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Research Article

Chemical Composition, Antioxidant Activity, and Antifungal Effects of Essential Oil from *Laurus nobilis* L. Flowers Growing in Morocco

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In this study, the chemical composition and the antioxidant and antifungal activities of essential oil from *Laurus nobilis* flowers were examined. The essential oil was prepared using steam distillation in a modified Clevenger-type apparatus. The chemical composition of the obtained essential oil and chemotypes was determined using gas chromatography coupled with mass spectrometry (GC/MS) and gas chromatography with flame ionization detection (GC-FID). Twenty-five volatile compounds were identified, which made up 92.07% of the total essential oil content. The essential oil yield was 1.06% and the most abundant compounds were 1.8-cineole (45.01%), α-caryophyllene (7.54%), germacradienol (6.13%), limonene (4.69%), α-pinene (3.04%), and germacrene D (3.14%). The antifungal activity of the obtained essential oil was tested against seven fungal strains: *Aspergillus clavatus*, *A. niger*, *Chaetomium globosum*, *Cladosporium cladosporioides*, *Myrothecium verrucaria*, *Penicillium citrinum,* and *Trichoderma viride*. The results indicated that essential oil from *L. nobilis* flowers exhibited significant antifungal activity against the tested fungal strains with minimum inhibitory concentrations (MICs) ranging from 0.05 to 0.46 mg/mL. The essential oil of *L. nobilis* also exhibited strong total antioxidant capacity (TAC) as indicated by its ability to scavenge free radical DPPH. Taken together, this study indicates that the essential oil from *L. nobilis* flowers possesses significant antifungal and antioxidant activities, possibly due to the high level of 1,8-cineole.

1. Introduction

Herbs and spices have been used for generations, not simply as food ingredients but also to treat a plethora of ailments and, in recent times, scientific data are accumulating that demonstrate, for many spices and related essential oils, medicinal properties useful in the prevention of diseases or the relief of their symptoms. In fact, many natural compounds extracted from plants have demonstrated biological activities, notably antibacterial, antifungal, and antioxidant properties. The antimicrobial properties of plant volatile oils and their constituents from a wide variety of plants have been documented [1, 2]. The Lauraceae comprises 32 genera and about 2.500 species. Laurel (*Laurus nobilis* L.) is a plant native to the southern Mediterranean region. It is an evergreen tree cultivated in many warm regions of the world, particularly in the Mediterranean countries (Turkey, Greece, Spain, Portugal, and Morocco). In Europe and the USA, *L. nobilis* is widely cultivated as an ornamental plant [3].

Essential oils are valuable natural products used as raw materials in perfumes, cosmetics, aromatherapy, phytotherapy, spices, and nutrition [4]. The leaves of *L. nobilis* have been used to treat Neuralgia and Parkinsonism [5], while the essential oil obtained from the leaves of this plant

has been used for relieving hemorrhoid and rheumatic pains and has been used to treat epilepsy [6]. The chemical composition of the volatile fraction of *L. nobilis* as well as the composition and bioactivities of the alcoholic and nonpolar extracts has been studied extensively [7–9]. Intensive research has been conducted on this species [10–12]. The antifungal activity of essential oil extracted from *L. nobilis* has been documented [13, 14], the oil presents a low minimum inhibitory concentration and is rich in monoterpenes and sesquiterpenes. Essential oils have been shown to possess antibacterial, antifungal, antiviral, insecticidal, antidiarrheal, and antioxidant properties [15–19]. There is currently an increased interest in looking at antimicrobial properties of extracts from aromatic plants particularly essential oils [20]. Essential oils are a rich source of biologically active compounds; they have been used in cancer treatment [21] and food preservation [22]. The chemical composition and antimicrobial properties of essential oils extracted from diverse plant species have been demonstrated using a variety of experimental methods [23, 24]. However, to the best of our knowledge, the chemical composition, antioxidant activity, and antifungal effects of essential oil from *L. nobilis* flowers in Morocco have not been studied.

