Hindawi
Journal of Chemistry
Volume 2020, Article ID 9728093, 12 pages
https://doi.org/10.1155/2020/9728093

Research Article

Plant Extract Valorization of *Melissa officinalis* L. for Agroindustrial Purposes through Their Biochemical Properties and Biological Activities

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Received 28 August 2019; Revised 9 December 2019; Accepted 9 April 2020; Published 5 June 2020

Guest Editor: S. M. Mahfuzul Islam

Lemon balm (*Melissa officinalis* L.) is one of the rare medicinal plants in Tunisia. It was found only in two sites in the north of Tunisia with a small number of plants. The study of germination under the NaCl and PEG effect showed that Tunisian lemon balm seeds were sensitive to saline and osmotic stress. Morphological and biochemical characterizations of Tunisian *M. officinalis* were performed. Results showed that the Tunisian populations presented plants with long, broad leaves and weak branching. The major constituent in leaf essential oil was germacrene-D with a percentage ranging from 29.17 to 24.6%, and the major fatty acids were polyunsaturated fatty acids, linoleic acid, ranging from 73.93 to 66.74%. The phenolic content of *M. officinalis* extract varied significantly among origins which could explain the high variation in antiradical scavenging activity. The evaluation of allelopathic activities showed that the extract of the lemon balm leaves presented an allelopathic effect with the majority of the tested seeds.

1. Introduction

During the last two decades, research in herbal medicine has become one of the greatest scientific concerns [1]. In the socioeconomic context, the study of plants can lead to the achievement of adequate and low-cost therapeutic responses, combining proven scientific efficacy and cultural acceptability [2]. Located in the Mediterranean basin with great climatic variations from North to South, Tunisia presents a favorite terrain for the development of a flora rich in medicinal and aromatic species. More than 500 species out of a total of 2,200 (about 25% of the total flora) are considered for therapeutic use [3]. *Melissa officinalis* L. or lemon balm is a very rare medicinal species in a spontaneous area in Tunisia, classified among the 48 species identified as endemic rare and endangered according to the *IUCN* classification [4]. It has been encountered in few sites with a reduced number of plants in the forest region of Kroumirie and Mogods, having the geographical coordinates 8°–8°30′ East for the longitude and 36°15′–36° 45 ′North for the latitude [5]. Lemon balm is sought after for its lemony scent as well as for its many therapeutic activities. Since ancient times, it is used in cases of nervousness and minor sleep disorders, as well as in case of gastrointestinal disorders such as flatulence and abdominal pain, especially antidepressants [6]. In recent years, other uses have been studied, particularly in the field of plant protection products [7]. It is empirical that these properties have been attributed to it [8].

In Tunisia, there are no reports conducted on *M. officinalis*. Therefore, the aims of this study were to investigate, for the first time, the germination rate under abiotic stress and the morphological and chemical characterization and to

evaluate their antioxidant and allelopathic activities which may provide data for suitable conditions for cultivation of the best populations and for their agroindustrial exploitation.

2. Experimental

2.1. Plant Material. The plant material was botanically characterized by Dr Ben Brahim N. (Laboratory of Science and Agricultural Techniques, National Agricultural Research Institute of Tunisia (INRAT)) according to the morphological descriptions in the Tunisian flora. Several surveys were carried out before and after flowering (May and August) on the Tunisian territory according to the data of the geographical distribution of El Mokni et al. [9]. Tabarka and Nefza (Figure 1) cover almost the entire area of *M. officinalis* in Tunisia. Tunisian seeds were harvested from sites found in northern Tunisia. French seeds of *M. officinalis* were provided by the National Institute for Agricultural Research, France, and the German seeds were provided by the Institute for Food and Resource Economics (ILR), University of Bonn. The introduced seeds were used as references.

2.2. Study of Germination under Abiotic Stress. Seed germination is an essential process in the development of the plant. It is influenced by many abiotic factors such as salt and drought stress, which are perhaps the two most important abiotic constraints limiting plant development [10, 11]. In order to eliminate the germination inhibitors, the seeds, which had the same age, were sterilized in 0.5% sodium hypochlorite solution for 1 min and then washed with distilled water solution. Germination was performed in incubators with a photoperiod of 12 h (Sylvania white fluorescent lamps, 25 $mM{\cdot}m^{-2}{\cdot}s^{-1}$ photons with photosynthetically active radiation). Each treatment consisted of three replicates of 50 seeds. The seeds were germinated in NaCl solutions (0; 50; 100; 200; and 300 mM) and PEG 600 (polyethylene glycol) (0; −2.3; −4.6; −9.3; −13.9 bar) under the optimum temperature of 20°C [12]. The seeds were considered germinated during the appearance of radicals [13]. Sprouted seeds were counted and eliminated every two days over a 14-day period [14, 15].

2.3. Culture. The seeds of *M. officinalis* were grown on an experimental site at the National Institute of Agronomic Research of Tunisia (Ariana, 36°50′, 10°11′E), at 10 m altitude, with alkaline soil (pH = 8.7), and 450 mm of rain and upper semiarid bioclimatic stage. The experimental setup included lines spaced 1m and 50 cm apart between the plants. Each line comprised 10 individuals at the rate of three replicates for each origin. The average temperature was 25°C; the average monthly temperature varied between 15°C in January and 35°C in August. The plants were developed under biological conditions (no pesticide and fertilizer, weeds were eliminated manually, and additional irrigation if necessary was made). The cultivation was followed until the collection of the seeds.

