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Research Article

Chemical composition and antimicrobial and antioxidant activities of Tunisian, France and Austrian *Laurus nobilis* (Lauraceae) essential oils

Mounira MKADDEM GUEDRI[1]*, Mehrez ROMDHANE[1], Ahmed LEBRIHI[2], Florence MATHIEU[2], Jalloul BOUAJILA[2]

[1]*Université de Gabès, Ecole National d'Ingénieurs de Gabès, Laboratoire d'Environnement, Catalyse et Analyse des Procédés, Rue Omar elkhattab-ZRIG-6029 Gabès, Tunisie; mounira.mkaddem@gmail.com (*corresponding author); mehrez.romdhane1965@gmail.com*
[2]*Université de Toulouse, Laboratoire de Génie Chimique, UMR 5503, CNRS, INPT, UPS, Toulouse, France; lebrihi@ensat.fr; florence.mathieu@ensat.fr; jalloul.bouajila@univ-tlse3.fr*

Abstract

Essential oil (EO) of *Laurus nobilis*, from Tunisian, France and Austrian were screened for their chemical composition, antioxidant and antimicrobial activities and compared. GC-MS analysis showed that leaves of Tunisian *L. nobilis* had camphor (34.43%), 1,8-cineole (20.21%) and α-terpineol (7%) as major components. France and Austrian EOs had a high content of 1,8-cineole (45.8% and 43.4%, respectively) followed by bornyl acetate (13.8% and 17.7% respectively) and methyl eugenol (7.7% and 10.9% respectively). Antioxidant potential was measured by ABTS and DPPH tests. Tunisian *L. nobilis* EO showed greater radical scavenging by ABTS activity (IC_{50}=44.8±0.1 mg/L) than the France and Austrian EOs (76.4±3.2 mg/L and 81.4±4.0 mg/L, respectively). However, for DPPH test system, French and Austrian EOs activities were excellent (IC_{50}=176.1±5.1 mg/L and 236.3±2.9 mg/L respectively) then Tunisian *L. nobilis* EO (IC_{50}=2859.7±99.0 mg/L). A good Antimicrobial activity was observed on the yeasts and fungi for all EOs. Tunisian laurel EO show a better antibacterial activity against gram-negative bacteria (*Klebsiella pneumonial, E. coli* and *Salmonella enterica* CMI: 0.004 mg/ml*)* than gram-positive ones (*Bacilus subtilis, Staphylococcus aureus* and *Listeria monocytogenes* CMI: 0.01 mg/ml). A significant antifungal activity of Tunisian EO was also observed against fungi and yeasts species (CMI: 0.004 mg/ml). France essential oil shows better activities against all organisms tested wail Austrian oil activity is more important against yeasts species tested and *Mucor ramannianus* (fungi). Chemical composition, antimicrobial and antioxidant activity of Tunisian *L. nobilis* essential oil, were different from that of France and Austrian and it give the opportunity for its uses in new pharmaceuticals and natural therapies of infectious diseases.

Keywords: antioxidant activity; antimicrobial activity; DPPH and ABTS, essential oil; GC-MS; *Laurus nobilis*

Introduction

Laurus nobilis (Lauraceae) is cultivated in temperate parts of the world, mainly in south Europe and Mediterranean area. It is more used as flavouring in the culinary preparations. Specie was reputed for his

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ownership particularly stimulating and sedative analgesic (Arvy and Galloin, 2003). The essential oil obtained from the leaves of this plant has been used for relieving haemorrhoid and rheumatic pains (Zargari, 1990). It also has diuretic (Zargari, 1990), healing (Nayak *et al.*, 2006), antifungal (Hassiotis, 2010) and antibacterial (Seyed *et al.*, 1991; Bouzouita *et al.*, 2003) activities which are interesting and useful for food preservation (Brahmi *et al.*, 2015). Nevertheless, species became rare in Tunisia and subsist only in some regions of the North West (Pottier Alapetite, 1979).

Many studies of chemical composition of essential oil extracted from different part of plant (steam, leaves, seeds, floral) of *L. nobilis* cultivated from different geographical region in the world was carried (Lawrence, 1983; Anaç, 1986; Pino *et al.*, 1993; Bouzouita *et al.*, 2001; 2009).

