Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Volatile and Non-Volatile Chemical Compounds and Biological Power of the genus Lavandula: Case of two Moroccan Lavenders *Lavandula angustifolia* Mill. (Cultivated Lavender) and *Lavandula pedunculata* (Mill.) Cav. (Spontaneous Lavender)

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Abstract

This work consists of the comparison of the chemical composition, the antioxidant activity, and the antimicrobial effect of two species of the genus *Lavandula* essential oils (EOs) and extracts (hydroethanolic, aqueous and decocted), that were collected from the region of Ifrane in Morocco "*Lavandula angustifolia* and *Lavandula pedunculata*". Gas chromatographic analyzes coupled with mass spectrometry of the studied EOs showed that *L. angustifolia* contains camphor and linalool as majority compounds with 20,17% and 12,41% respectively, while camphor (41,15%) and fenchone (15,70%) are the major compounds of *L. pedunculata*. The dosage of hydroethanolic, aqueous and decocted extracts showed that the content of polyphenols and flavonoids is higher in the hydroethanolic extracts of *L. pedunculata*, while *L. angustifolia* contains the highest content of tannins. The determination of the antioxidant activity was carried out using FRAP and DPPH methods, the last method revealed that the EO of *L. angustifolia* is less effective (IC_{50}=7,98 mg/ml) than that of *L. pedunculata* (EC_{50}=7,47 mg/ml) while, there was no great divergence concerning the IC_{50} values of the hydroethanolic and decocted extracts of the two species. As for the aqueous extract, the value recorded by *L. pedunculata* was slightly higher than that of *L. angustifolia*. Finally, tests of the antimicrobial activity of EOs and extracts of the two species were carried out on six bacteria and five fungal strains whose antimicrobial power was remarkable. As a conclusion, the two Lavenders' extracts can be considered as alternative sources of powerful natural antioxidants, antifungals and antibiotics.

Keywords: *L. angustifolia*, *L. pedunculata*, chemical composition, antimicrobial activity, antioxidant activity.

1. Introduction

For centuries, MAPs continue to provide most natural remedies through their bioactive molecules. The genera of these plants are characterized by a special type of active principles existing in most of their species; some of these principles may be identical or only have similar structures [1].

On the other hand, in several aromatic and medicinal species, although grouped in the same genus under the same vernacular or common name, differences are observed both at the intra and interspecific levels in their quantitative and qualitative chemical compositions. This can affect their biological activity, which undoubtedly correlates with this chemical composition. The genus *Lavandula* is an important member of the Labiateae (Lamiaceae) family, and consists of approximately 39 species, many hybrids, and nearly 400 registered cultivars [2]. Lavender species are of great market value due to their pleasant aroma. The plant material and its essential oil are mainly used in perfumery, cosmetics and in the food industry. The medicinal importance of the plant is well documented, and the extracts prepared from this plant are registered in several pharmacopoeias [3].Lavender is used in herbalism and aromatherapy. It is considered a medicinal plant because of its essential oil effects. Indeed, it is used to treat superficial wounds, and burns and it has sedative, antibacterial, antifungal, antidepressant [4] and anti-inflammatory [5] effects. The medicinal properties and fragrance of lavender's essential oils (EOs) are mainly attributed to their volatile organic compounds,

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EJCHEM use only: Receive Date: 23 June 2021, Revise Date: 05 August 2021, Accept Date: 23 September 2021
DOI: 10.21608/ejchem.2021.82036.4053
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which means that the monoterpenes and sesquiterpenes are responsible of the lavender's characteristic fragrance [6] and of the EOs therapeutic properties.

In Morocco, lavender exists in a cultivated form; in particular *L. officinalis* and hybrid lavandin (the lavender cultivated in the region of Oulmes is a lavandin, a hybrid resulting from the cross between officinal lavender and aspic lavender), in addition to other wild species in several regions, especially in Rif, Middle and High Atlas [7]. Biographical resources allude to several species of this genus in Morocco, commonly called lavender, with diversified ecology and phytogeography. The taxonomic distinction of these species is quite delicate, while the possibility of a different use depending on the localities is not well recognized.

From this perspective, the present work takes place. Indeed, our major objectives are to characterize the diversity of volatile and non-volatile compounds as well as the biological power of two species of the genus *Lavandula*, and to enhance both spontaneous lavender (*Lavandula angustifolia* Miller) and cultivated one (*Lavandula pedunculata* (Mill.) Cav).

2. Materials and methods

2.1. *Plant Material*

L. pedunculata aerial parts (stems, leaves and flowers L) were collected in May 2016 in Azrou region (Middle-Atlas of Morocco), while those of *L. angustifolia* were collected in June 2016 in Dayet Aoua (Middle-Atlas of Morocco). The identification of the two plant species was carried out at the Botanical and Plant Ecology Laboratory of the Scientific Institute in Rabat (Morocco). The aerial parts of the two plants were dried at room temperature for 15 days in the dark, then processed and stored away from *light and moisture.*

2.2. *Extraction of the essential oils*

The extraction of the two plants EOs was carried out by hydrodistillation using the Clevenger type apparatus [8].

One hundred grams (100 g) of the plant material are placed in a two-liter flask of the Clevenger apparatus. 1 liter of distilled water is added, and then the flask is installed in a flask heater. After three hours, at the end of the extraction, the EO is collected at the surface using a Pasteur pipette. It is dried using anhydrous magnesium sulfate, then introduced into a brown bottle and stored in a dark cool place.