2.4. Morphological Characterization. Eleven morphological characters were measured from 26 plants for each population. These characters relate to vegetative and reproductive developments. The choice of these characters was inspired by previous work done on several Lamiaceae plants such as *Lavandula* species [16].

2.5. Isolation of Essential Oil and GC/MS Analysis. The essential oils were obtained from 100 g (dry weight) of plant material using a Clevenger-type apparatus for 3 h. The hydrodistillation was performed in three replicates, and the oils were stored at 4°C until analysis by GC/MS. The average oil yields were estimated on the basis of the dry weight of the plant material. The oils were analyzed with a Hewlett-Packard 6890N/5975B inert GC-MSD system (Agilent, USA) equipped with two cap. columns, a HP-INNOWAX (30 m × 0.25 mm i.d., film thickness 0.25 μm), and a HP-5MS (30 m × 0.25 mm i.d., film thickness 0.25 μm) column (J&W Scientific, USA). The oven temperature was programmed isothermal at 50°C for 1 min, then increased from 50 to 250°C at 28/min, and finally held isothermal at 250°C for 15 min (injector temperature, 250°C; ion source temperature, 230°C; carrier gas, He (high purity 99.99%; 1.2 $ml{\cdot}min^{-1}$); injection volume, 1 μl; split ratio, 100 : 1). The electron impact ionization mode was used with an ionization voltage of 70 eV. Total ion chromatograms were obtained over the scan range of 30–800 a.m.u. in the full-scan acquisition mode, and the compounds were identified using the NIST05 and Wiley 7 databases with a resemblance percentage above 85%. Semiquantitative data were calculated from the GC peak areas without using correction factors and were expressed as relative percentage (peak area %) of the total volatile constituents identified. Retention indices (RIs) were determined for all the detected compounds based on the retention times (tr) of a homologous series of *n*-alkanes (C8-C30) [17, 18].

2.6. Fatty Acid Methyl Ester Preparation. Triplicate samples of 1 g of *M. officinalis* leaves were subjected to lipid extraction using a modified version of the Bligh and Dyer [19] method. Thus, leaf samples were kept in boiling water for 5 minutes and then ground manually using a mortar and pestle; chloroform/methanol mixture (2 : 1 v/v) was used for lipid extraction. After washing using fixation water, the organic layer containing lipids was recovered and dried under a nitrogen stream. Total fatty acids (TFAs) of total lipids were transmethylated using sodium methoxide solution (3% in methanol) [20]. Methyl heptadecanoate (C17 : 0) was used as an internal standard. The fatty acid methyl esters (FAMEs) obtained were subjected to GC analyses.

2.7. Phenolic Content and DPPH Radical Scavenging Assay. *M. officinalis* extracts were prepared with two solvents of ethanol and water. 20 g of plant powder was stirred in the presence of 200 ml of solvent for 72 h and then filtered. The filtrate was evaporated in the rotavapor [21]. The yield was calculated by the following formula:

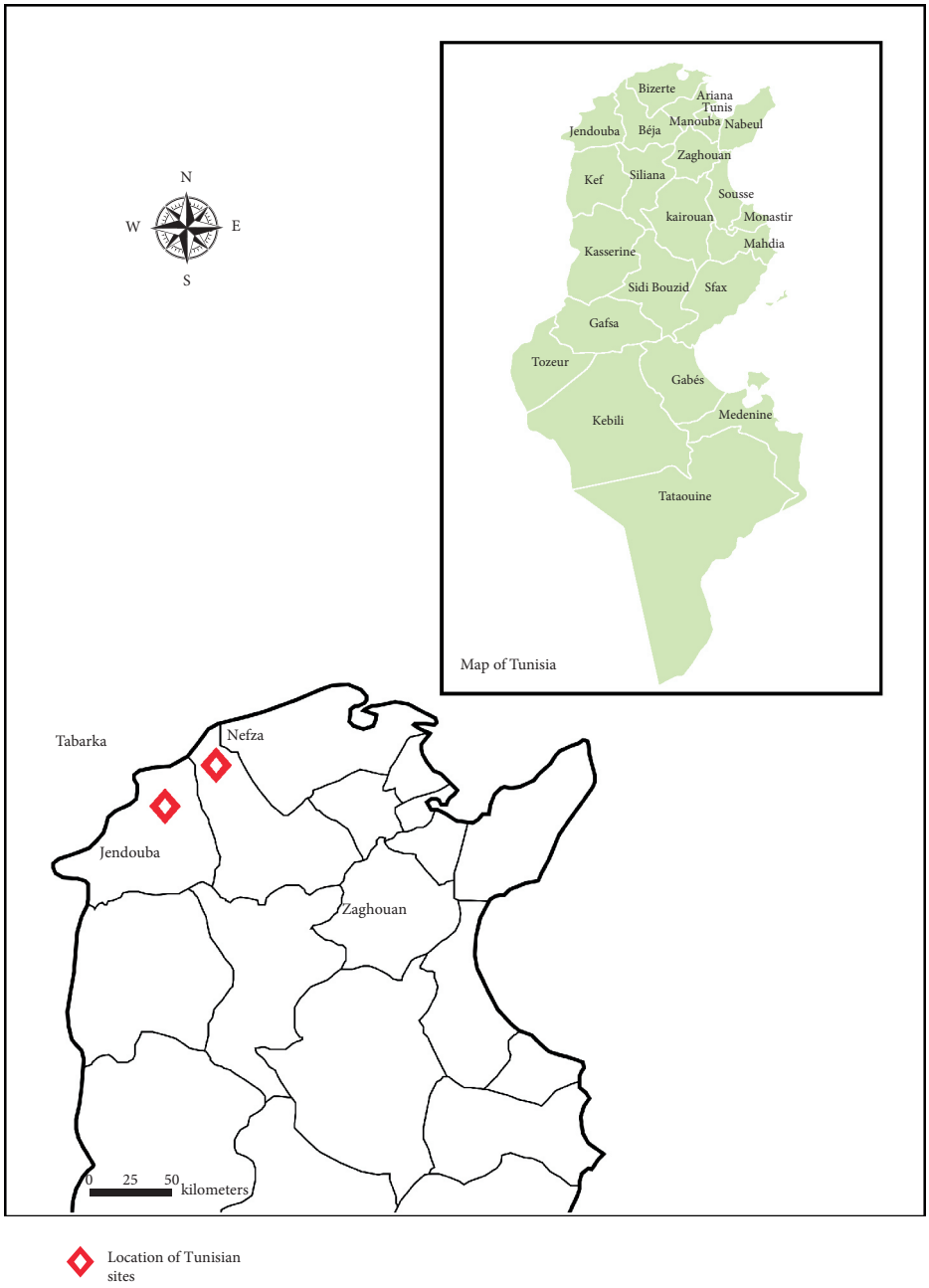

Figure 1: Location of *M. officinalis* sites in Tunisia.

$$\% \quad \text{yield} = \frac{\text{mass of the extract obtained}}{\text{mass of plant material before the extraction}} \times 100. \tag{1}$$

The total phenolic content of ethanolic and aqueous extracts was determined according to the method described by Lowman and Box and slightly modified by Moghaddam and Miran [22, 23]. About 100 μL of each sample was mixed with 46 mL of distilled water and 1 mL Folin–Ciocalteu reagent. The mixture was thoroughly shaken and left for 3 min before adding 2.9 mL of Na2CO3 (2%). After incubation for 2 hr in dark, the absorbance was measured at 760 nm. A standard curve of gallic acid was prepared. Total phenolic contents were expressed as milligrams gallic acid equivalents per gram of the essential oil (mg GAE/g DW) through the calibration curve with gallic acid. All samples were analyzed in triplicate.

The antioxidant activity was determined using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method according to Tohidi and Rahimmalek [24]. About 100 mL of each sample at different concentrations (between 50 and 800 μg/mL) was added to 2,500 mL DPPH ethanolic solution. The mixtures were shaken vigorously and then placed in the dark for 30 min. The absorbance of the solutions was measured at 517 nm. All samples were analyzed in triplicate. Butylated hydroxytoluene (BHT) was used as

positive control. The antiradical activity was expressed as IC_{50} (in mg/mL) which was defined as the concentration of the sample required to inhibit the formation of DPPH radicals by 50%. The percentage inhibition of the DPPH radical was calculated according to the following equation:

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

where A_{control} is the absorbance of the DPPH solution without sample solution and A_{sample} is the absorbance of the sample after 30 min at 517 nm.

2.8. Allelopathic Activity. The extracts of lemon balm have been studied for their herbicidal effect. These extracts were tested on four seeds: radish (*Raphanus sativus* L.), fenugreek (*Trigonella foenum-graecum* L.), wild chicory (*Cichorium intybus* L.), medicago (*Medicago polymorpha* L.). The germination tests are carried out in an oven set at 25°C. For each test, four concentrations were used (0; 1; 3; and 5 mg/ml). The percentage of germination was observed daily for five days. Measurements of root length and shoot length were reported only at the end of the test (total germination of controls). The results represent the average of three replicates of 50 seeds for each treatment. To ensure their germination capacity, they were sterilized with 70% ethanol for 30 seconds, washed with sterile water, and then soaked in sodium hypochlorite for 20 minutes. Germination indices were determined by counting the number of seeds germinated at 24 hour intervals.

2.9. Statistical Analysis. All parameters were statistically described to determine means, standard deviations, and coefficients of variation by population. ANOVA (SAS version 9 software) was performed using the Duncan 5% test for each parameter. Principal component analysis (PCA) is one of the factor analysis methods that reduce data by defining the main axes (or principal components) from the monitored parameters. The observations are represented in relation to the main axes. Group analysis is performed by the ascending hierarchical classification (ACH) method. The graph representing the classification is a dendrogram of dissimilarity with standardized distances representing the nearest population in homogeneous groups. This analysis was performed using JMP®11.0 statistical software (SAS Institute, Inc., Cary, NC) with component analysis procedure.