Anticonvulsant activity, aflatoxigenic, insecticides and repellent activity of the leaf essential oil was reported in previous works (Sayyah *et al.*, 2002; Erler et *al.*, 2006; Atanda *et al.*, 2007). Antioxidant activity of *L. nobilis* essential oil was also being studied (Baratta *et al.*, 1998) Therefore, considering the importance of toxicogenetic investigation of plant the possible effects on Central Nervous System of laurel essential oil (Caputo *et al.*, 2017) and cytotoxic and genotoxic potential of the aqueous extract of *L. nobilis* leaves was recently prouved (Silva *et al.,* 2020). Tunisian *L. nobilis* floral buds' oil was evaluated for their antioxidant activity by two methods: β-carotene bleaching (BCB) test and 2.2-diphenyl-β-picrylhydrazyl (DPPH) assay (Bouzouita *et al.*, 2009). The antimicrobial properties against some microorganisms (Seyed *et al.*, 1991; Bouzouita *et al.*, 2003; Marzouki *et al.*, 2009) and antifungal activity were reported (Christos and Evanthia, 2011). Quantitative and qualitative changes in Tunisian *L. nobilis* L. leaves essential oil as affected by different drying methods and total fatty acids laurel seed was recently studied (Sellami *et al.*, 2011; Dhifi *et al.*, 2018). Some previous study reports significant antimicrobial activities in Tunisian *L. nobilis* essential oil (Bouzouita *et al.*, 2003; Marzouki *et al.*, 2009). However, no earlier study reported the comparison of Tunisian essential oil composition in relation with his biological activities with the most commercialised laurel essential oil to better oriented industrials markets.

Here we reported the results of chemical composition, antioxidant, and antimicrobial activity of Tunisian *L. nobilis* essential oil compared with those of France and Austrian *L. nobilis* essential oil commercialized. Our objective is to present the Tunisian laurel essential oils as a new natural and effective antioxidant and antimicrobial agent and to study consequences of chemical composition variability associated with concept of chemotype in terms of therapeutic use of essential oils.

Materials and Methods

Essential oil isolation

Fresh leaves of *L. nobilis* cultivated species were collected from region of Mannouba, Tunisia. About 100 g of the fresh leaves of *L. nobilis* was placed in 400 mL of distilled water and subjected to hydrodistillation for 3 hours, using a Clevenger-type apparatus (British Pharmacopoeia, 1980) (1.5% yield). Anhydrous sodium sulphate was used to eliminate water traces from essential oil. The essential oil is stored at + 4 °C until tested and analysed. Condition of culture, extraction method and stockage were the same of that indicated for commercialized essential oils from France and Austrian *L. nobilis*.

Chemicals used

All chemicals used were of analytical reagent grade. All reagents were purchased from Sigma-Aldrich-Fluka (Saint-Quentin France).

GC and GC/MS analysis

The essential oil composition was determined by a VarianStar3400 (Les Ulis, France) Cx gas chromatograph equipped with a flame ionisation detector (FID) and DB-5 (30 m × 0.25 mm; film thickness

0.25 µm) capillary column. Oven temperature gradually rose from 60 °C to 260 °C with a gradient of 5 °C/minute and 15 minutes isothermal at 260 °C and finally rose to 340 °C at 40 °C /min. Helium (purity 99.99999%) was used as the carrier gas at 1 mL/minute. Total analysis time was 57 minutes. 1 µ of diluted sample (1/100 in petroleum ether, v/v) was injected in the split mode (ratio 1:10). The injector was operated at 200 °C. Quantitative data were obtained electronically from FID area percent data without the use of correction factors. Peak integration and quantification were performed automatically with Saturn 2100 Workstation software. A checking of the integration of each peak is carried out and corrected manually if necessary.

Essential oil analysis was performed also with GC chromatograph equipped with a Varian Saturn GC/MS/MS 4D mass selective detector in the electron impact mode (70 ev). MS was adjusted for an emission current of 10 µA and electron multiplier voltage between 1400 and 1500 V. The trap temperature was 150 °C, and that of the transfer line was 170 °C. Mass scanning was from 40 to 650 amu. The components were identified based on the comparison of their Kovats indices (KI), co-injection of standards, MS experimental data with those contained in commercial or literature libraries (NIST 02 version 2.62, Adams, 2001). Alkanes (C5-C24) were used as reference points in the calculation of KI.

GC and GC-MS analysis results are given in Table 1. All determinations were performed in duplicate and averaged.