2.3. *Moisture rate of dry matter*

The method used is that recommended by the AFNOR standard, (NF V 18-1095). Moisture is determined by the mass loss resulting from drying at a temperature of 103 ° C for 24 hours. For this, 5 g of the sample is weighed, in crucibles previously dried in a desiccator and tared. The full crucibles are placed in an oven at 103 °C for 24 hours. Then they are cooled in a desiccator and weighed.

The result is expressed as the percentage of dry matter:

$$\%\mathbf{DM} = \frac{\mathbf{Mf} - \mathbf{M0}}{\mathbf{PE}} \boldsymbol{X}\mathbf{100}$$

M0: The empty crucible's mass, in grams.
Mf: Mass of the crucible containing the dry residue, in grams.
PE: Mass of the test sample, in grams.

2.4. *Mineral matter (ash) and organic matter content*

The method used is that described by the AFNOR standard [9]. Crude ash is obtained after destruction of the organic material by incineration. In a desiccator, nickel crucibles are oven dried for 1 hour, then they are tared. After cooling, each crucible is tared to 0.1 mg. About 3 g of sample ground to 1 mm is weighed into the crucibles to 0.1 mg. The full crucibles are placed in the muffle furnace under the extractor hood and their contents are calcined for 4 h at 550 °C. At the end, the temperature is dropped to 100 °C. and the crucibles are taken out, cooled in a desiccator, and then weighed to 0.1 g.

Total ash is calculated as follows:

$$\%\mathbf{MM} = \frac{\mathbf{Mf} - \mathbf{M0}}{\mathbf{PE}} \boldsymbol{X}\mathbf{100}$$

MM: Mineral matter content (ash content) expressed as a percentage of the raw product.
M0: the empty crucible's mass, in grams.
Mf: Mass of the crucible containing the dry residue in grams.
PE: Mass of the test sample in grams.

The difference between the mass of the dry matter and that of the mineral matter is the organic matter content of the sample; it is expressed as a percentage.

$$\mathbf{OM} = \boldsymbol{DM} - \boldsymbol{MM}$$

2.5. *Determination of the chemical composition of the EOs extracted by Gas Chromatography coupled with Mass Spectrometry (GC/MS)*

The chromatographic analysis of the EOs from the aerial parts of the two plants was carried out on a gas chromatograph of the Thermo Electron type (Trace GC Ultra) coupled to a mass spectrometer of the Thermo Electron Trace MS system type (Thermo

Electron: Trace GC Ultra; Polaris Q MS).Fragmentation was carried out by electronic impact of intensity 70 eV. The chromatograph is equipped with a DB-5 (5% phenyl-methyl-siloxane) type column (30m x 0.25mm x 0.25µm film thickness), a flame ionization detector (FID) powered by a mixture of H2 / Air gas. The temperature of the column is programmed at a rate of an increase of 4°C / min from 50 to 200°C for 5 min. The injection mode is split (leakage ratio: 1/70, flow rate ml/ min), the carrier gas used is nitrogen with a flow rate of 1 ml/ min.

The identification of the chemical composition of the EOs of the plants was carried out based on the comparison of their Kovàts (IK) and Adams indices, with those of the reference products known in literature [10,11], that are supplemented by a comparison of indices and mass spectra with different references [11,12].

Kovàts numbers compare the retention time of any product with that of a linear alkane with the same carbon number. They are determined by injecting a mixture of alkanes (standard C_7-C_{40}) under the same operating conditions.

The Kovàts retention index is calculated using the formula below:

$$\mathbf{IK} = \left[\frac{(\mathbf{TRx} - \mathbf{TRn})}{(\mathbf{TRn} + \mathbf{1} - \mathbf{TRn})} + \mathbf{n}\right] * \mathbf{100}$$

KI : Kovàts retention Index.
TRx: Retention time of the compound X to be identified.
TRn: Retention time of the hydrocarbon (of which the number of carbon atoms is n) eluted before compound X.
TR_{n+1}: Retention time of the hydrocarbon (the number of carbon atoms of which is n + 1) eluted after compound X.
n: Number of carbon atoms of the eluted hydrocarbon before compound X.

2.6. Determination of the physico-chemical constituents that are characteristic of the two lavenders EOs

2.6.1. Refractive Index

The refractive index of an essential oil is a measurement that tests how the speed of light is altered when passing through the oil. It is the ratio of the speed of light in vacuum to the speed of light in oil. It is generally measured at 20°C.

The method consists of putting a drop of EO on the flat section of a glass prism, the device is turned on then the refractive index value is given directly.

2.6.2. Degree Brix

The oil's Brix principle is based on the measurement of the concentration (%) of all solids dissolved in the oil (sugar, salts, proteins, fatty acids, etc.).

A few drops of oil are placed on the vessel of the refractometer, which uses a polarized beam of light that is deflected differently depending on the nature of the medium, in which it is propagated. The deviation of the light by the sample varies and indicates, by a colored delimitation, the degree Brix.

2.6.3. Density

The density is measured using a hydrometer, which is a cylindrical, hollow, graduated glass tube ballasted with lead shot.

2.7. *Phytochemical screening*

The phytochemical screening is a qualitative analysis that is based on coloring and/ or precipitation reactions. The different chemical families are determined according to the indications of Bruneton [13], Edeoga et al.[14] and Karumi et al.[15].

2.8. Extraction and determination of polyphenols

2.8.1. Extraction of total phenolic compounds using soxhlet method

30 g of plant material in the form of powder are placed in a cartridge inside the extraction chamber of the soxhlet. Two extracts were prepared: The first is extracted with 300 ml of solvent consisting of a 70/30 ethanol/ water mixture. The second is extracted with 300 ml of distilled water. A total of eight cycles is required for the depletion of plant material. After filtration, the solvent is removed by evaporation under vacuum, and the residue obtained constitutes the crude extract of polyphenols.