The objective of this study was to identify the chemical compounds and examine the antioxidant and antifungal activities of essential oil from *L. nobilis* flowers.

2. Materials and Methods

2.1. Chemicals and Standards. All solvents used in this study were of analytical grade unless otherwise specified. Hexane solution, methanol, sulfuric acid, anhydrous sodium sulfate**,** series of alkanes (C_4–C_{28}) standards, and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical were purchased from Sigma-Aldrich (St. Louis, MI, USA). The fungal strains were obtained from the Microbiology Laboratory, Faculty of Medicine and Pharmacy, Fez, Morocco.

2.2. Collection and Preparation of the Plant Material. The flowers of *L. nobilis* were collected in July 2019, from the Middle Atlas mountains in Morocco, a region where people frequently use this plant for therapeutic uses [25]. The region is located 15 km southeast of Boulemane city (latitude: 25°31′11″; longitude: 5°22′21″; altitude: 2100 m); it is characterised by semidesertic climate with strong continental influence, and an annual average temperature of 20°C. The collected flowers were air-dried for sixteen days and stored in the laboratory, at the Faculty of Sciences, University Sidi Mohamed Ben Abdellah, Fez, Morocco.

2.3. Essential Oil Extraction. The essential oil was extracted by hydrodistillation using an apparatus of Clevenger type as previously described [26, 27]; briefly, 200 g of *L. nobilis* flowers were mixed in 1.4 ml of distilled water for 2.5 hours. The yellowish oil (0.5 ml) was dissolved in hexane and then dried over anhydrous sodium sulfate. After filtration, the solvent was eliminated by pressure distillation reduced in a rotary evaporator at 35°C, and the extracted oil was stored at 4°C in obscurity until analysis.

The amount of oil obtained was calculated as follows:

$$\text{oil}\,(\%\,\text{v/w}) = \frac{\text{observed volume of oil}\,(\text{ml})}{\text{weight of the sample}\,(\text{g})} \times 100. \quad (1)$$

2.4. Gas Chromatography (GC/MS and GC-FID) Analysis. The chemical composition of the oil extracted from *L. nobilis* flowers was determined by GC-MS (Trace GC ULTRA, Thermo Fischer, France) using a Varian capillary column (CP-Sil 5CB, 50 m length, 0.32 mm of diameter, and Film thickness 1.25 μm). The column temperature was programmed from 40°C to 280°C for 5°C/min. The temperature of the injector was fixed to 250°C and one of the detectors (FID) to 260°C. The debit of the gas vector (nitrogen) was fixed to 1 ml/min. The volume of the injected specimens was 0.5 μl of diluted oil in hexane solution (10%). The percentage of each constituent in the oil was determined by area peaks.

The essential oil was subjected to GC-FID analysis (Polaris Q, Thermo Fischer, France) using the same type of column used for GC-MS (the constituents of essential oil were identified in comparison with their Kovats index, calculated concerning the retention time of a series of linear alkanes (C_4–C_{28}) with those of reference products and in comparison with their Kovats index with those of the chemical components gathered in [28], in comparison with their specters of mass with those gathered in a library of (NIST-MS) type.

2.5. Fungal Strains. The antifungal activity of the essential oil prepared from *L. nobilis* flowers was evaluated by measuring the zone of inhibition and the minimal inhibition concentration (MIC). Values were calculated according to published procedures [29, 30] with minor modifications.

Antifungal tests were then carried out by the disc diffusion method [31], using 50 μl of suspension containing 52 spores/ml of fungi spread on potato dextrose agar (PDA). The discs (6 mm in diameter) were impregnated with 10 μl of essential oil and placed on the inoculated agar. Negative controls were prepared using the same solvents used to dissolve the plant extract. Ofloxacin (20 μg per disc) and sulbactam (30 μg) + cefoperazone (70 μg) (100 μg/disc) were used as positive reference standards to determine the sensitivity of one strain/isolate in each microbial species tested. The inoculated plates were incubated at 27°C for 72 h. The fungal strains tested were *Aspergillus clavatus*, *A. niger*, *Chaetomium globosum*, *Cladosporium cladosporioides*, *Myrothecium verrucaria*, *Penicillium citrinum,* and *Trichoderma viride*., and assays were performed in duplicate.