DPPH radical scavenging activity

The 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH) quenching ability of plant essential oil was measured according to Blois (1958) with some modifications. 1.5 mL of various dilutions of samples (extracts or essential oil [d=1.1]) were mixed with 1.5 mL of 0.2 mM methanolic DPPH solution. After an incubation period of 30 minutes at 25 °C, the absorbance at 520 nm was measured. The wavelength of maximum absorbance of DPPH, was recorded as $A_{(sample)}$, using Helios spectrophotometer (Unicam, Cambridge UK). A blank experiment was also carried out applying the same procedure to a solution without the test material and the absorbance was recorded as $A_{(blank)}$. The free radical-scavenging activity of each solution was then calculated as percent inhibition according to the following equation:

$$\% \text{ inhibition} = ((A_{(blank)} - A_{(sample)})/A_{(blank)}) \times 100$$

Antioxidant activity of standard or samples was expressed as IC_{50}, defined as the concentration of the test material required to cause a 50% decrease in initial DPPH concentration. Ascorbic acid was used as a standard. All measurements were performed in triplicate.

ABTS radical-scavenging activity

The ABTS (2, 2'-azinobis-3-ethylbenzothiazoline-6-sulphonate) quenching ability of plant essential oil was determined as described by Re et al. (1999). ABTS was generated by mixing a 7 mM of ABT Sat pH 7.4 (5 mM NaH_2PO_4, 5 mM Na_2HPO_4 and 154 mM NaCl) with 2.5 mM potassium persulfate (final concentration) followed by storage in the dark at room temperature for 16h before use. The mixture was diluted with ethanol to give an absorbance of 0.70 ±0.02 units at 734 nm using spectrophotometer (Helios, Unicam, Cambridge UK). For each sample, diluted methanol solution of the essential oil (100 µL) could react with fresh ABTS solution (900 µL), and then the absorbance was measured 6 min after initial mixing. Ascorbic acid was used as a standard. A blank experiment was also carried out applying the same procedure to a solution without the test material and the absorbance was recorded as A (blank). The free radical-scavenging activity of each solution was then calculated as percent inhibition according to the following equation:

$$\% \text{ inhibition} = ((A \text{ (blank)} - A \text{ (sample)})/A \text{ (blank)}) \times 100$$

Antioxidant activity of standard or samples was expressed as IC50, defined as the concentration of the test material required to cause a 50% decrease in initial DPPH concentration. Ascorbic acid was used as a standard. All measurements were performed in triplicate.

Antimicrobial activity

Essential oils were individually tested against a panel of microorganisms (Table 1). Six bacteria including three Gram-positive: *Bacilus subtilis* ATCC 6633 (Bs), *Staphyloccocus aureus* CIP7625 (Sa) and *Listeria monocytogenes* Scott A724 (Lm) and three Gram-negative, *Escherichia coli* ATCC10536 (E coli), *Klebsiella pneumonaea* CIP8291 (Kp) and *Salmonella enterica* CIP833 (Se) were used. Two yeasts *Sacharomyces cerevisiae* ATCC 4226A (Sc) and *Candida albicans* IPA 200 (Ca) and three fungi *Mucor ramannianus* ATCC 9314 (Mr), *Aspergillus parasiticus* CBS 100926(Ap) and *Fusarium culmorum* NRRL 3288(Fc) were also tested.

All strains were obtained from the Laboratory of Chemical Engineering, Bioprocess Systems Microbial Department of High National School of Agronomy, Toulouse, France.

Discs diffusion method

The paper disc diffusion method was employed for the determination of essential oil antimicrobial activity (Ennajar *et al.*, 2009). A suspension of the tested microorganism (concentration to 4 10^6 cells/mL) was spread on nutrient agar. We take 600 µL/200 mL for the bacteria and yeasts and 1 mL/200 mL for the fungi. The filter discs (9 mm in diameter) were imbibed with 15 µL of essential oil and placed on the inoculated plates. The plates were then covered with parafilm, finely punched to allow the circulation of air without allowing the mites to escape.

Ampicillin and nalidixic acid (0.5-20 mg/L) were used as positive reference standards to determine the sensitivity of Gram-positive, Gram-negative bacterial species tested, respectively. Nystatin (0.5-20 mg/L) was used as positive reference standards to determine the sensitivity of fungi and yeasts species.