2.8.2. Extraction of total phenolic compounds by decoction

30 g of plant material in the form of powder are placed in a 1 L Erlenmeyer flask, and then 300 ml of distilled water are added, the mixture is brought to the boil using a hot plate for one hour. After cooling and filtration, water is removed by evaporation under vacuum and then dried in an oven at 40°C.

2.8.3. Determination of total phenols

The total phenol content of the extracts of the two lavenders was determined by the method of Folin-Ciocalteu [16]. In a 100ml volumetric flask, a quantity of each extract (to obtain a blue color) is mixed with 1.5ml of Folin-Ciocalteu reagent (10%), and 1.5ml of sodium carbonate (Na_2CO_3) to 7.5% (m/v), then the flask is made up with distilled water. The whole is left for two hours at room temperature,

and the reading is taken against a blank using a spectrophotometer (UV mini-1240) at 765 nm. The calibration curve is established under the same operating conditions using gallic acid as a positive control. The results are expressed in milligrams equivalent of gallic acid per gram of extract (mg GAE/g E).

2.8.4. Determination of the total flavonoids of the two lavenders

The quantification of the flavonoids was carried out by the colorimetric method described by Djeridane et al. [17]. In a 50 ml volumetric flask, 100 µl of each extract are mixed with 20 ml of distilled water. After 5 min, 100 µl of 10% (m/v) aluminum trichloride ($AlCl_3$) are added. Then the solutions are adjusted to 50ml with pure methanol, shaken immediately and kept in the dark for 30min at room temperature, and the reading is taken against a blank using a spectrophotometer (UV mini-1240) at 333 nm. The calibration curve is performed in parallel under the same operating conditions using quercetin as a positive control. Results are expressed as milligrams of quercetin equivalent per gram of extract (mg GAE/g E).

2.8.5. Condensed tannins of the two lavenders

The amount of condensed tannins was determined using the vanillin method in an acidic medium [18]. A volume of 3 ml of the vanillin / methanol solution (4% m/v) is added at different concentrations prepared from the catechin solution (2 mg/ ml). Then, a volume of 1.5ml of concentrated hydrochloric acid is added to each concentration. The mixtures obtained are left to react at room temperature for 20 min, and then the absorbance is measured at 499nm against a blank using a spectrophotometer. The amount of condensed tannins in our samples (extracts) was determined following the same procedure used to plot the calibration curve by replacing catechin with our samples. The concentration of tannins is expressed in milligrams of catechin equivalents per gram of the weight of dry matter from the calibration curve.

2.9. Antioxidant activity of the extracts of the two lavenders

2.9.1. DPPH* free radical scavenging test

The anti-free radical activity of the various extracts and EOs of the two plants is determined using DPPH method (1,1-diphenyl-2-picrylhydrazyl) as a relatively stable radical. The DPPH* solution is prepared by dissolving 2,4 mg of DPPH* in 100 ml of ethanol. The extracts are prepared by dissolution in ethanol at a rate of 1 mg/ ml; this stock solution will then undergo a series of dilutions to have the following concentrations: (from 0,05 to 0,55mg/ml). The EOs are prepared by dissolution in ethanol at a rate of 2 mg/ml, this stock solution will undergo a series of dilutions to have the following concentrations: (from 0,2 to 140mg/ml). The test is carried out by mixing 2 µl of the compound to be tested and 2.8 ml of DPPH* solution. These same concentrations were prepared with ascorbic acid (vitamin C) to serve as positive controls. Also a blank was made with absolute ethanol alone. The samples are then left in the dark for 30 minutes and the discoloration against the negative control containing only the DPPH* solution is measured at 517 nm. The results are expressed as a percentage reduction in DPPH* (AA%):

$$AA\% = \frac{\mathbf{A_{control}} - \mathbf{A_{Sample}}}{\mathbf{A_{control}}} \times \mathbf{100}$$

AA% : Percentage of antioxidant activity.
A $_{control}$: Absorbance of the solution containing only the DPPH* radical.
A $_{sample}$: Absorbance of the samples to be tested in the presence of DPPH*.

The graph plotting Absorbance versus the extract concentration was used to determine IC_{50}.

The values of the concentrations to inhibit or reduce 50% of the initial concentration of DPPH* (IC_{50}) were determined graphically by linear regression. Since there is no absolute measure of a compound's antioxidant capacity, the results are often compared to a benchmark antioxidant, such as ascorbic acid.

2.9.2. Iron reduction test: FRAP (Ferric Reducing Antioxidant Power)

The power of phenolic extracts to reduce ferric iron (Fe^{3+}) present in the potassium ferricyanide complex to ferrous iron (Fe^{2+}) is determined according to the method described by Koncic et al. [19]. In test tubes, 0,5 ml of extracts (aqueous, hydroethanolic and decocted) and EOs at different concentrations (for extracts a concentration range from 0,1 to 100 mg/ml and for EO a concentration range from 1 to 10 mg / ml) are mixed with 2,5 ml of a 0,2 M phosphate buffer solution (pH= 6,6) and 2.5 ml of a 1% solution of potassium ferricyanide $K_3Fe(CN)_6$.