3. Results and Discussion

3.1. Study of Germination under Abiotic Stress. The seed germination under different concentrations of sodium chloride showed that the germination rates of Tunisian origins and those introduced decrease when the sodium chloride concentration increases (Table 1). Exposure to osmotic conditions during the germination shows that Tunisian seeds are clearly sensitive to variations in water potential. The germination rate of Tunisian *M. officinalis* seeds decreases slightly at −2.3 bars and disappears from −4.6 bars. On the other hand, introduced seeds are relatively more tolerant (Table 2). Seeds from Germany have a germination rate above 40% at −4.6 bars. Introduced seeds, from Germany and France, show similar responses for the two treatments, tolerating the variation of NaCl and PEG than the Tunisian lemon balm seeds. We have little data about the tolerance of lemon balm seeds to abiotic stress. Germination of Tunisian seeds is studied for the first time in order to analyze the influence of salinity and osmotic potential on germination. Significant differences were observed between Tunisian and introduced seeds. Although the data were obtained from seeds sprouted in Petri dishes, the result may be related to in situ performance. Seeds from Tabarka and Nefza are more sensitive to salinity and to the osmotic potential, which explains the reduced number of *M. officinalis* plants in situ, where climatic constraints exert a natural selection pressure [25]. The germination rate of the lemon balm seeds studied is similar to the germination rate observed by Chartzoulakis and Klapaki, which demonstrated that 50 mM of salinity in the external environment delays germination in some plants but did not reduce the percentage of germination observed at the end of the experiment [26]. Similar results have been reported for *Atriplex griffithii* and *Cressa cretica* [27, 28]. Indeed, Iranian lemon balm seeds presented a germination rate that reaches 90% at 0 bar and 81% at −2 bar [29]. The reduction of germination rate under salt stress and their toxic and osmotic effects involved in germination have been demonstrated by several authors [25, 30]. In the case of Tunisian lemon balm seeds based on the results obtained, it is suggested that the osmotic effect of NaCl is responsible of the disturbance of seed germination. So, the osmotic effect, which leads to reduced water absorption, may be responsible for the inhibition of germination seeds from Tunisia.

3.2. Morphological Characterization. The morphological characterization of *M. officinalis* from the four origins, Tabarka, Nefza, Germany, and France, was addressed by statistical analysis carried out on 11 agro-morphological characters measured on plants cultivated under homogeneous conditions. One-way analysis of variance (population effect) showed significant differences for most of the traits studied (Table 3). To understand the variance sources of *M. officinalis*, a principal component analysis (PCA) was performed by grouping together the seven significant morphological characters in the first two axes describing 79.3% of the total variation (Figure 2(a)). The plot drawn along the two axes showed three distinct groups of plants (Figure 2(b)). The first group consisted of the plants of the German population, which was characterized by plants with good vegetative development presenting the tallest plants (43.34 cm), the most branched, and the highest number of leaves and flowers. The Tunisian populations (Tabarka and Nefza) formed the second group, which was characterized by plants with long, broad leaves and weak branching. The third group was formed by the plants from France population, which was distinguished by a weak vegetative development. Hierarchical analysis based on morphological characters

Table 1: Effect of NaCl on seed germination of *M. officinalis*.

NaCl (mM)	0	50	100	200	300
Tabarka	98.33a	85b	0b	0	0
Nefza	100a	88.33b	10b	0	0
France	100a	93.33b	48.33a	0	0
Germany	100a	100a	58.33a	0	0

Means in each column followed by the same letters are not significantly different ($P > 0.05$).

Table 2: Effect of PEG on the germination rate of *M.officinalis* seeds.

Bars	0	−2.3	−4.6	−9.3	−13.9
Tabarka	100a	91.66a	0c	0	0
Nefza	100a	90a	0c	0	0
France	100a	86.66b	13.33b	0	0
Germany	100a	63.33c	41.66a	0	0

Means in each column followed by the same letters are not significantly different ($P > 0.05$).

using the Euclidean square method allowed establishment of the relationship between lemon balm of different origins. The morphological study showed a large phenotypic diversity between the populations for the majority of the agromorphological characters. This indicated the existence of a wide range of genetic variability for the traits studied and highlighted the potential from genetic improvement using such a gene pool. Sari and Ceylan also observed a great variability of morphological characters in 11 populations of *M. officinalis* from different regions of Turkey and European countries [31]. This may be due to cross pollination of lemon balm.

3.3. Essential Oil. The *M. officinalis* samples cultivated under the climatic conditions of the INRAT yielded a small amount of essential oil. The oils were analyzed by GC/MS. The chemical composition of the four *M. officinalis* from different origins was reported in Table 4. Chemical analysis of the essential oil samples conducted according to their retention time (RT) revealed the presence of 42 compounds, representing about (86.11%, 83.1%, 96.72%, and 71.88%) of the total oils obtained from Nefza, Tabarka, Germany, and France, respectively. In addition to the differences in the essential oil yield, the GC/MS analyses revealed qualitative and quantitative differences in the composition between the oils of the four origins. GC/MS analysis showed that the oils of the German population were characterized by the presence of 39.31% of geranial and 27.71% neral, which were the dominant components, and 12.23% of β-caryophyllene. The sesquiterpene caryophyllene oxide (27.06%) was found to have the highest value in the French population, which exhibited lower levels (7.12–4.29%, respectively) of geranial and neral. Germacrene-D (32.08–27.06%) was the highest in the Tunisian samples from Tabarka and Nefza, together with the sesquiterpene caryophyllene (16.4–14.7%, respectively). Considering the fact that minor components of the essential oils were less important for a comparison, only the major components representing more than 2% of the essential oil composition were taken into account to illustrate the variation of the components content from different origins [17].

In order to investigate the similarity and relationship between essential oil compositions of our studied samples, a hierarchical cluster analysis (HCA) was performed based on the components. The dendrogram of HCA classified the oils of the Tunisian populations of Tabarka and Nefza in the same group. This group was characterized by the predominance of germacrene-D as the major compound and emphasized the distinctiveness of the French oil, rich in caryophyllene oxide. The third group was formed by the German oil characterized by citral (neral and geranial) (Figure 3).