2.6. In Vitro Free Radical Scavenging Assays

2.6.1. DPPH Radical Scavenging Activity. The ability of the essential oil to scavenge the DPPH radical was tested as previously described [32]; 100 μl of different concentrations of the essential oil was added to 750 μl of an ethanolic

solution containing 0.1 mmol of DPPH (2,2-diphenyl-1 picrylhydrazyl). Mixtures were incubated at room temperature for 30 min in dark. The absorbance of the mixture was then measured at 517 nm using a spectrophotometer (UV-2005. J.P. SELECTA. s.a) and the percentage of inhibition (PI) was calculated using the following equation:

$$\mathrm{PI}(\%) = \left(1 - \left(\frac{A_s}{A_c}\right)\right) * 1000, \quad (2)$$

where A_c is the absorbance of the negative control and A_s is the absorbance of the sample. Butylated hydroxytoluene (BHT) and ascorbic acid served as positive controls for maximal radical quenching. The IC_{50} values were calculated as the concentration causing 50% inhibition of DPPH radical.

2.6.2. Total Antioxidant Capacity (TAC). Total antioxidant capacity was measured as previously described [33]. Different volumes (100 μl, 50 μl, and 25 μl) of the essential oil were mixed in 1 ml of reactive solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate.) After incubation at 95°C for 90 minutes, the optical density at 695 nm was measured using a spectrophotometer, with a blank containing methanol instead of the essential oil. The antioxidant capacity was expressed in milligrams of ascorbic acid equivalent per gram of extracts (Mg EAA/g extracts). Ascorbic acid was used to make a standard curve.

3. Results

3.1. Phytochemical Analysis. The constituents of essential oil *L. nobilis* flowers are listed in order of their elution on the CP-Sil 5CB column (Figure 1). The yield of the obtained essential oil was 1.06% and contained 25 compounds, which made up 92.07% of the total essential oil. The most abundant constituents were 1.8-cineole (45.01%), α-caryophyllene (7.54%), germacradienol (6.13%), limonene (4.69%), α-pinene (3.04%), and germacrene D (3.14%) (Table 1).

3.2. Antifungal Activity. The antifungal activity of the essential oil extracted from the flowers of *L. nobilis* was tested against *Aspergillus clavatus*, *A. niger*, *Chaetomium globosum*, *Cladosporium cladosporioides*, *Myrothecium verrucaria*, *Penicillium citrinum,* and *Trichoderma viride*. The obtained results are presented in (Table 2).

The disc diffusion assay indicated that the inhibition zone of the essential oil ranged between 6.05 mm and 19.25 mm, depending on the fungal strain tested. The strongest inhibition zone was observed for *A. clavatus* followed by *A. niger* and *C. globosum*, indicating the sensitivity of these strains to the essential oil from *L. nobilis* flowers. The MIC values obtained for these strains were 0.05, 0.14, and 0.18 mg/mL, respectively. The other fungal strains tested were also sensitive to the essential but to a lesser extent.

3.3. Antioxidant Activity

3.3.1. DPPH Radical Scavenging Activity. The antioxidant activity of the extracted essential oil was determined based on its ability to reduce the free radicals DPPH. The essential oil from *L. nobilis* flowers exhibited strong power of reducing DPPH ($IC_{50} = 82.01 \pm 0.002$ μg/ml), compared to BHT ($IC_{50} = 7.71 \pm 0.0001$) and ascorbic acid ($IC_{50} = 1.16 \pm 0.0001$ μg/ml) (Table 3). A low inhibitory concentration is an indication of a high scavenging activity. A substance can be considered as an antioxidant if its IC_{50} does not exceed 5 mg/ml [34]. In this study, the essential oil of *L. nobilis* has proven its ability as a strong antioxidant; this is accordance with previous reports [35, 36]. In fact, the major compound of the essential oil, 1.8-cineole (45.01%), has been shown to have strong antioxidant capacity [37].

