ORIGINAL ARTICLE

CHEMICAL COMPOSITION, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY OF ESSENTIAL OIL OF LAVANDULA DENTATA GROWING WILD IN MOROCCO

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ABSTRACT

Backgound: Lavandula species are one of the most popular aromatic plants and are well-known as herbal medicines with a number of useful biological properties. **Objective**: To investigate the chemical composition, antioxidant and antibacterial activity of essential oil of lavandula dentate a number of tests were carried out. **Methods**: The essential oil, was obtained by hydrodistillation, with a 1.4±0.01 (w/w) yield, then the chemical composition was analyzed by gas chromatography coupled with mass spectrometry (GC-MS). The antimicrobial activity of the essential oil evaluated against sex microorganisms (E. Coli, Listeria, S. Aureus, B. Ceraus, P. Aeruginosa and Salmonella Enteritidis) using paper disc diffusion method. The anti-free radical activity was assayed using DPPH• and ABTS•+ methods and phosphomolybdenum methods. **Results**: The results of the GC-MS showed the presence of 31 components, The main components are Carvacrol (44.38%), beta-Bisabolene (24.82%), Caryophylleneoxide (3.36%), Camphene (2.92%) and Spathulenol (2.44%). The essential oil inhibited the growth of all tested bacteria except for P. Aeruginosa, and the minimum inhibitory concentration was determined to be around 4.6–92µg/mL. The level of antioxidant activity estimated by DPPH was (IC50 = 70±1.03µl/ml) and ABTS was (0.032±0.0006 Equi mg trolox/ml DW) and Phosphomolybdenum was (4.66±0.007 Equi TROLOX mg/ml). **Conclusion**: The results of this study showed that essential oil of lavandula dentate present an interesting antioxidants and antimicrobial activity, which could be used as a potential source of natural antioxidants and bioactive molecules and could present a very promising alternative in the field of antimicrobial applications.
Keywords: *lavandula dentata, Essential oil, Chemical Composition, Antioxidant activity, Antimicrobial activity.*

1. INTRODUCTION

The therapeutic virtues of aromatic essences have been known since antiquity [1, 2]. However, interest in the scientific study of the power of aromatic and medicinal plants has only increased in recent decade. Essential oils can be defined as complex mixtures of volatile organic compounds produced as secondary metabolites of plants [3]. Monoterpenes and sesquiterpenes are the main constituents of essential oils. They are synthesized through condensations of the universal five-carbon precursors isopentenyldiphosphate (IPP) and dimethylallyldiphosphate (DMAPP), which derive from two independent pathways [4]. Several studies have demonstrated the different biological activities of these aromatic and medicinal plants, especially antifungal, antibacterial, antioxidant and insecticidal [5-9]. Therefore, this research is set up with the aim of seeking alternatives to chemicals that present risks to human health and to the environment. Antioxidants have been widely used as food additives to provide protection against oxidative degradation of foods by free radicals [10, 11].

Lavandula (lavender) genus (Lamiaceae) is an aromatic plant that contain considerable amount of essential oil, It is native to the Mediterranean region south to tropical Africa and to the southeast regions of India. The genus includes annuals, herbaceous plants and small shrubs, having aromatic foliage and flowers. It is cultivated in France, Spain and Italy. Among them, the most common species believed to have medicinal value are Lavandula dentata, Lavandula angustifolia, Lavandulalatifolia, Lavandula intermedia, Lavandula stoechas and Lavandula dhofarensis [12]. Qualitative studies on composition of lavender oil have shown that in contains considerable amounts of linalool, linalyl acetate, 1,8-cineole and camphor, with a number of useful biological properties [13, 14]. lavender il is predominantly used in aromatherapy as a relaxant and as a carminative and sedative agent [14].

The aim of this study is to extend our knowledge about chemical constituents of antioxidant and antimicrobial activity of essential oil from lavandula dentate.

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MATERIALS AND METHODS

1.1. Plant material

The aerial part of Lavandula dentata was harvested in May 2016 in the wild in Al Hoceima National Park in the Nord of Morocco. A voucher specimen was deposited in the herbarium of the Faculty of Sciences, Oujda, Morocco.

1.2. Chemicals: Chemicals DPPH (1,1-diphenyl-2-picrylhydrazyl), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and ABTS(2,2_-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid)) were purchased from Sigma–Aldrich (St. Louis, MO). All solvents and reagents used were of the highest purity.

1.3. Essential oil isolation: The dried vegetal material was submitted to hydrodistillation for 3 h using Clevenger-type apparatus. Anhydrous sodium sulfate was used to dry the essential oil, which was stored at 4°C in the dark tell analysis.