3.4. Fatty Acids. To the best of our knowledge, the foliar fatty acid composition of *M. officinalis* was reported herein for the first time. The proportion of the majority of fatty acids did not show differences according to the origin of samples (Table 5). *Melissa officinalis* leaves were characterized by a high proportion of polyunsaturated fatty acid (PUFA), linoleic acid (73.93–66.74%), versus (16.25–13.32%) saturated ones (SFA), oleic acid, and (6.29–4.26%) of monounsaturated (MUFA) palmitic acid. The comparison between different lemon balm evidenced a similarity, at least with reference to the presence of the main fatty acid constituents. It is noteworthy that previous findings showed that the genera *Satureja, Origanum*, and *Thymus* of the Lamiaceae family had some minor variations in fatty acid composition, which were dominated by the chemotypes of linoleic acid, palmitic acid, and linolenic acid [32].

3.5. Total Phenolic Content and Antioxidant Activity. Total phenolic contents in lemon balm leaves from different origins are shown in Table 6. The amounts of total phenolics in *M. officinalis* varied significantly among populations. Ethanolic extract from Tunisian populations Tabarka and Nefza showed the highest amount of phenolic compounds (0.94 mg GAE/g DR and 0.87 mg GAE/g DR, respectively). However, the lowest content was observed in the aqueous extract from German population (0.2 mg GAE/g DR) (Figure 4).

The extraction yield was influenced by the polarity of the solvent; according to Bourgou, the water (5.2) is more polar than ethanol (9.0), which is why the yield ethanol extraction is lower than the water [33]. When comparing values in this study to the literature, there was considerable variation in total phenolic contents reported of lemon balm from different origins. Boneza and Niemeyer indicated that the average total phenolic contents for the five lemon balm cultivars from the USA ranged from 5.50 ± 1.03 mg GAE/g DW to 26.87 ± 1.93 mg GAE/g DW [34]. Wojdyło et al. and Dastmalchi and Dorman reported that unspecific lemon balm showed total phenolic contents from 0.13 to 269 mg GAE/g DW [35, 36]. Boneza and Niemeyer noted much higher total phenolic contents for the lemon balm cultivars, with values ranging from 359 mg GAE/g DW for the "Lorelei" cultivar to 427 mg GAE/g DW for "Gold Leaf" [34]. This variability in lemon balm total phenolic contents across studies was therefore most likely due to differences in plant

Table 3: Analysis of variance in *Melissa officinalis* populations based on agromorphological traits.

	Mean of square (MS)										
Source of variation	Plant height (PH)	No. of branches/ plant (nB)	Stem diameter (SD)	Leaf length (LL)	No. of leaves (nL)	Leaf width (LW)	No. of nodes (nN)	No. of flowers (nF)	Fresh weight (FW)	Dry weight (DW)	Seed yield/ plant (SY)
Populations	511.36974**	295.07169***	1.05966***	8.09158***	6327.01563*	3.24630***	1.58592ns	12655.95356***	70.89806 ns	7.56814 ns	0.51593 ns

*, **, and *** denote statistical significance at 0.05, 0.01, and 0.1 levels, respectively. ns: not significant.

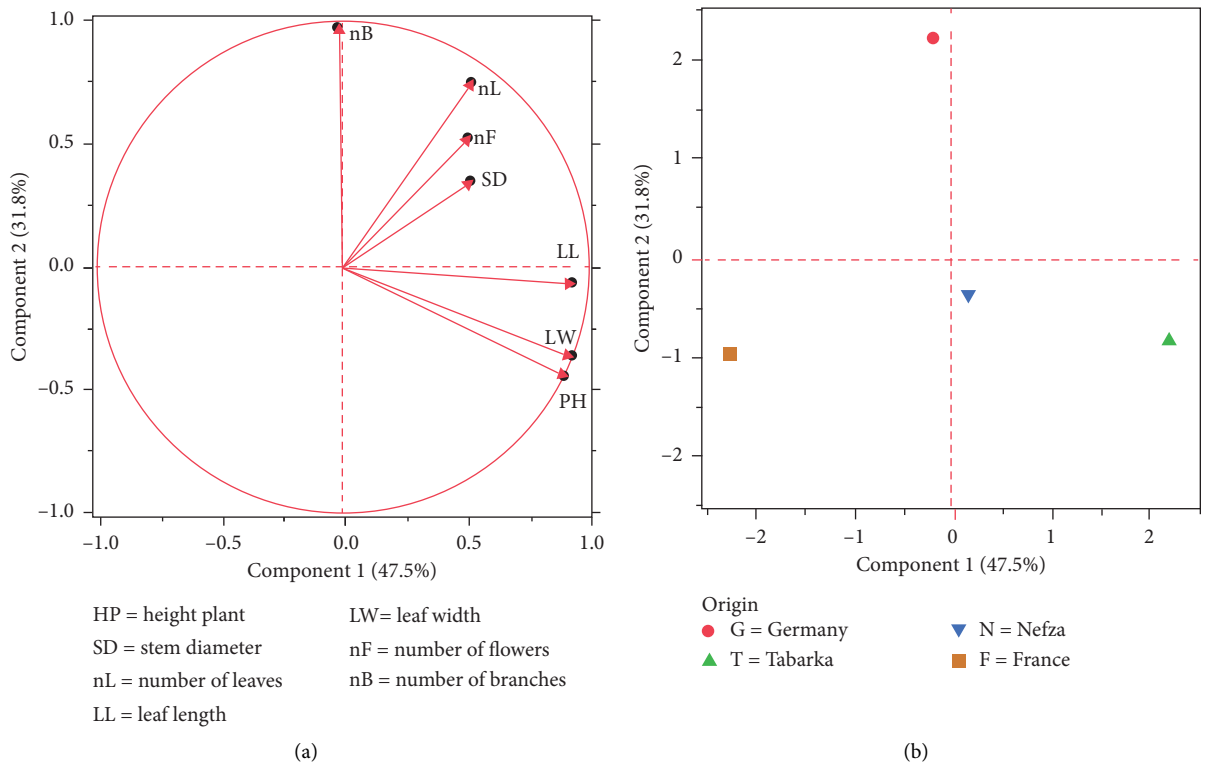

Figure 2: Plot of the first two principal components (C1 and C2) for the PCA of *M. officinalis*.