3.3.2. Total Antioxidant Activity. The total antioxidant capacity was determined by the method of phosphomolybdenum, based on the reduction of Mo (VI) to Mo (V) by the antioxidant substance [38], and the values were expressed as ascorbic acid equivalents per gram (AAE/g) (Figure 2). For the different volumes of the essential oil used (100 μl, 50 μl, and 25 μl), the total antioxidant activities obtained were 796.74 ± 3.53 AAE/g), (568.91 ± 96.16 AAE/g), and (440.91 ± 10.37 AAE/g), respectively, indicating a positive correlation between the volume of the essential oil used and the level of antioxidant activity.

4. Discussion

4.1. Phytochemical Analysis. In this study, the yield of the essential oil obtained from the hydrodistillation of the flowers of *L. nobilis* was high (1.06%) compared to other plants such as *Artemisia herba-alba* (0.59%), *A. absinthium* (0.57%), *A. pontica* (0.31%) [39], *Pseudotsuga menziesii* (0.67%), *Pistacia lentiscus* (1.02%) [40], *Lavandula angustifolia* (0.8–2.8%), *Mentha spicata* (0.5–1%), and *Citrus aurantium* (0.5–1%) [41], but lower compared to *Eucalyptus grandis* (4.7%) [42] and *E. globulus* (1.21%) [43]. These differences might be due to harvest time and local, climatic, and seasonal factors as well as the storage duration of the medicinal plant used. 1,8-Cineole was the main component present in the essential oil, with other compounds being present in low percentage or even in traces; this is in accordance with the previously published report. 1.8-Cineole was identified as the major component in essential oils obtained from *L. nobilis* in different countries [44–52]. In addition, 1.8-cineole was also identified as the major component in other plants, such as *Origanum minutiflorum* [53] and *Callistemon speciosus* [54]. The essential oil composition showed a pattern similar to those published for other geographical regions, including a high level of 1.8-cineole [55–60]. The essential oil content varies depending on the geographical origin of the plant, but also on the plant part used for extracting the essential oil [61].