The whole is incubated in a water bath at 50°C for 20 min, then 2.5ml of 10% trichloroacetic acid is added to stop the reaction. The whole is centrifuged at 3000 rpm for 10 min, then 2,5 ml of the supernatant from each concentration is mixed with 2,5 ml of distilled water and 0,5 ml of the 0,1% aqueous $FeCl_3$ solution. The absorbance of the

reaction medium is read at 700nm against a similarly prepared blank, replacing the aqueous extract with distilled water which makes it possible to calibrate the device (UV-VIS spectrophotometer). The positive control is represented by a standard solution (Ascorbic acid), which absorbance is measured under the same conditions as the samples. An increase in absorbance corresponds to an increase in the reducing power of the tested extracts. The graph plotting absorbance versus the extract concentration was used to determine the EC50 by linear regression.

2.10. Antibacterial and antifungal activities

2.10.1. Sensitivity test

Six bacterial strains (*Escherichia coli*, *Staphylococcus aureus*, *Acinetobacter baumanii*, *Shigella dysenteria*, *Salmonella thyphi*, and *Enterobacter cloacae*) were chosen for their pathogenicity and their incrimination in human infections. The six bacteria belong to both, the Provincial Laboratory of Epidemiology and Environmental Hygiene in Ifrane, and the medical laboratory of the 20th August Provincial Hospital in the province of Ifrane. The antibiotic and antifungal sensitivity profiles of the tested strains were carried out according to the recommendations of the French Society of Microbiology and EUCAST.

To determine the antifungal activity, five fungal strains including three yeasts (*Candida albican*, *Candida glabrata*, and *Candida* ssp) and two molds (*Aspergillus fisheri*, and *Fusarum solani*) were tested. The five strains belong to the collection of the Laboratory of Ecology and Biodiversity of Wetlands, Moulay Ismail University, Faculty of Sciences in Meknes.

2.10.2. Disc diffusion method

To explore the antibacterial and the antifungal effects of the two lavenders Eos and extracts, we used the solid diffusion method using Muller-Hinton agar for bacterial strains, and Sabouraud chloramphenicol agar for fungal strains. The used methods to determine the antibacterial and the antifungal activities are those described by Remmal et al. [20] and Boukili et al.[21].

At first, our oils were filtered through a Millipore filter with a pore diameter of 0.45µm. Next, dimethyl-sulfoxide amide (DMSO) was used as an emulsifying agent (E) because of its effectiveness as a solubilizing agent for essential oils with a low concentration (2µl) which does not influence the antibacterial quality of the tested essential oil.

The bacterial and fungal suspensions were prepared from pure cultures in the exponential phase of growth, that were suspended in sterile physiological saline (EρS).The turbidity of the suspensions was adjusted to a standardized inoculum (10^5 CFU/ml). Then from the prepared suspensions, 1 ml of each bacterial and fungal suspension was taken, then spread by flooding on the surface of a Petri dish containing Muller Hinton Agar (MHA) for bacteria and Sabouraud chloramphenicol agar for fungal, then the remaining liquid is sucked out. the Petri dish is kept in the septic zone of the Bunsen burner until it becomes dry, then sterile discs of blotting paper are placed in the center and at the peripheries of the petri dish where varying volumes of the EO to be tested are deposited (2 µl, 4 µl, 6 µl, 8 µl, 10 µl and 12 µl) or 10 mg, 20 mg, 30 mg and 40 mg of the extracts to be studied. For each test, we carried out two repetitions. For bacteria, a sterile disc flooded with 6 µl of sterile physiological saline was used as negative control as well as another negative control disc impregnated with 2 µl of DMSO.

The dishes are then incubated at a temperature of 37°C for 18 to 24 hours. After incubation, the inhibition zone diameters are measured.

For antifungal tests, each sterile Wattman paper disc of 6 mm is impregnated with 20 µl of each Essential Oil (EO) or 20 mg of each extract, then put on the surface in the middle of the petri dish. A sterile disc impregnated with 20 µl of sterile physiological saline was used as a negative control for fungal strains.

2.10.3. Minimum inhibitory concentration (MIC)

This technique consists of the inoculation, with a standardized inoculum (10^5 CFU/ml), of a range of increasing volumes of essential oils. After incubation, the observation of the range makes it possible to determine the Minimum Inhibitory Concentration (MIC), which corresponds to the lowest concentration of essential oils and extracts for which bacterial growth is no longer visible in vitro (no growth but 100% of surviving bacteria). The method consists of transferring 2 µl of dimethyl sulfoxide (DMSO) into 10 hemolysis tubes containing the culture medium (1 ml) for each bacterial strain, then each volume of essential oil (EO) is introduced into each hemolysis tube. For the extracts, only the extract is introduced into the hemolysis tube (without DMSO). The 10 chosen volumes of EOs were: 2 µl, 4 µl, 6 µl, 10 µl, 12 µl, 14 µl, 16 µl, 18 µl, 20 µl and 22µl with two repetitions made for each volume. For the extracts, the 10 chosen volumes are 10 mg, 20 mg, 30 mg and 40 mg. Then a volume of 6 µl of the bacterial suspension with a concentration of 10^5 CFU/ml is deposited in each of the previous tubes. For fungal strains, the chosen volumes of EOs were 10 µl, 20 µl, 30 µl, 40 µl, 50 µl and 60 µl and the chosen extracts amounts were 10 mg, 20 mg, 30 mg, 40, and 50 mg. For each EO and extract, two controls were carried out; one containing the culture medium (1ml) and the bacterial strain and the other containing

the culture medium (1ml) and 2µl of the essential oil alone or 10 mg of the extract.