After staying at 4 °C for 2 h, plates were incubated for 48 h (at 37 °C for bacteria and at 30 °C for yeasts and fungus). Antimicrobial activity was evaluated by measuring the zone of inhibition (in millimeters) against the test organism. Tests were carried out in triplicate. Values are presented as means± SD of three parallel measurements.

Oils minimal inhibitory concentration (MIC) values were determined based on the agar dilution method. 15 concentrations of each essential oil were essayed (0.5, 1, 2, 3, 4, 5, 10, 15, 20, 30, 40 and 50 µL/mL). The dilutions of essential oils were prepared in the methanol, the Tween 80 to 10% and 3 mL of nutrition's solid media. Bacterial suspensions of10^6 bac/mL and of 10^5 of yeasts and fungi were prepared and 1 µL was deposed on the solid environments already inoculated by the various microorganisms' tests and then incubated for 48 h (at 37 °C for bacteria and at 30 °C for yeasts and fungi). The same concentrations of every witness and of pure methanol were prepared and dissolved in the Tween 80 to 10% before tested.

Statistical analysis

All data were expressed as means± standard deviations of triplicate measurements. The confidence limits were set at $P < 0.05$. Standard deviations (SD) did not exceed 5% for most of the values obtained.

Results

Chemical composition

Analysis of the Tunisian *L. nobilis* essential oil revealed forty-two components representing 99.30% of the total volatiles (Table 1). The main components were oxygenated monoterpenes (73.1%). Camphor (34.43%) was the major compound followed by 1,8-cineole (20.21%), α-terpineol (7.19%), borneol (6.75%) and α-pinene (6.47%). Monoterpene hydrocarbons represent 10.8% of the total oil. However, sesquiterpenes hydrocarbons and sesquiterpenes oxygenated presented only by 4.42% and 2.19% of total essential oil, respectively (Table 1).

Table 1. Chemical composition (peak area (%)) of essential oils from *L. nobilis* leaves

Compounds	KI	Tunisia oil	France oil	Austria oil
β-pinene	974	0.3	0.1	0.1
α-pinene	936	6.5	1.7	2.0
α-fenchene	951	0.5	-	-
camphene	954	0.5	-	-
Sabinene	976	2.7	0.3	0.3
β-myrcene	985	0.1	-	-
p-cymene	1025	0.3	-	-
Limonene	1028	-	2.9	3.8
α-phellandrene	1030	-	0.1	0.2
1,8-cineole	1030	20.2	45.8	43.4
eucalyptol	1038	0.5	-	-
γ-terpinene	1057	0.3	0.3	0.3
d-terpinene	1057	-	0.1	0.1
α-terpinolene	1086	-	1.0	0.4
Linalool	1094	1.4	-	-
Fenchol	1108	0.1	-	-
α-campholenal*	1128	0.2	-	-
cis-verbenol	1140	0.1	-	-
trans-2- menthenol	1141	-	0.1	0.1
Camphor	1143	34.4	-	-
β-terpineol	1159	0.1	0.2	0.2
Borneol	1166	6.7	0.3	0.2
pinocarvone	1168	0.3	-	-
terpinen-4-ol	1178	1.6	1.7	1.3
α-terpineol	1185	7.2	5.7	2.1
verbenone	1210	0.2	-	-
Geraniol	1242	-	0.1	0.1
bornyl acetate	1285	5.0	13.8	17.7
Thymol	1290	0.6	-	-
carvacrol	1302	0.4	-	-
4-terpenyl acetate	1340	-	1.0	0.4
Eugenol	1350	0.1	2.8	2.9
neryl acetate	1376	0.4	0.8	0.5
α-copaene	1388	-	0.2	0.3
β-elemene	1389	0.1	1.5	0.8
methyl eugenol	1406	2.0	7.7	10.9
β-caryophyllene	1415	0.4	0.2	1.4
β-gurjunene	1432	0.3	-	-
terpinyl propionate	1435	0.1	3.8	3.2
α-guaiene	1440	-	0.4	0.6
α-himachalene	1450	0.1	1.3	1.1
Ethyl*trans*-cinnamate	1460	-	0.4	0.3
γ-muurolene	1480	0.2	0.2	-
germacrene D	1480	0.2	-	-
β-selinene	1482	2.6	1.7	1.0
calamenene*	1512	0.2	-	-
Cubenol	1514	0.3	-	-
δ-cadinene	1524	0.1	0.2	1.1
α-cadinene	1534	0.2	0.5	0.4
β-caryophyllene oxide	1580	1.6	0.2	-
elemicine*	1582	-	0.7	1.1
α-cedrol	1596	0.3	-	-
β-eudesmol	1650	-	1.4	0.4
nootkatone*	1804	-	0.2	-
Total		**99.3**	**99.7**	**98.9**
Monoterpene hydrocarbons		10.8	6.7	7.2
Monoterpenes oxygenated		73.1	54.0	47.4
Sesquiterpenes hydrocarbons		4.4	6.3	6.7
Sesquiterpenes oxygenated		2.2	1,8	0.4
Phenolics		1.1	2.8	2.9
Others		7.8	28.2	34.2