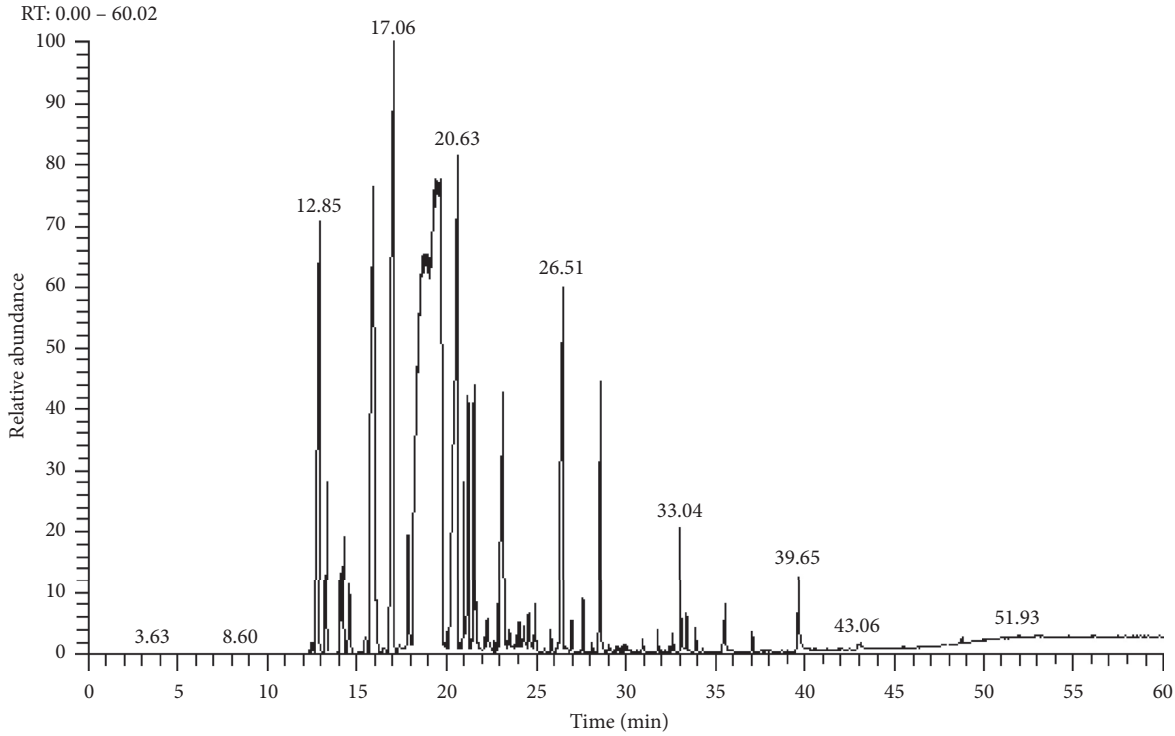


Figure 1: Chromatogram of essential oil from *L. nobilis* flowers.

Table 1: Phytochemical analysis of essential oil from *L. nobilis* flowers.

Peak	Compounds	Chemical formula	∗RT (min)	∗∗KI	Identification	Area (%)	∗∗∗Mass range (*m*/*z*)
1	Terpinolene	C10H16	12.85	1042	GC-MS, IK	0.10	(136),93,121,91,136,79,77,105,39,41,107
2	Germacradienol	C2H6O	15.75	1476	GC-MS, IK	6.13	(204),161,105,91,41,119,79,81,93,77,80
3	1,8-Cineole	C10H18O	17.06	1059	GC-MS, IK	45.01	(154),43,93,81,71,69,84,68,108,41,55
4	α-Phellandrene	C10H16	19.45	964	GC-MS, IK	1.02	(136),93,77,91,136,79,94,41,80,92,39
5	α-Terpinene	C10H16	20.46	1112	GC-MS, IK	2.69	(154),71,111,93,43,86,41,69,55,68,154
6	α-Caryophyllene	C15H24	20.63	1494	GC-MS, IK	7.54	(136),93,77,91,136,79,94,41,80,92,93
7	Germacrene D	C15H24	21.60	1505	GC-MS, IK	3.14	(204),161,105,91,41,119,79,81,93,77,27
8	Linalool	C10H18O	22.14	1093	GC-MS, IK	1.04	(136),93,41,69,39,91,77,79,27,92,53
9	α-Terpineol	C10H18O	25.10	1177	GC-MS, IK	1.05	(154),59,93,121,136,81,43,68,95,67,41
10	Limonene	C10H16	26.51	1018	GC-MS, IK	4.69	(136),93,92,91,77,79,41,39,121,27,105
11	Sabinene	C10H16	27.56	983	GC-MS, IK	3.01	(136),93,41,91,77,79,39,27,69,94,43
12	α-Pinene	C10H16	28.50	938	GC-MS, IK	3.04	(153),71,41,43,93,55,69,80,39,121,27
13	Methyl-eugenol	C11H14O2	30.03	1376	GC-MS, IK	1.11	(178),178,136,147,103,91,107,179,151,41,77
14	α-Phellandrene	C10H16	37.45	954	GC-MS, IK	1.28	(136),93,77,91,136,79,94,41,80,92,39
15	β-Pinene	C10H16	38.57	924	GC-MS, IK	3.01	(136),93,91,136,121,77,92,79,43,41,105
16	Myrcene	C10H16	40.40	948	GC-MS, IK	1.56	(136),41,93,69,39,27,53,79,77,67,91
17	(Z)-3-Hexenol	C6H12O	41.24	862	GC-MS, IK	0.85	(100),67,41,39,55,82,31,69,53,54,27
18	Camphene	C10H16	42.07	933	GC-MS, IK	0.15	(136),93,79,91,77,41,121,80,94,107,39
19	Caryophyllene oxide	C10H18O	43.50	1506	GC-MS, IK	0.42	(220),43,41,79,93,91,95,69,55,67,81
20	p-Cymene	C10H14	44.80	1032	GC-MS, IK	0.10	(134),119,134,91,120,117,41,77,39,65,115
21	Carene	C10H16	48.04	973	GC-MS, IK	0.01	(136),93,41,91,77,79,39,27,69,94,43
22	3-Carene	C10H16	49.13	1004	GC-MS, IK	0.98	(136),93,91,79,77,92,121,80,136,94,105
23	Isobornyl acetate	C12H20O2	50.26	1262	GC-MS, IK	0.25	(196),95,43,121,93,136,41,108,110,55,82
24	β-Elemol	C15H26O	55.34	1545	GC-MS, IK	0.10	(208),208,193,209,91,65,133,79,77,177,105
25	α-Thujene	C10H16	51.25	973	GC-MS, IK	0.65	(136),93,41,91,77,79,39,27,69,94,43
		Total	**92.07**				
		Yields (%)	**1.06**				