Table 4: Comparison of the essential oils isolated from different origins of *M. officinalis*.

No	Components	RI	Content (%)			
			Nefza	Tabarka	Germany	France
1	Camphene	954	—	—	—	1.29
2	£-3-Carene	1011	0.32	—	—	—
3	(Z)-α-Ocimene	1026	—	0.5	—	—
4	Citronellol	1229	—	—	1.88	—
5	Neral	1240	—	—	27.71	4.29
6	Geranial	1267	—	—	39.31	7.12
7	Thymol	1290	—	—	0.4	—
8	α-Ylangene	1372	0.42	—	—	—
9	α-Copaene	1376	0.72	0.54	—	—
10	Geranyl acetate	1381	—	—	1.42	—
11	Dehydro-ar-ionene	1389	0.84	—	—	—
12	(E)-α-Bergamotene	1412	0.55	—	—	1.24
13	(E)-Caryophyllene	1419	1.36	1.25	—	1.06
14	B-Caryophyllene	1420	14.7	16.4	12.23	8.92
15	α-Cedrene	1432	0.52	0.27	—	—
16	Alloaromadendrene	1439	—	0.59	—	—
18	Aromadendrene	1441	0.3	—	—	—
19	α-Cubebene	1475	1.45	1.34	1.23	0.42
20	Germacrene D	1468	27.06	32.08	1.67	2.0
21	Bicyclogermacrene	1495	0.18	—	—	—
22	cis-Calamenene	1521	0.75	—	—	—
23	B-Sesquiphellandrene	1522	2.75	0.4	—	0.97
24	delta-Cadinene	1523	0.73	—	—	—
25	α-Cadinene	1524	0.34	—	—	—
26	gamma-Cadinene	1538	4.96	—	0.76	1.77
27	α-Calacorene	1542	0.71	1.23	—	0.76
28	Nerolidol	1559	0.9	—	—	—

Table 4: Continued.

No	Components	RI	Content (%)			
			Nefza	Tabarka	Germany	France
29	Globulol	1568	0.42	—	—	—
30	Caryophyllenol	1572	0.91	1.47	0.5	2.23
31	Germacrene D-4-ol	1574	0.72	—	—	—
32	Caryophyllene oxide	1576	9.54	16.61	8.76	27.06
33	Spathulenol	1578	0.49	—	—	—
34	Humulene oxide II	1606	0.56	1.01	0.26	1.29
35	α-Cadinol	1654	4.61	3.24	—	5.64
36	t-Muurolol	1627	—	—	0.59	—
37	Isoaromadendren epoxide	1641	0.77	—	—	0.46
38	Farnesol	1743	—	0.49	—	—
39	(β-Z) Curcumen-12-ol	1756	0.53	—	—	—
40	Phytol	1949	6.96	5.68	—	3.64
41	Epi manoyl oxide	1993	0.22	—	—	—
42	(E-E)-geranyl linalool	2027	0.82	—	—	1.59
	Total compound (%)		86.11	83.1	96.72	71.88

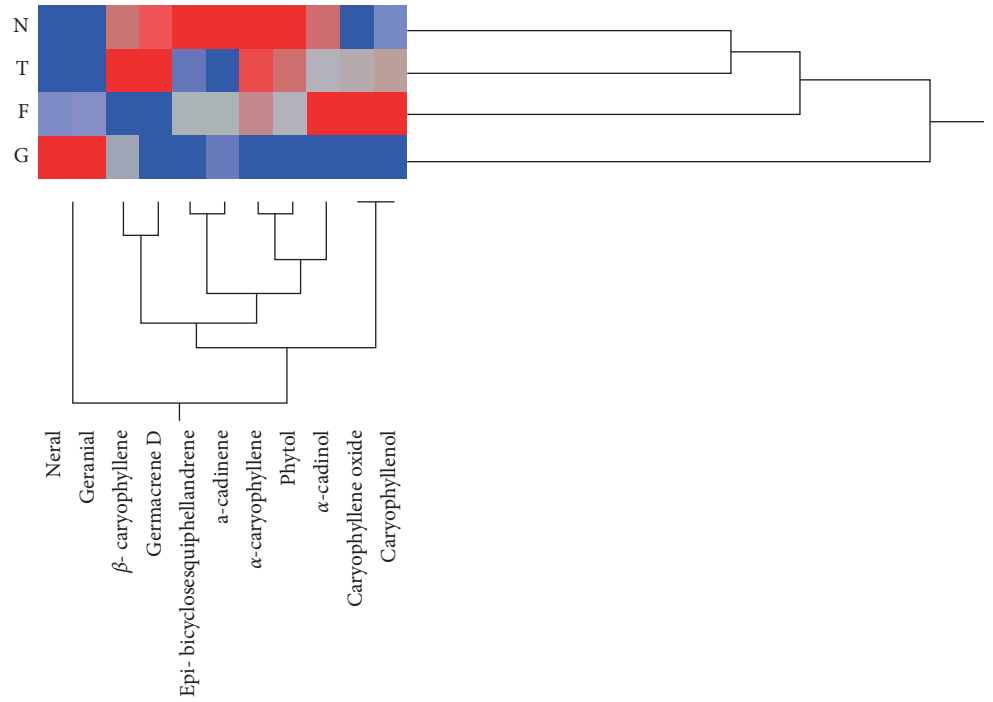

Figure 3: Total phenolic content according to the origin of *M. officinalis*. N: Nefza, T: Tabarka, F: France, and G: Germany.