*: Tentatively identified supported by good match of MS. -: Not determined.

Thirty-five components representing 99.7% of the total volatiles were identified for France *L. nobilis* essential oil. The main components were oxygenated monoterpenes (54%). 1,8-cineole (45.8%) was the major compound followed by bornyl acetate (13.8%) and methyl eugenol (7.7%). Monoterpene hydrocarbons represented only 6.7% of the total, when sesquiterpenes hydrocarbons and sesquiterpenes oxygenated were lowly represented (6.3 and 1.8%, respectively) (Table 1).

Thirty-one components, representing 98.9% of the total volatiles, were identified for Austrian *L. nobilis* essential oil and oxygenated monoterpenes (47.4%) were the abundant. The prominent component in *L. nobilis* essential oil from Austrian was 1,8-cineole (43.4%), bornyl acetate (17.7%) and methyl eugenol (10.9%). Monoterpene hydrocarbons (7.2%) and sesquiterpenes hydrocarbons were not mostly present (Table 1).

Antioxidant activity

We have investigated free radical scavenging activity, of the three *L. nobilis* essential oils by ABTS assay (Table 2). The ABTS was not tested for *L. nobilis* leaves. Tunisian *L. nobilis* essential oil (mixture of compounds) included a high activity (IC_{50}= 44.8± 0.1 mg/L) to scavenge ABTS radical cation comparing with standards (1.9± 0.1 mg/L). Although the ABTS radical scavenging activity of France and Austrian essential oils (IC_{50}= 76.4± 3.2 mg/L and IC_{50}= 81.4± 4.0 mg/L, respectively) were significant but less important compared to Tunisian essential oil.

Table 2. Antioxidant activity of *L. nobilis* leaves essential oils

Samples	ABTS IC_{50} (mg/L)	DPPH IC_{50} (mg/L)
L. nobilis - Tunisia	44.8± 0.8	2859.7± 99.0
L. nobilis - France	76.4± 3.2	176.1± 5.1
L. nobilis - Austria	81.4± 4.0	236.3± 2.9
Vitamin C	1.9± 0.1	4.4± 0.2

By DPPH assay, results were in opposition to those obtained with ABTS assay (Table 2). Therefore, Tunisian *L. nobilis* essential oil (IC_{50}=2859.7± 99.0 mg/L) were feebly active and a moderate antioxidant activity were determined for France (IC_{50}= 176.1± 5.1 mg/L) and Austrian (IC_{50}= 236.3± 2.9 mg/L) essential oils compared to activity of standard (4.4± 0.2 mg/L).

Antimicrobial activity

Antimicrobial activity of *L. nobilis* essential oils was evaluated against 3 Gram-positive bacteria, 3 Gram-negative bacteria, 3 fungi and 2 yeasts species by zone diameter (Table 3). These micro-organisms are morphologically and physiologically different.

Tunisian *L. nobilis* essential oil (15 µL) exhibited a great potential for antimicrobial activities against the tow fungi: *M. ramamnianus* (43 mm of inhibition zone) and *A. parasiticus* (24 mm of inhibition diameter) and yeasts species (*C. albicans* and *S. cerevisiae* with 26 mm of inhibition zone) (Table 3). The strongest activity for bacterial strains sensitive to Tunisian laurel oil was observed against *S. aureus* (gram+bacteria) with 23 mm of inhibition zones more than that of antibiotic (Table 3). Activity against *B. subtilis* and *L. monocytogenes* (Gram+ bacteria) is moderate and it attends respectively 20 and 17 mm of inhibition zone. A low sensitivity of *K. pneumonia* and *S. enterica* (Gram-negative bacteria) (Table 3) comparing with standard one (10 mm of inhibition zone). However, *E. coli* (gram-bacteria) is insensitive to Tunisian *L. nobilis* essential oil. We can say that Gram positive bacteria are significantly more sensitive to Tunisian *Laurus nobilis* oil. However, gram negative bacteria are low sensitive to this oil as perhaps their resistance mechanism that manifests itself in the structure of their bacterial envelope (Moghtader and Farahmand, 2013) as well as in their enzymatic arsenals.