∗RT: retention time obtained by chromatogram (Figure 1). ∗∗KI: Kovats index was determined by GC-FID on a CP-Sil 5CB column. ∗∗∗Mass range (*m*/*z*): determined by mass spectrometry (PlarisQ).

Table 2: Minimum inhibitory concentration (MIC) of essential oil from *L. nobilis* flowers (mg/mL).

Microorganisms	Disc diffusion assay (inhibition zone in mm)	MIC (mg/mL)
Aspergillus clavatus	19.25 ± 0.12	0.05
Aspergillus niger	14.35 ± 0.04	0.14
Chaetomium globosum	12.45 ± 0.34	0.18
Cladosporium cladosporioides	8.25 ± 0.02	0.25
Myrothecium verrucaria	7.35 ± 0.35	0.29
Penicillium citrinum	7.15 ± 0.01	0.36
Trichoderma viride	6.05 ± 0.03	0.46

Disc diameter 6 mm average of two consecutive trials. MIC: minimal inhibitory concentration; concentration range: 0.05–0.46 mg/mL.

Table 3: IC_{50} values of DPPH radical scavenging activity.

	IC_{50} (μg/ml)
Essential oil	82.01 ± 0.002
BHT	7.71 ± 0.0001
Ascorbic acid	1.16 ± 0.0001

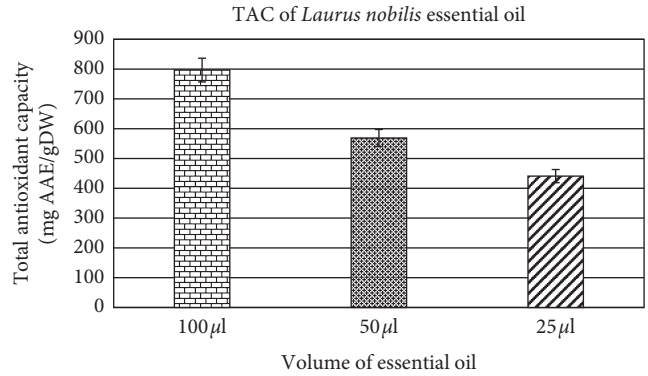


Figure 2: Total antioxidant capacity of different volumes of essential oil from *L. nobilis* flowers. Results are expressed as mg AAE/g.