Table 5: Fatty acid percentage content of *M. officinalis* leaves.

Fatty acid	Tabarka	Nefza	France	Germany
C16: 0 (palmitic acid)	15.82	13.32	16.25	15.77
C16: 1 (palmitoleic acid)	1.00	1.47	—	1.71
C18: 1$n-9$ (oleic acid)	5.89	4.26	4.62	6.29
C18: 2$n-6$ (linoleic acid)	70.75	66.74	73.93	74.08
C20: 0 (arachidic acid)	1.19	1.31	1.60	1.06

Table 6: Yield of aqueous and ethanolic extracts of *Melissa officinalis*.

Origin	Aqueous extract (%)	Ethanolic extract (%)
Tabarka	31a	6b
Nefza	29.05a	7b
France	28.5a	7.5b
Germany	26.5a	7.5b

Means in each column followed by the same letters are not significantly different ($P > 0.05$).

materials and extraction conditions. Few studies within the literature have evaluated the effect of seed source on plant phenolic levels. Antioxidant capacities of lemon balm were determined using the DPPH assays, and results are shown in Table 7. There was significant variation in free radical scavenging activities among populations, and the inhibitory concentrations IC50 ranged from 123,29 to 541,81 mg/mL

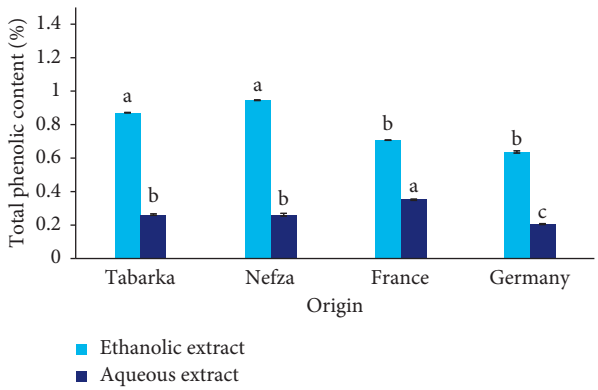

Figure 4: IC_{50} of the ethanolic extract according to the origin of *M. officinalis* and seeds. Values followed by the same letters are not significantly different ($P > 0.05$).

Table 7: DPPH radical scavenging capacity (IC_{50}) of ethanolic and aqueous extract.

Origin	IC_{50} DPPH (mg/mL)	
	Ethanolic extract	Aqueous extract
Tabarka	$123.29^{d} \pm 0.22$	$514.69^{b} \pm 0.20$
Nefza	$175.64^{c} \pm 0.16$	$496.54^{c} \pm 0.45$
France	$350.85^{b} \pm 0.16$	$340.28^{d} \pm 0.27$
Germany	$541.81^{a} \pm 0.33$	$645.57^{a} \pm 0.32$

Means in each column followed by the same letters are not significantly different ($P > 0.05$).

Table 8: IC_{50} of the aqueous extract according to the origin of *M. officinalis* and seeds tested.

IC_{50}	Medicago	Wild chicory	Radish	Fenugreek
Tabarka	3.82^{a}	5.67^{c}	2.92^{c}	21.32^{a}
Nefza	3.63^{a}	5.34^{c}	2.79^{c}	18.33^{a}
France	2.66^{b}	6.55^{b}	6.05^{b}	5.71^{c}
Germany	2.59^{b}	7.85^{a}	9.77^{a}	16.26^{b}

Means in each column followed by the same letters are not significantly different ($P > 0.05$).

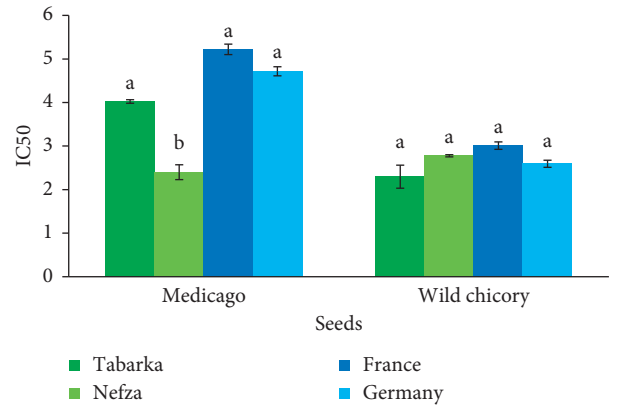

Figure 5: IC_{50} of the ethanolic extract according to the origin of *M. officinalis* and seeds. Values followed by the same letters are not significantly different ($P > 0.05$).

for the ethanolic extract and from 340,28 to 645,57 for the aqueous extract.

In accordance with the data obtained from this study, Aissi et al. reported the effect of geographic origin on antioxidant activity of essential oils [37]. Indeed, these variations were probably due to differences in phenolic compound content [38, 39].