France commercialized essential oil reacted efficiency with fungi microorganisms: *M. ramamnianus* (43 mm of inhibition zone), *A. parasiticus* (28 mm of inhibition diameter) and *F. Culmorum* (ZI: 26 mm) compared to standard test (Table 3). The maximal inhibition zones for bacterial strains sensitive to the France laurel oil was also *S. aureus* (gram- positive bacteria) (30 mm of inhibition zone >20 mm standard zone). Activity against the two others gram positive bacteria as *L. monocytogenes* (ZI: 20 mm) and *B. subtilis* (ZI: 12 mm) are less important. The weakest activity was also observed against gram-negative bacteria (ZI: 10-13 mm) with a feebly sensitivity to E coli (ZI: 10mm). Activity of France laurel oil against yeasts species (ZI: 17- 20mm) were relatively low then that of standard and that noted for Tunisian laurel leaves oil.

Important activity of Austrian laurel leaves oil against fungi*: M. ramamnianus* (ZI: 38 mm) and *F. culmorum* (ZI: 20 mm) are also distinguished compared to standard (31 and 24mm respectively). However, *A. parasiticus* (fungi) is insensitive to this oil. Yeasts activities were also important then standard ones for *C. Albicans* (with ZI: 35) and *S. cerevisiae* (23 mm).

Table 3. Antimicrobial activity of *L. nobilis* essential oil

Zones of inhibition (mg/mL)				
Laurus nobilis	**Tunisia**	**France**	**Austria**	**Standards**
Gram positive bacteria				Ampicillin
B. subtilis	20	12	17	54
S. aureus	23	30	23	20
L. monocytogenes	17	20	16	31
Gram negative bacteria				Nalidixic acid.
Echerchia coli	0	10	10	30
K. pneumoniae	10	12	10	28
S. enterica	10	13	10	28
Yeast				Nystatin
S. cerevisiae	26	17	23	29
C. albicans	26	20	35	30
Fungi				Nystatin
M. ramamnianus	43	43	38	31
A. parasiticus	24	28	0	24
F. culmorum	0	26	20	30

"–": Absence of inhibition zone detected. Essential oil concentration:
15 µL/disc; reference standards concentration: 0.33 mg/L.

The maximal inhibition zones for bacterial strains sensitive to the Austrian oil was also noted for *S. aureus* (gram-positive bacteria) (23 mm of inhibition zone) while activity against *B. subtilis* and *L. monocytogenes* (ZI: 17 and 16 mm respectively) are not significant compared to standard ones (25 and 35 mm). Activity against gram-negative bacteria is relatively insignificant as France and Tunisian oils in comparison with standard (ZI: 10 mm).

CMI of gram-positive bacteria of Tunisian *L. nobilis* essential oil was very important (0.01 mg/ml) compared to standard ones (0.02 mg/ml) and to France and Austrian activities (Table 4) and they confirm result obtained by screening test.

Although gram-negative bacteria tested found to be very sensitive to Tunisian *L. nobilis* oil (CMI: 0.004 mg/ml) in comparison with standard test (0.01 to 0.02 mg/ml) they present a non-significant screening test. CMI noted for yeasts species (*C. albicans* and *S. cerevisiae*) (0.004 and 0.005 mg/ml respectively) and fungi tested (*M. ramamnianus* and *A. parasiticus*) (0.003 to 0.004 mg/ml) proved a stronger activity confirm result obtained by screening test. *F. culmorum* and *E. coli* exhibit important value of CMI (0.004 mg/ml) nevertheless they appeared to be inactive in screening test (Table 3).

France essential oil exhibited an interesting antimicrobial activity against all microorganisms tested proved by CMI tests (0.003 to 0.004 mg/ml) (Table 4). These results prove the screening test only for *M. ramamnianus* (fungi) and *S. aureus* (gram-positive bacteria).