2.10.4. Minimum Bactericidal Concentration (MBC)

Previously prepared Nutrient and Sabouraud chloramphenicol mediums are inoculated with contents of tubes having a concentration greater than or equal to the MIC in the series of previous concentrations. MBC is determined after a 24 hours incubation at 37°C for bacteria, and 72 hour incubation at 24°C for fungal strains. The MBC is defined as the smallest concentration that totally inhibits growth.

2.11. Statistical study

The effects of the essential oils and extracts (inhibition diameter) against fungal and bacterial strains were evaluated by analysis of variance (ANOVA); then, means and standard deviations were calculated using the Origine 8.5 pro software, and the Tukey test at the 5% probability threshold was used for the comparison of the means.

3. Results and discussion

3.1. *Essential oils' yields of the two lavenders*

The study performed using samples of *L. angustifolia* provided an EO yield of approximately 7,11% ± 0,01; which is considerably higher than that obtained with *L. pedunculata* (1,70% ± 0,03) (Table 1).In this study, the obtained essential oil yield of *L. angustifolia* is superior to those of other previous works. In Morocco, the results obtained by Chahboun et al. [22] indicated that the dry flowers of L. *angustifolia* have an essential oil content of around 1,12%. Laib et al. [23] as well as Mohammedi et al. [24] indicated that dry lavender flowers from two Algeria regions (Skikda and Costontene) have essential oil contents of the order of 1,36% and 2,01% respectively. Moreover, Verma et al. [25] and Elharas et al. [26] reported essential oil yields reached 1,5% and 2.3% respectively.

Table1: Essential oils' yields of selected Lavenders

EO	*L. angustifolia*	*L. pedunculata*
Density	0,871	0,980
Refractive index	1,47795	1,47009
Brix degree (%)	71,92	75,06

For *L. pedunculata*, SORO et al. [27] obtained a yield of 1,97% that is slightly higher than our result. These variations in EO yields can be explained by several factors including the degree of maturity of the flowers, the interaction with the environment (type of climate, soil), the time of harvest and the extraction method [28].

A study of the coefficient of variation was applied to the data obtained concerning the essential oils' yields, which is a relative measure of the dispersion of the data around the average. The coefficient of variation (CV) is calculated as the ratio of the standard deviation to the average. And it is expressed as a percentage. The lower the CV is (close to 0), the more homogeneous the data is, and therefore the more representative the average is. The CV values for the two yields are low (near 0), so the data is homogeneous and the averages are representative.

3.2. Chemical composition and physico-chemical constants characteristic of the EOs of *L. angustifolia* and *L. pedunculata*

3.2.1. Determination of the physicochemical constants

The determination of the refractive index is usually used to have a quick and reliable check of the purity of a substance. The refractive index, like the density, depends on either the chemical composition of the oil or its temperature. It grows with unsaturation and the presence of secondary functions on fatty chains.

According to Table 2, the refractive index of *L. angustifolia* is slightly higher (1,47795) than that of *L. pedunculata* (1,47009). The low refractive indices of essential oils (1,47009 and 1.47795) indicate their low refraction of light, which could promote their use in cosmetic products [29]. On the other hand, the density of *L. pedunculata* (D = 0,980) is higher than that of *L. angustifolia* (D = 0,871), and these two densities are lower than that of water. According to the European Pharmacopoeia, the density of the lavender EO is between 0,878 and 0,892, on average of 0,885[30].

Harvest site	Species	EO yield (%)	Variation Coefficient (CV)%
Dayet Aoua	***L. angustifolia***	**7,11 ± 0,01**	**0,14**
Azrou	***L. pedunculata***	**1,70 ± 0,03**	**1,76**

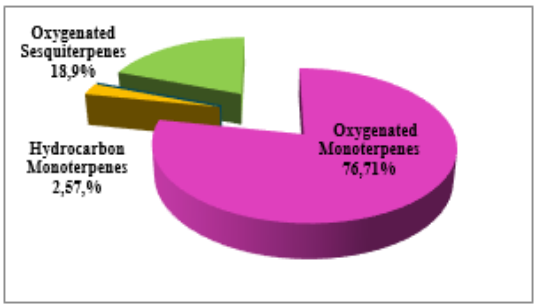
Table 2: Physicochemical constants of the EOs of *L. angustifolia* and *L. pedunculata*

3.2.2. Chemical composition of the lavenders EOs

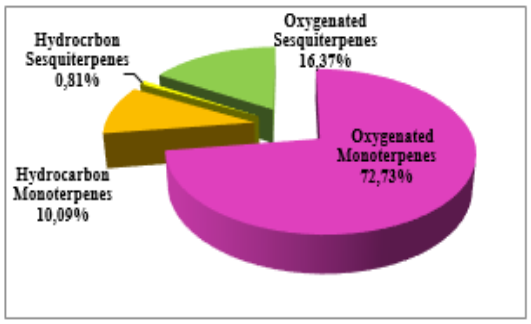
The qualitative and quantitative analysis of the essential oil of *L. angustifolia* carried out by GC/SM made it possible to identify 35 compounds which represent a total of 98,18% (Table 3), with 76,71% of