In fact, phenolics, due to their hydroxyl groups that allow them to donate hydrogen to DPPH free radicals, were considered as the major factor contributing to antioxidant activity of plants [40]. Several studies reported by Hammoudi et al., Habellah et al., and Tlili et al. showed a correlation between total phenolic content and antioxidant activity [41–43]. Cultivar also influenced DPPH antioxidant capacity ($P = 0.002$) with "Lemonella" ($45.2 \pm 11.2\ \mu$mol TEAC/g DW) having significantly lower DPPH free radical scavenging than all other cultivars in the study. "Lime" balm also had the highest DPPH antioxidant capacity, $189.5 \pm 19.7\ \mu$mol TEAC/g DW. Samples having higher phenolic levels typically exhibit greater antioxidant capacity [44].

3.6. Allelopathic Activity. The inhibitory effect of the aqueous extract of *Melissa officinalis* from different origins on seed germination was clearly visible from the lower concentrations. In the case of Medicago, the aqueous extract of lemon balm had the highest inhibition levels (Table 8). The ethanol extract was tested only on the seeds of medicago and chicory, given the low yield of extract. The results showed that the rate of germination was strictly concentration dependent (Figure 5). The study of IC_{50} of ethanolic extract from the four origins showed close concentrations with chicory seeds. However, with the medicago seeds, the ethanol extract from Tunisian *M. officinalis* showed the lowest IC_{50}. To follow the effect of the ethanolic extract of Tunisian Lemon balm on the development of seedlings, the length of the root and the shoot were measured under the effect of different concentrations. The longest root was recorded in untreated seeds, and the shortest length was recorded in seeds treated with 5 mg/ml. For the shoot, the ethanolic extract of lemon balm significantly reduced its length depending on the concentration in both species. Variance analysis showed significant differences ($P < 0.05$) between treatments (Tables 9 and 10). From a physiological point of view, germination begins with the imbibitions of the seed and ends with the beginning of growth marked by the lengthening of the root [45]. The germination of a seed can take place only if certain favorable conditions are met: oxygen, temperature, and water. Moreover, it is well known that natural substances produced by plants are able to delay or inhibit seed germination and seedling growth. This explains the effects of aqueous and ethanolic extracts of *M. officinalis* on the germination of certain seeds. The results obtained from the germination tests showed the existence of an allelopathic phenomenon under experimental conditions. This provided evidence that some plants contain chemical compounds with herbicidal activity. The aqueous extract of lemon balm from Tunisia was shown as the most inhibitor against the majority of seeds tested. The ethanolic extract showed a greater allelopathic power against the Medicago seeds than on the chicory seeds. The allelopathic effect may depend on the species of seeds tested, which was provided by

Table 9: Effect of ethanol extract on the germination of medicago seeds.

	Nefza			Tabarka		
	Inhibition rate (%)	Shoot	Root	Inhibition rate (%)	Shoot	Root
0 mg/ml	0	3.57a	3.25a	0	3.57a	2.62a
1 mg/ml	25	2.45b	2.55b	10	2.37b	2.80a
3 mg/ml	31.66	1.97c	2.52b	30	1.47c	2.05a
5 mg/ml	38.33	1.47d	1.77c	78.33	0.92c	1.20b
IC50	4.06			2.3		

Means in each line followed by the same letters are not significantly different ($P > 0.05$).

Table 10: Effect of ethanol extract on the germination of wild chicory seeds.

	Nefza			Tabarka		
	Inhibition rate (%)	Shoot	Root	Inhibition rate (%)	Shoot	Root
0 mg/ml	0	2.02a	3.25a	0	1.90a	3.35a
1 mg/ml	56.66	0.70b	1.07b	60	0.95b	0.70b
3 mg/ml	61.66	0.25c	0.45c	73.33	0.67b	0.55b
5 mg/ml	68.33	0.22c	0.30c	76.66	0.20c	0.25b
IC50	2.39			2.65		

Means in each line followed by the same letters are not significantly different ($P > 0.05$).

Serghini et al. [46], where sunflower extract (*Helianthus annus* L) had an effect on the germination of *Orobanche ramose (Phelipanche ramose* L.) but had no effect on the germination of *Orobanche cernua* Wallr. The results obtained corroborated with those found by Kato-Noguchi and Ino in which certain fractions of a hydroacetonic extract of *M. officinalis* inhibited the germination and the growth of roots and shoots of A*maranthus caudatus* L., *Lepidium sativum* L., *Digitaria sanguinalis* L., *Phleum pratense* L, *Lactuca sativa* L., and *Lolium multiflorum* Lam. [6]. The aqueous extract is the most effective with an IC_{50} being 0.14 mg/ml.

4. Conclusion

Exploration of native plant genetic resources is one of the main objectives of the Medicinal and Aromatic Plants Program. It also aims to evaluate and conserve rare spontaneous plants, genetic resources, and especially threatened species in their natural environment. The seeds of Tunisian lemon balm were the most sensitive to salinity and the variation of the osmotic pressure, which explains the reduced number of plants in situ. The morphological study showed that Tunisian lemon balm was characterized by small plants with a weak branching. The aqueous and ethanolic extracts of this species had an interesting allelopathic activity, which allowed the use of lemon balm in the field of phytosanitary products as weed killers.

Data Availability

All data generated or analyzed during this study are included in this published article.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

This research was funded by the National Institute of Agronomical Research of Tunisia.

Supplementary Materials

Supplement 1: morphological characters; Supplement 2: raw data of morphological studies; Supplement 3: dendrogram established from Euclidean squared for standard variables using the average method based on agromorphological traits among four populations of *M. officinalis*. (*Supplementary Materials*)

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