Table 4. Antimicrobial activity (MIC (mg/mL)) of *L. nobilis* essential oils

Zones of inhibition (mg/mL)				
Laurus nobilis	**Tunisia**	**France**	**Austria**	**Standards**
Gram-positive bacteria				*Ampicillin.*
B. subtilis	0.01	0.004	0.005	0.02
S. aureus	0.01	0.004	0.01	0.02
L. monocytogenes	0.01	0.004	0.01	0.02
Gram-negative bacteria				*Nalidixic acid*
Escherichia coli	0.004	0.004	0.005	0.02
K. pneumoniae	0.004	0.004	0.004	0.02
S. enterica	0.004	0.004	0.01	0.02
Yeast				*Nystatin*
S. cerevisiae	0.005	0.004	0.004	0.02
C. albicans	0.004	0.003	0.004	0.02
Fungi				*Nystatin*
M. ramamnianus	0.003	0.003	0.004	0.02
A. parasiticus	0.004	0.004	0.01	0.02
F. culmorum	0.004	0.004	0.004	0.015

The antimicrobial properties of essential oils from Austrian were significant also by CMI concentration which ranged from 0.01 mg/ml (for *S. aureus, L. monocytogenes* and *S. enterica)* to 0.005mg/ml (*B. subtilis and E. coli*) antifungal activity are also important (0.004 mg/ml for *M. ramamnianus* to 0.01 mg/ml *for A. parasiticus*). These results agree with screening test only for *S. aureus, C. albicans* and *F. culmorum.*

CMI concentration of all microorganisms are relatively effective in comparison with standards ones (Table 4), so we can conclude that essential oil of *L. nobilis* from Tunisian, France and Austrian have a good antimicrobial activity (Table 4).

Discussion

Tunisian *L. nobilis* essential oil was richer in terpenes (42 components) for the most part by oxygenated monoterpenes (73.1%) then France (54%) and Austrian (47.4%) essential oils. Monoterpene hydrocarbons were also relatively higher in Tunisian laurel oil (10.8%) then France (6.7%) and Austrian (7.2%) ones. In Tunisian laurel oil, camphor is the major component added to 1,8-cineole. This result is original and it make a difference with France and Austrian oil how the 1,8-cineole only is the more represented component. This difference can be attributed to bioclimatic and ecological conditions of three different provenances. camphor has a long tradition of use as antiseptic, antipruritic, rubefacient, abortifacient, aphrodisiac, contraceptive and lactation suppressant (Ashish and Aggarwal, 2007). It has also antifumigant and insecticidal effect (Quintai and Yongcheng (1998). The natural form of camphor (D-camphor) was not harmful (Belzt *et al.*, 2003). A few Studies of pharmacological interactions between camphor and other compounds are present in the literature (Tassell *et al.*, 2010).

L. nobilis leaves essential oils from all over the world were richer in 1,8-cineole (chahal et al., 2017). However, Linool, α-terpinol, α- terpenyl acetate and thymol were also revealed as major component (chahal et al., 2017). Camphor is present in essential oil of Tunisian *Laurus nobilis* (Ben Jemâa *et al*, 2012) bat it was not previously found as a major component.

Many studies have determined the potential antioxidant activity of various extracts (lives and seeds) (Chahal *et al.*, 2017) but a little information is available about the antioxidant leaves essential oil (Bouzouita *et al.*, 2009; Saab *et al.*, 2012). Antioxidant activities for Tunisian laurel are essayed only in floral buds' essential oil by B-carotene bleaching test and DPPH radical scavenging method (Bouzouita *et al.*, 2009).

In many reports, the antioxidant performance of essential oils was due to the presence of phenolic compounds (Bouzouita *et al.*, 2009; Mkaddem *et al.*, 2010; Ozen *et al.*, 2011 ; Goudjil *et al.*, 2015).The lowest antioxidant activity of Tunisian *Laurus nobilis* essential oil with DPPH test (IC_{50}= 2859.7 ± 99.0 mg/L) can be explained by the fact that the phenolic compounds in the essential oils are low (1.1% of total oil represented by carvacrol, thymol and eugenol).While the ABTS assay involved other anti-radical cationic compounds. The percentage of components with hydroxyl function (aromatic and non-aromatic) in Tunisian *L. nobilis* essential oil is 21.6% (Table 1). In fact, especially hydroxyls of tertiary carbon (terpinen-4-ol, linalool, β-terpineol…) forms a radical presenting an acceptable stability even if it is less stable that the phenolic hydroxyls. In France essential oil, components with hydroxyl function represent 20% of total essential oil when they represent only 18.1% in Austrian essential oil (Table 1).