1.4. Gas chromatography–mass spectrometry: GC–MS analysis methods were used to determine the composition of the essential oils. The analysis was carried out using a GC/MS-QP 2010 (Shimadzu, Kyoto, Japan) and a Shimadzu GC-2010 Plus gas chromatograph.BPX25 capillary column (30mm.0,25 mm, ef = 0,25µm film thickness, Restek, Bellefonte, USA)was used. Helium was the carrier gas at 1 mL/ minutes with following temperature program: at 50 °C for 1 minute, increased to 250 °C at the rate of 10 °C /minutes held for 1 minute. Samples were injected at a temperature of 250 °C with a split ratio of 1/50 during 1 minute. An electron impact ionization system with ionization energy of 70 eV and electron ionization spectra with a mass scan range of 30-500 m/z was used.

The identification of the compounds was based on the comparison of their retention indices (KI), their retention times (RT) and mass spectra with those from the NIST147 and NIST27 libraries and the pure standard.

1.5. Antioxidant activity

1.5.1. Free radical-scavenging activity: The ABTS radical cation (ABTS•+) bleaching assay was used. ABTS•+ was produced by reacting equal volumes of 7 mM of ABTS with 2.5 mM potassium persulfate and leaving the mixture in the dark at room temperature for 12–16 h before use. The ABTS•+ solution was diluted (1:100) with ethanol to 0.035 mM with an absorbance of 0.7 ± 0.01 at 734 nm. After reaction at room temperature for 6 min, the absorbance at 734 nm was measured using Rayleigh - Model UV1800 V/VIS – Spectrophotometer. Trolox was used as a reference to express the results in (mg Trolox /ml DW). [15]

The DPPH (1,1-diphenyl-2-picrylhydrazyl) assay were used for determination of the antiradical activity of the essential oil. 600µL of various dilutions of the test essential oil were mixed with 2600µL of a methanolic DPPH solution (0.004 %). After an incubation period of 30 min at 25 °C, the absorbance at 517 nm was recorded as $\mathbf{A}_{(\mathbf{sample})}$. A control experiment was also carried out by applying the same procedure to a solution without the test material and the absorbance recorded $\mathbf{A}_{(\mathbf{blank})}$. The free radical scavenging activity of each solution was then calculated as percentage inhibition according to the following Eq(1):

$$\%\ \mathbf{inhibition} = \mathbf{100} \times \left[\left(\mathbf{A}_{(\mathbf{blank})} - \mathbf{A}_{(\mathbf{sample})}\right)/\mathbf{A}_{(\mathbf{blank})}\right] \qquad (1)$$

Extract antioxidant activity was expressed as IC50, defined as the concentration of the test material required to cause a 50% decrease in initial DPPH concentration. Values were estimated using linear regression. Galic acid was used as a reference.

1.5.2. Molybdenum method

The total antioxidant capacity was evaluated by the method of Prieto, Pineda, and Aguilar (1999).An aliquot of 0.3 ml of sample solution was combined with 1 ml of reagent solution (0.6Msulfuric acid, 28mMsodium phosphate and 4 mM ammonium molybdate). For the blank, 0.3 ml of methanol was used in place of sample. The tubes were incubated at 95 °C for 90 min. After the samples had cooled to room temperature, the absorbance measured at 695 nm against a blank in a Rayleigh - Model UV1800 V/VIS - Spectrophotometer. Antioxidant capacity was expressed as equivalents of TROLOX.

1.6. Antibacterial activity

1.6.1. Test microorganisms and preparation of inoculum: Antibacterial activity of essential oils was tested by the paper disc diffusion method using 6 strains of Gram-positive and Gram-negative bacteria: E. Coli ATCC 25922, S. Aureus ATCC25923, Listeria, B. Ceraus ATCC1177B, P. Aeruginosa ATCC 27853 and Salmonella Enteritidis ATCC13076. Reference strains originated from the American Type Culture Collection (ATCC, Manassas, VA, USA). Streptomycein 10µg (MST-S10C), Gentamicin 10µl(BIO-RAD), Erythromycin 15µg (MST-E15C), were used as positive controls.

The culture mediums we used are Mueller Hinton. It was melted in a water bath at 95◦C, and then a was poured into Petri dishes of 90 mm diameter at a rate of 20 ml per box and was cooled and solidified on the bench.
The bacteria were grown on PCA (Plate Count Agar) for 18–24 h. Then the isolated colonies of each bacteria were transferred to tubes containing sterile saline (0.85%), the bacterial suspension was homogenized to achieve a density equal to 0.5 McFarland.