4.2. Antifungal Activity. The antimicrobial activity of essential oil from *L. nobilis* flowers against microorganisms was determined qualitatively and quantitatively. In Table 2, we report the widths (mm) of the inhibition halos exhibited by the essential oil against different pathogens (*A. clavatus*, *A. niger*, *C. globosum*, *C. cladosporioides*, *M. verrucaria*, *P. citrinum,* and *T. viride*). The results indicate that the essential oil of *L. nobilis* flowers exhibits strong antifungal activity especially against *A. clavatus*, *A. niger*, and *C. globosum*. Previous studies have reported the antimicrobial activity of members of the genus *Laurus*. This activity might be due to 1.8-cineole and α-caryophyllene and their precursors [62–65]. In fact, 1.8-cineole alone has been shown to exhibit a better antifungal activity than the whole essential oil [66].

The antifungal activities, in general, have been mainly explained through terpenes with aromatic rings and phenolic hydroxyl groups able to form hydrogen bonds with active sites of the target enzymes, although other active terpenes, as well as alcohols, aldehydes, and esters can contribute to the overall antifungal effect of essential oils [67]. Pinene-type monoterpene hydrocarbons are well-known chemicals having antimicrobial properties [68]. The difference in antifungal efficacy is a result of higher concentrations of the same chemical or a result of different chemical composition between plants. Several studies have been conducted to understand the mechanism of action of plant extracts and essential oils; however, it is still unclear [69]. It has been suggested that components of the essential oils and extracts cross the cell membrane interact with the enzymes and proteins of the membrane producing a flux of protons towards the cell exterior, causing cell death [66]. Pinene-type monoterpene hydrocarbons (α-pinene and β-pinene), limonene, and linalool are well-known chemicals having antimicrobial properties [67, 70, 71].

4.3. Antioxidant Activity

4.3.1. DPPH Radical Scavenging Activity. The strong free radical scavenging capacity of the essential oil of *L. nobilis* may be due to different chemical compounds present in the oil, especially the relatively high percentage of 1.8-cineole (45.01%) and α-caryophyllene (7.54%), although other compounds such as germacradienol (6.13%), limonene (4.69%), α-pinene (3.04%), and germacrene D (3.14%) may be involved.

Furthermore, the antioxidant activity may be altered by synergistic and antagonistic effects between some components of the essential oil [72]. The synergistic activity of 1.8-cineole and α-caryophyllene has been documented [73]. Our

results are in accordance with those found by other researchers [74–77], further confirming the antioxidant capacity of essential oil from *L. nobilis* and its potential as a natural preservative in food and pharmaceutical industries.

4.3.2. Total Antioxidant Activity. The essential oil of *L. nobilis* flowers exhibited higher total antioxidant capacity compared to previous reports [72], and this can be explained by the variation in the chemical composition of the essential oil due to the difference of regions affected by environmental factors, and also by the redox power of this substance and its electron/hydrogen exchange capacity.

These results represent a basis for further studies that could lead to the utilisation of this essential oil as a natural antioxidant agent, in both food and pharmaceutical fields.

5. Conclusion

This study revealed the chemical composition of the essential oil of *L. nobilis* flowers originated from the Middle Atlas mountains in Morocco. The essential oil was characterised by GC-MS and GC-FID, and twenty-five volatile compounds were identified which made up 92.07% of the total essential oil. The essential oil yield of the studies was 1.06%, and the major constituents were 1.8-cineole, α-caryophyllene, and germacradienol (6.13%). The essential oil of *L. nobilis* flowers exhibited important antioxidant and antifungal activities especially against *A. clavatus*, *A. niger*, and *C. globosum*, suggesting its potential as a source of antifungal compounds identification. However, further studies should be conducted to evaluate its efficacy against phytopathogenic fungi under field conditions.

These results constitute an important step in the search for biologically active natural substances. Additional tests will be necessary to find formulations for the use of the essential oil from *L. nobilis* flowers in food, pharmaceutical, and cosmetic industries.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare no conflicts of interest.

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