oxygenated monoterpenes, 18,90% of oxygenated sesquiterpenes and 2,57% of hydrocarbon monoterpenes (fig 1a). The main components are camphor (20,07%), 1,8-cineole (16,68%), linalool acetate (13,66%), linalool (12,34%), linalool acetate (10,65%), borneol (9,69%). Compared to the chemical compositions published in the literature, the chemical profile described here shows similarities but also reveals differences. In Algeria, a study by Boughendjioua [31] on *L. angustifolia* in the region of skikda identified 20 compounds, the major constituents according to this study are: linalool acetate (32,98%), linalool (28,92%), β-caryophyllene (4,62%), lavandulyl acetate (4,52%), Z-β-ocimene (4,44%), terpinene-4-ol (4,32%), E-β-ocimene (3,09 %), β-farnesene (2,73%) totaling about 85,62%. Studies also carried out in Algeria by Laib et al. [32] on *L. angustifolia* in the Constantine region identified: linalool acetate (15,26%), linalool (10,68%), 1,8-cineole (10,25%), γ-terpinene (11,2%) and camphor (11,25%). Our results and those of Laib et al. [32] are dissimilar to those shown by some other authors. Kulevanova et al. [33] analyzed the chemical composition of the essential oil of *L. angustifolia* flowers from the Kozjak mountain (Macedonia), they found 32 constituents with a predominance of linalool (25,7%), linalool acetate (23,2%) and lavandulyl acetate (12,4%) and a dominance of monoterpene components and the presence of sesqueterpene hydrocarbons and its oxygenated derivatives. Verma et al. [25] studied the essential oil composition of *L. angustifolia* flowers cultivated in Uttarakand (India), they identified 37 monoterpenes. The major compounds were: linalool acetate (47,56%), linalool (28,06%), lavandulyl acetate (4,34%) and α-terpineol (3,7%).

The analysis of the EO of *L. pedunculata* carried out by CG/SM, identified 34 compounds representing 100% of the total composition, with 72,73% of oxygenated monoterpenes, 16,37% of oxygenated sesquiterpenes, 10,09% of the hydrocarbon monoterpenes and 0,81% of the hydrocarbon sesquiterpenes (figure 2b). This chemical composition is dominated by camphor (41,15%), fenchone (15,70%), borneol (5,72%), cubenol <1,10-di-epi> (7,53%), camphene (5,77%), muurol-5-en-4-one- <cis-14-> (2,27%) and α-pinene (2,06%). Our results coincide with those obtained by Soro et al. [34] 2015, which revealed a chemical composition dominated by monoterpenes (88,43%), including: α-pinene (10.74%), fenchone (13,19%) and camphor (46,36%). According to Bellakhdar [7], the composition of *L. pedunculata* is generally dominated by fenchone and camphor. Their percentages vary from 47 to 83% with an alternation of the dominant compounds. Harborne et al. [35] confirm this information but consider camphor (24%) as the dominant compound and fenchone (20%) in second place. Costa et al. [36] identified camphor 40,6% and fenchone 38% in the EO of the species from Portugal. The EO of *L. stoechas* is dominated by α-thujone, L-camphor and 1,8-cineole [37] or by camphor and fenchone [38]. The chemical composition of the EOs of *L. pedunculata* change very slightly and great similarities are observed between the essential oils of *L. stoechas* and *L. pedunculata*.



(a)



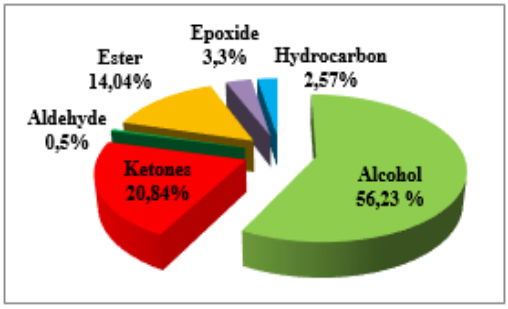
(b)

Figure 1: Distribution of terpenes families in the EOs of *L. angustifolia* (a) and *L. pedunculata* (b)

The comparison of the chemical composition of the EOs of the two lavenders reveals that both of them contain variable proportions of oxygenated monoterpenes and oxygenated sesquiterpenes. *L. angustifolia* EO contains a significant percentage of these two terpenes (figure 1a), and it is dominated mainly by alcohols (56,23%) (Figure 2b). while, *L. pedunculata* EO is composed mainly of ketones (figure 2a).

Table 3: Chemical composition of the EOs of *L. angustifolia* and *L. pedunculata*