1.6.2. Disc diffusion method (method aromatogram): A sterile disc of 6 mm was laid down on the surface of agar inoculated previously with 0.1 ml of microbial suspension. Then, a drop of 20 µl of essential oil was put on the disks, and was left to disseminate for 30 min. The petri dishes were incubated at 37◦C for 18–24 h. The reading is taken by measuring the diameter of the inhibition zone around each disc using a caliper. The results were expressed by the diameter of the inhibition zone depending on the sensitivity of the strains against essential oil.

1.6.3. The minimum inhibitory concentration (MIC): The minimum inhibitory concentration (MIC) value was defined as the lowest concentration of essential oil, in which no visual growth of bacteria was noted. The MIC of the essential oil was determined using the microdilution broth method, in96-well microplates. Inocula of the different strains were obtained from a preculture of 18 hour the microbial load was adjusted to 10^6CFU mL^{-1}using a 0.5 McFarland turbidity standard. Plates containing 96 wells were prepared by dispersing, into each well, volumes from 175 to 194 µl of Mueller Hinton broth, and from 1 to 20 µl of sample of essential oil, then 5 µL of inoculum were poured into the wells to obtain a final volume of 200 µL, in each well. The microplates were covered with parafilm and incubated at 37 °C for 24 h. Inhibition of bacterial growth was confirmed by the addition of 20 µl of the aqueous solution of resazurin (0.02%) and re-incubation for 3 h. A change of colour from blue to red indicated the presence of live microorganisms [16].

1.7. Statistical analysis: The experiment was carried out with three replications for each test. Statistical analysis was performed using SPSS17 at the significance level $P < 0.05$.

2. RESULTS AND DISCUSSION

2.1. Yield and composition of Essential oil

The yield of lavandulala dentate essential oil was 1.4 ± 0.01% (w/w). A total of 31 components were identified using GC-MS. The main compounds were Carvacrol (44.38%), beta.-Bisabolene (24.82%), Caryophyllene oxide(3.36%), Camphene (2.92%) and Spathulenol (2.44%). The constituents identified by GC–MS analysis, their retention indices and area percentages (concentrations) are summarized in Table 1.

The main components in the oil of the aerial part and flowers of *L. dentata* in eastern Morocco were , respectively, β-pinene (27.08 %, 30.06 %), pinocarveol (14.77%, 8.59 %), myrtenal (8.18 %, 6.81 %), α-pinene (7.78 %, 1,8%), cineole (5.53 %, 5.47 %), linalool (4.7 %, 4.46 %), pinocavone (2.36 %, 2.44 %) and borneol (2.56 %, 1.66 %). In Tunisian, the main Constituents for *Lavandula dentata essential oil* were 1,8-cineole, camphor and L - fenchone, accounting for 33.54, 18.89 and 8.36 % in the leaf oils and 19.85, 23.33 and 7.13 % in the flower oils, respectively. A study of the influence of plant growth regulators on the essential oil content, concluded that, quantitative changes in the major monoterpene components (1,8-cineole, fenchol, borneol and camphor) and sesquiterpene content of plantlet oil, were observed in response to the effect of varying growth regulator concentration in the culture medium of cultured Lavandula dentata plantlets [17].

Tableau 1: Table presents the chemical constituents of the essential oil of L. dentate.

	Compound	Kovats RI	Composition (%)
1	alpha.-Pinene	385	1.97
2	Camphene	466	2.92
3	beta.-Pinene	563	1.10
4	beta.-Myrcene	592	2.23

5	3-Carene	638	0.60
6	D-Limonene	707	2.18
7	cis-.beta.-Ocimene	736	1.01
8	beta.-Cymene	747	0.73
9	Cineole	755	1.49
10	Linalool	927	1.36
11	Fenchone	966	0.46
12	2-Phenyldodecane	1010	0.21
13	Camphor	1131	0.44
14	alpha Terpineol	1182	0.60
15	Methylthymylether	1248	0.59
16	Carvacrol	1417	44.38
17	Caryophyllene	1518	1.25
18	beta.-Damascenone	1545	0.29
19	beta.-Bisabolene	1653	24.82
20	Undecane, 2,8-dimethyl	1685	0.16
21	delta.-Cadinene	1699	0.27
22	Phenol, 2,4-di-tert-butyl-	1718	0.28
23	Spathulenol	1859	2.44
24	Caryophylleneoxide	1870	3.36
25	Farneseneepoxide, E	1888	0.51
26	alpha.-Cadinol	1979	0.22
27	alpha.-Bisabolol	1994	1.48
28	Isobutylphthalate	2312	0.26
29	Dibutylphthalate	2431	0.29
30	Tetracosamethyl-cyclododecasiloxane	2518	1.95
31	Cyclononasiloxane, octadecamethyl	2546	0.18

