camphor, 1,8-cineole, limonene, α-caryphellene, and β-caryphellene. On the contrary, microwave-assisted extraction interacted, on the negative side of PC2, with higher levels of ROEO yield, α-pinene,

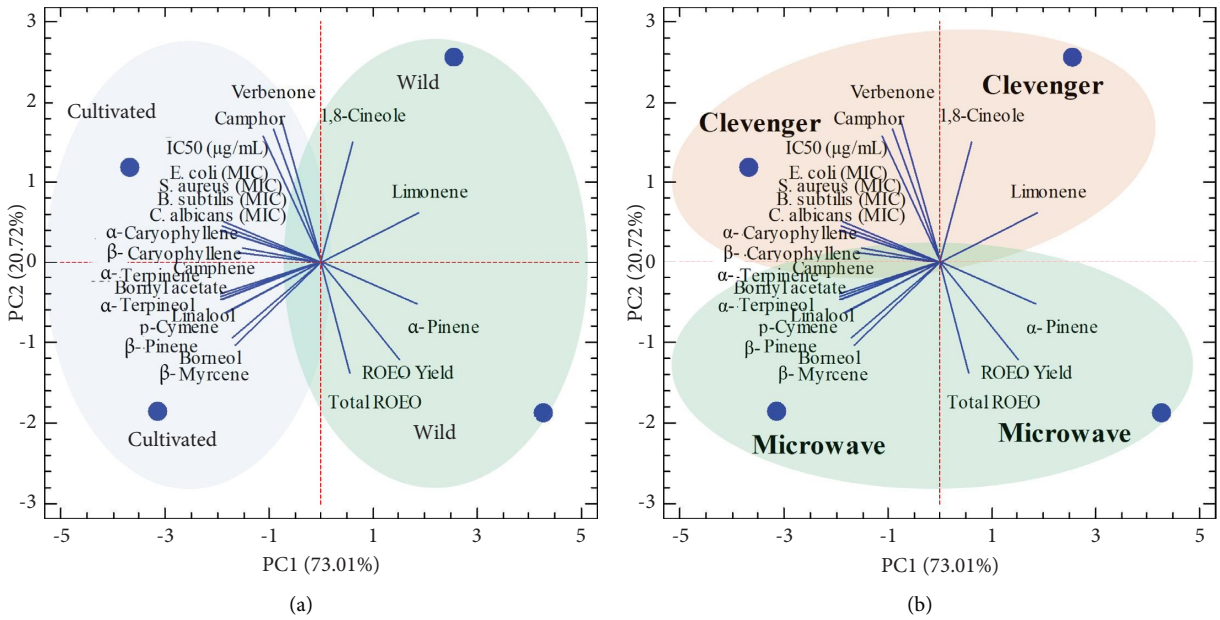

Figure 2: (a) Principal component projections on PC1 and PC2. The blue points plotted are the mean values associated with plant domestication (wild and cultivated rosemary). Blue segments are linked to *Rosmarinus officinalis* essential oil (ROEO) yield, its phytocompounds, antioxidant activity (IC_{50}), and minimal inhibitory concentration (MIC) as antimicrobial activity against various microbial strains. (b) Principal component projections on PC1 and PC2. Blue points plotted are mean values associated with essential oil isolation techniques (Clevenger hydrodistillation and microwave-assisted extraction). Blue segments are linked to *Rosmarinus officinalis* essential oil (ROEO) yield, its phytocompounds, antioxidant activity (IC_{50}), and minimal inhibitory concentration (MIC) as antimicrobial activity against various microbial strains.

camphene, α-terpinene, bornyl acetate, α-terpineol, linalol, β-pinene, p-cymene, borneol, and β-myrcene. These outcomes confirm those reported in the comparison of mean values and correlation analysis.

4. Conclusions

This is the first report on Moroccan rosemary domestication and its effects on essential oil in Taounate Province (central-northern Morocco). Our outcomes demonstrate that rosemary aerial parts (leaves) are an important source of various compounds, with large variations between wild and cultivated samples on the one hand and between both isolation techniques. *R. officinalis* domestication results in differentiated effects on ROEO traits, fostering a better accumulation of some compounds but reducing yield as well as other compounds and therefore antioxidant along with antimicrobial activity. ME as a green method was proven to be more efficient in terms of ROEO traits as well as antioxidant and antimicrobial activity. Our outcomes revealed that ROEO from wild rosemary performed better in terms of ROEO yield, some compounds (limonene, 1,8-cineole, and camphor), MIC, and IC_{50}. The rest of the major compounds were better expressed in cultivated rosemary. Microwave-assisted extraction allowed higher records of most of the studied ROEO traits except for limonene, camphor, and verbenone, whose great content was obtained by Clevenger hydrodistillation. These outcomes were confirmed by principal component analysis, which allowed the separation of wild and cultivated rosemary as well as isolation techniques through the first two components with over 93% of data variability. Further investigations are needed to optimize the production of biomass and phytochemicals of interest under different cultivation conditions such as water regimes and soil fertility.

Data Availability

The data that support the findings of this study are available from the corresponding author, E.H. Sakar, upon a reasonable request.

Additional Points

Statement of Novelty. There is an increasing demand on the market for medicinal and aromatic plants (MAPs). This may lead to unsustainable harvesting of MAPs. In such a situation, the cultivation of some wild MAPs of interest becomes an important strategy to meet market demand. Rosemary (*Rosmarinus officinalis* L.) is an important industrial crop widely grown in the Mediterranean basin and abroad, and little is known about its domestication. In this paper, we aimed at comparing the effects of domestication and extraction technique on essential oil yield, chemical profiling, and antioxidant and antimicrobial activities of *R. officinalis*.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Conceptualization, methodology, formal analysis, investigation, resources, and writing - original draft was done by El Hassan Sakar. Conceptualization, resources, and writing - review & editing was provided by Ahmed Zeroual. Data acquisition, interpretation, and writing - review & editing was done by Ayoub Kasrati Data acquisition, interpretation, and writing - review & editing was performed by Said Gharby.

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