Compounds	IK	Molecular Formula	*L. angustifolia*	*L. pedunculata*
Tricyclene	921	$C_{10}H_{16}$	-	0,46
α-pinene	939	$C_{10}H_{16}$	0,25	2,06
Camphene	954	$C_{10}H_{16}$	0,62	5,77
thuja-2,4 (10) -diene	960	$C_{10}H_{14}$	-	0,28
Verbenene	967	$C_{10}H_{14}$	-	0,22
β-pinene	979	$C_{10}H_{16}$	0,25	-
Myrcene	1021	$C_{10}H_{16}$	0,28	-
Cymene <p>	1024	$C_{10}H_{14}$	0,38	0,36
Cymene <O>	1026	$C_{10}H_{14}$	-	0,69
Limonene	1029	$C_{10}H_{16}$	0,52	0,25
Cineole <1.8>	1031	$C_{10}H_{18}O$	16,68	-
Ocimene <(E) -β>	1050	$C_{10}H_{16}$	0,27	-
Linalool oxide <cis>	1072	$C_{10}H_{18}O_2$	3,46	0,38
Camphenilone	1082	$C_9H_{14}O$	-	0,25
Fenchone	1086	$C_{10}H_{16}O$	-	**15,70**
Linalool oxide <trans>	1086	$C_{10}H_{18}O_2$	2,56	0,51
Linalool	1096	$C_{10}H_{18}O$	**12,41**	0,58
Octen-3-ylacetate <1>	1112	$C_{10}H_{18}O_2$	0,32	-
Fenchol <exo>	1121	$C_{10}H_{18}O$	-	1,57
Pinocarveol <trans>	1139	$C_{10}H_{16}O$	-	0,18
Verbenol <cis>	1141	$C_{10}H_{16}O$	-	0,29
Verbenol <trans>	1144	$C_{10}H_{16}O$	0,29	1,81
Camphor	1146	$C_{10}H_{16}O$	**20,17**	**41,15**
Nerol oxide	1158	$C_{10}H_{16}O$	0,30	-
Borneol	1169	$C_{10}H_{18}O$	9,71	5,72
Cryptone	1185	$C_9H_{14}O$	0,39	-
Linalool oxide <cis>	1174	$C_{10}H_{18}O_2$	0,36	-
Terpinen-4-ol	1177	$C_{10}H_{18}O_2$	1,06	-
Cymen_8-ol <m>	1179	$C_{10}H_{14}O$	-	0,75
Cymen_8-ol <p>	1182	$C_{10}H_{14}O$	-	1,25
Thuj-3-en-10-al	1184	$C_{10}H_{14}O$	-	0,28
Terpineol <α>	1188	$C_{10}H_{18}O$	2,70	-
Verbenone	1205	$C_{10}H_{14}O$	0,28	1,68
Carveol <trans>	1216	$C_{10}H_{16}O$	-	0,42
Cumin aldehyde	1241	$C_{10}H_{12}O$	0,50	
Geraniol	1252	$C_{10}H_{18}O$	0,26	-
Linalool acetate	1257	$C_{12}H_{20}O_2$	10,67	-
Bornyl acetate	1285	$C_{12}H_{20}O_2$	0,41	1,34
Thymol	1290	$C_{10}H_{14}O$	0,60	-
Lavandulyl acetate	1290	$C_{12}H_{20}O_2$	2,09	-
Carvacrol	1299	$C_{10}H_{14}O$	4,66	0,46
Geranyl acetate	1381	$C_{12}H_{20}O_2$	0,55	-
Calamenene <cis>	1529	$C_{15}H_{12}$	-	0,81
Caryophyllene oxide	1583	$C_{15}H_{26}O$	3,30	-
Cubenol <1,10-di-epi>	1619	$C_{15}H_{26}O$	-	**7,53**
Cadinoll <epi-α>	1640	$C_{15}H_{26}O$	0,73	0,60
Cadinoll <α>	1654	$C_{15}H_{26}O$	-	0,91
Guaia-3,10 (14) dien-11-ol	1677	$C_{15}H_{24}O$	0,54	-
Ishwarane	1681	$C_{15}H_{22}O$	-	**0,34**
Bisabolol <α>	1685	$C_{15}H_{26}O$	0,61	-
Muurol-5-en-4-one- <cis-14-nor->	1689	$C_{14}H_{22}O$	-	2,27
Calamenene <5-hydroxy-cis>	1713	$C_{15}H_{22}O$	-	1,67
Nootkatol	1715	$C_{15}H_{24}O$	-	0,97
Widdrol hydroxyether	1740	$C_{15}H_{26}O_2$	-	0,49
Oxygenated monoterpenes			**76,71**	**72,73**
Hydrocarbon monoterpenes			**2,57**	**10,09**
Hydrocarbon sesquiterpenes			-	**0,81**
Oxygenated sesquiterpenes			**18,90**	**16,37**
Total			**98,18 %**	**100%**



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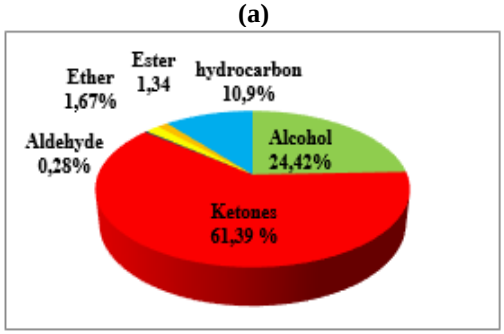

(b)

Figure 2: Distribution of the chemical compounds families contained in the EOs of *L. angustifolia* (a) and *L. pedunculata* (b)

In addition, there are other families of chemical compounds that characterize the two plants such as: hydrocarbons, ethers, esters, aldehydes and epoxides in varying proportions.

3.3. Phytochemical screening

The preliminary tests or "screening", always represent the first step in the chemical study of the plant. Moreover, phytochemical screening makes it possible to conduct further researches to better understand the chemical functions and biological activity of the plant in question. The results of the phytochemical tests of the two studied plants are grouped together in Table 4. The results show similarities and differences between the two species, both at the qualitative (different compounds) and the quantitative levels (different proportions of certain compounds).

The phytochemical screening revealed the presence of five major chemical groups in both species of *Lavandula*: tannins, flavonoids, sterols and triterpenes, alkaloids and mucilages. As for saponosides, they are present only in *L. angustifolia*. Coumarins, however, were absent. A study conducted by Benyagoub et al. [39], showed that the phytochemical screening carried out on *L. angustifolia* revealed the presence of tannins, coumarins, alkaloids, terpenoids, sterols and steroids, quinones and saponosides, as well as the absence of flavonoids, anthracenosides, starch, emodols, fatty acids and reducing compounds. The phytochemical screening of *L. pedunculata* extracts carried out by Bachiri et al. [40], revealed the existence of polyphenolic substances including catechetical and gallic tannins, flavonoids (anthocyanins, flavones and catechols), sterols and triterpenes, in addition to combined anthracenics (C-heterosides and Oheterosides) and reducing compounds (Oses and holosides).