2.2. Antioxidant activity

Table.2 illustrated the antioxidant capacity determined by DPPH and ABTS and Molybdenum method. The level of antioxidant activity estimated by DPPH was (IC50 = 70±1.03µl/ml) and ABTS was (0.032±0.0006 Equi mg trolox/ml DW) and Phosphomolybdenum (4.66±0.007 Equi TROLOX mg/ml).The total antioxidant capacity, based on the ABTS, DPPH assays and Phosphomolybdenum method revealed that the essential oil of L. dentata exhibit a moderate inhibitory potential.

The level of antioxidant activity estimated by DPPH for the aerial part of *L. dentata* oil in eastern Morocco was IC 50 value of 32.12µL/mL. [17].

Tableau 2: table presents the total antioxidant capacity determined by DPPH and ABTS and molybdenum method. Values are given as mean ± SD.

	DPPH IC50 (µl/ml)	**ABTS (Equi mg trolox/mlDW)**	**Phosphomolybdenum (Equi TROLOX mg/ml)**
Essential oil of lavandula dentata	70 ± 1.03	0.032 ± 0.0006	4.66 ± 0.007

DPPH: 1,1-diphenyl-2-picrylhydrazyl; **ABTS:** 2,2_-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid

2.3. Antibacterial activity

The essential oil was evaluated for antimicrobial activity against gram positive and gram negative bacteria. It was found to be active against most of the tested microbes. The essential oil was very active against S. Aureus, B. Ceraus and Salmonella Enteritidis moderately active against Listeria and E. Coli and showed no activity against P. Aeruginosa. The essential oil showed a highest or similar biological activity to antibiotic discs. The minimum inhibiting

concentration (MIC) of essential oil ranged from 4.6 to 23g/ml. These differences in the susceptibility of the test organisms to essential oil could be attributed to a variation in the rate of the essential oil constituent's penetration through the cell wall and cell membrane structures. The ability of essential oil to disrupt the permeability barrier of cell membrane structures and the accompanying loss of chemiosmotic control are the most likely reasons for its lethal action.

The oil of the aerial part of *L. dentata* in eastern Morocco had a substantial inhibitory effect on all assayed bacteria strains [17]. Gram-positive *Listeria monocytogenes* was the most sensitive strain followed by *Streptococcus* sp. The oil also exhibited high antimicrobial activity against *Streptococcus pneumoniae* but modest activity against *Staphylococcus aureus.* Gram-negative strains also displayed variable degree of susceptibility against investigated oil. Maximum activity was observed against *Neisseria meningitides* and *Haemophilus infl uenzae*, followed by *Klebsiella pneumoniae*, *Salmonella* sp., *Proteus mirabilis*, *Pantoea* sp. *and Enterobacter cloacae*. Modest activities were observed against important food pathogens such as *Escherichia coli* [17].

Tableau 3: Table presents the antibacterial activity of L. dentata essential oil estimated by diameter of inhibition zone (including the disc diameter, 6 mm) and minimum inhibitory concentration (MIC).

	E. Coli	Listeria	S. Aureus	B. Ceraus	P. Aeruginosa	SalmonellaEnteritidis
HE L. dentate	15	22	68	40	-	35
Streptomycein	24	30	32	20	17	28
Gentamicin	26	42	38	26	31	35
Erythromycin	12	43	40	12	32	09
MIC HE L. dentate (µg/ml)	23	4.6	23	4.6	-	23

MIC: minimum inhibitory concentration.

3. CONCLUSIONS

In this study, we investigate the chemical profile and biological activity of the essential oil of lavandula dentate, the major components were Carvacrol (44.38%), beta-Bisabolene (24.82%), Caryophylleneoxide (3.36%), Camphene (2.92%) and Spathulenol (2.44%).The antioxidant activity estimated by DPPH was (IC50 = 70 ± 1.03µl/ml) and ABTS was (0.032 ± 0.0006 Equi mg trolox/ml DW) and Phosphomolybdenum (4.66 ± 0.007 Equi TROLOX mg/ml). The essential oil was active against E. Coli, Listeria, S. Aureus, B. Ceraus and Salmonella Enteritidis. Therefore, this plant is potentially a good source as antibacterial and antioxidant agent.

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