Our sample shows an important variation in the essential oil composition in comparison with other sample from Tunisia due to geographical bioclimatic factors, age of plant and the season of harvest. It represents a new chemotype.

We noted a various antimicrobial activity due to the nature of microorganisms tested and to origins of plants and composition of the essential oil. In general, all laurel oils tested in our work were more active against Gram-positive than Gram-negative bacteria. *S. aureus* (gram+bacteria) is the most sensitive strain as proved by literature (Derwich *et al.*, 2009) and *M. ramamnianus* was the most sensitive fungi to laurel oil. However, the most resistant strains were *E. coli* (gram- bacteria). These results are different from those obtained forTunisian laurel leaves essential oil with 1,8 cineole as major component how E*. coli* was revealed the most sensitive bacteria (Bouzouita *et al.*, 2003).

This is the first study to report activities of *L. nobilis* essential oil against yeasts species. *C. albicans* and *S. enterica* (26 mm and CMI ringing from 0.004 to 0.005 mg/ml) and fungi species *M. ramamnianus* and *A. parasiticus* (43 and 24 mm and CMI of 0.003 and 0.004 mg/ml respectively). These activities were important and it may be related to major compounds of the essential oil (camphor and 1,8-cineole) or to synergic effect between them. However, 1,8- cineole, has been known to exhibit antimicrobial activity against the bacterial strains (*E. coli, P. aeruginosa, S. typhi, Staphylococcus aureus, Staphylococcus intermedius, Bacillus subtilis*) (Saab *et al.*, 2012) but there is no previous study that reported the activity of purified camphor components.

Conclusions

In recent years, the international demand for labelled and certified products is growing. The objectives of this study were: to orient seriously the choice of the essential oils replaying to the requirements of the markets, amplify their usage domains and envisaged conservation and improvement of variety with new specific essential oil composition suitable to pharmaceutical and industries food processing.

The results in this work showed that Tunisian laurel leaf essential oil represented a new chemotype of camphor and 1,8 cineole. Essential oils from France and Austrian were reached by 1,8-cineole. Ecological factors (climatic and soil conditions) have strong influence on the essential oil content (Bisio *et al.*, 1999; Sangun *et al.*, 2007)

A good antioxidant activity to scavenge ABTS radical cation was determined for Tunisian essential oil (IC_{50}= 44.8± 0.1 mg/L). This result is cited for the first time and it gives the opportunity for uses Tunisian *L. nobilis* essential oil in pharmaceuticals industries. Therefore, Tunisian *L. nobilis* essential oil was feebly active against DPPH radical cation and moderate antioxidant activities were determined for France and Austrian leaves essential oils.

Tunisian *L. nobilis* essential oil exhibited a great potential for antimicrobial activities against fungi (*M. ramamnianus* and *A. parasiticus*) and yeasts species (*C. albicans* and *S. cerevisiae*). Gram (+) bacteria (*S. aureus* and *B. subtilis*) is more sensitive to all *L. nobilis* essential oil tested then gram (-) ones. This result was proved by screening test and CMI concentration.

France commercialized essential oil shows good activities against fungi (*M. ramamnianus*). However, sensitivity of gram (+) bacteria (S*. aureus* and *L. monocytogenes*) was more important than that of gram (-) ones. Important activity of Austrian leaves essential oil against *F. culmorum* (fungi), *C. albicans* (yeasts) and *S. Aureus* (gram (+) bacteria) are also noted by screening and CMI testes.

Austrian essential oil is similar to Tunisian and France essential oils to inhibit activity of gram (+) bacteria (essentially *S. aureus*) more than antibiotics.

We concluded that Tunisian *L. nobilis* essential oil has a good activity against fungi, yeasts, and bacteria globally different from France and Austrian commercialized essential oil. This significant difference is probably due to variability in their composition (major and minor components) (Derwich *et al*., 2009; Dhifi *et al*., 2018). Results concerning antimicrobial and antioxidant activities of Tunisian *L. nobilis* were important and different from commercialized essential oil and it give the opportunity to better use Tunisian *L. nobilis* essential oil as a new natural and effective antimicrobial agent in conservation, pharmaceuticals, natural therapies of infectious in human, and management of plant diseases.

Authors' Contributions

Conceptualization: MR; Visualization, Methodology and Supervision: JB; Methodology and formal analysis AL and FM. All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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