Table4: **The chemical groups of the different samples of *L. angustifolia,* and *L. pedunculata***

Chemical group	*L. angustifolia*	*L. pedunculata*
Tannins	+++	+++
Flavonoids	++ (flavone)	+ (flavonole)
Gallic tannins	+++	+++
Catechic tannins	+++	+
Sterols and triterpenes	+++	+++
Mucilage	++	++
Coumarins	-	-
Saponosides	+	-
Alkaloids	+	+

(+++): Abundance; (++): Average presence; (+): Low presence; (-): Absence

Therefore, this qualitative chemical difference of the two lavenders may influence their biological activities. In addition to the effect of the biotope (climate and soil), the effect of the different origins (two species that are from two different localities), the genotype may in turn be responsible for such a difference.

3.4. Dry matter and moisture rate

Table 5 presented below together the humidity and dry matter levels of *L. angustifolia* and *L. pedunculata*. According to these results, it is quite clear that the dry matter and moisture levels are very similar for the two lavenders, with a slight difference of 0.60% between the two species.

Table 5: Dry matter and Moisture rate of *L. angustifolia* and *L. pedunculata*

	L. angustifolia	*L pedunculata*
Dry matter rate (DM%)	92,3	92,9
Moisture rate (M%)	7,70	7,10

3.5. Mineral and organic matter (ash) contents

The results in Table 6 indicate that the contents of organic constituents are important and almost similar for both plants. Indeed, they present 92,15% and 92,57% for *L. pedunculata* and *L. angustifolia* respectively. Likewise, the mineral content is almost

similar for the two plants, with a rate of 7,43% for *L. angustifolia* and 7,85% for *L. pedunculata*.

Table 6: Mineral and organic matter contents of *L. angustifolia,* and *L. pedunculata*

	L. angustifolia	*L. pedunculata*
Mineral matter %	7,43	7,85
Organic matter %	92,57	92,15

The small variations in mineral and organic matter contents can be explained by the soil's reserves of mineral matter, the efficiency of their root capture and the movement of the mineral matter into the aerial parts of the plant [41].

3.6. Quantification of the phenolic compounds of the two lavenders

3.6.1. Extraction yield

The extraction yield is defined as the ratio of the mass of the extract to that of the dry plant powder. It is represented as a percentage (%), and it is calculated by the following formula:

$$\text{Yield (\%)} = 100\ m/m_0$$

Yield: Extraction yield (%).
m : Mass of crude extract.
m_0 : Mass of the dry plant powder.

Both the used extraction method and the solvent (the percentage of alcohol contained in the solvent) are responsible for the variations in the extraction yield percentage of each plant (Table 7).

Table 7: Yields of different extracts of *L. angustifolia* and *L. pedunculata*

Species	Extraction method/Solvent	Yield (%)	(CV)* %
L. pedunculata	Soxhlet (Ethanol/Water)	17,36± 0,12	0,69
	Soxhlet (Water)	14,40± 1,15	7,98
	Decocted	12,80± 0,21	1,64
L. angustifolia	Soxhlet (Ethanol/Water)	24,36± 2,13	8,74
	Soxhlet (Water)	15,40± 1,2	7,79
	Decocted	15,28± 0,32	2,09

*Coefficient **of** Variation

According to the obtained results, for both lavender species, the soxhlet-ethanol/water method gave high yields compared to those obtained by soxhlet-water and by decoction.

For the three extraction techniques, *L. angustifolia* recorded the highest yields (24,36% (Soxhlet - Ethanol/Water) 15.40% (Soxhlet-Water) and 15.28% (Decocted)) compared to *L. pedunculata* (17,36% (Soxhlet- Ethanol/Water), 14,40% (Soxhlet-Water) and 12,80% (Decocted). However, the extraction method, by soxhlet and the solvent Ethanol/Water, gave the best yields for both plants. It is worth mentioning that other factors can influence the yield of secondary metabolite extraction such as the drying time of the plant material, the particle size of the crushed plant material, the volume of solvent to mass of the crushed plant material ratio, and the duration of extraction [42]. The CV values for the different extraction methods with different solvents are low (near to 0), which means, that the data is homogeneous and the average is representative.

3.6.2. Determination of the phenolic compounds of the two lavenders

In order to evaluate the total content of phenolic compounds in the different extracts of the two studied species, calibration curves of gallic acid, quercetin and vanillin were established (Figure 3). The concentrations of polyphenols, flavonoids and tannins condensed in the extracts were then respectively estimated to be equivalent per g of dry matter of gallic acid (GAE/ g DM), equivalent per g of dry matter of quercetin (QE/ g DM) and equivalent per g of dry matter of vanillin (VE/ g DM).

The determination of the total polyphenols, flavonoids and tannins in the various extracts of the flowering tops of the two lavenders revealed a strong heterogeneity of the contents of the phenolic compounds, which vary according to the species, the method of extraction and the polarity of the used solvent (Figure 4). The obtained results showed that the contents of these compounds vary considerably between the two species. In fact, the obtained results of the three extracts of each plant showed that *L. pedunculata* is richer in total polyphenols than *L. angustifolia* (800,08 mg GAE/ g DM against 227,42 GAE/ g DM (hydroethanolic extracts), 69,65 mg GAE/ g DM against 65,16 mg GAE / g DM (aqueous extract) and 0,75 mg GAE/ g DM against 0,49 mg GAE/ g DM (decocted)). These levels are much better than those observed in the methanolic extract of *L. angustifolia* from Lithuania [43], the aqueous extract of officinal lavender from Iran, the methanolic extract of *L. stoechas* from Turkey, and the ethanolic extract of *L. dentata* from Tunisia [44].

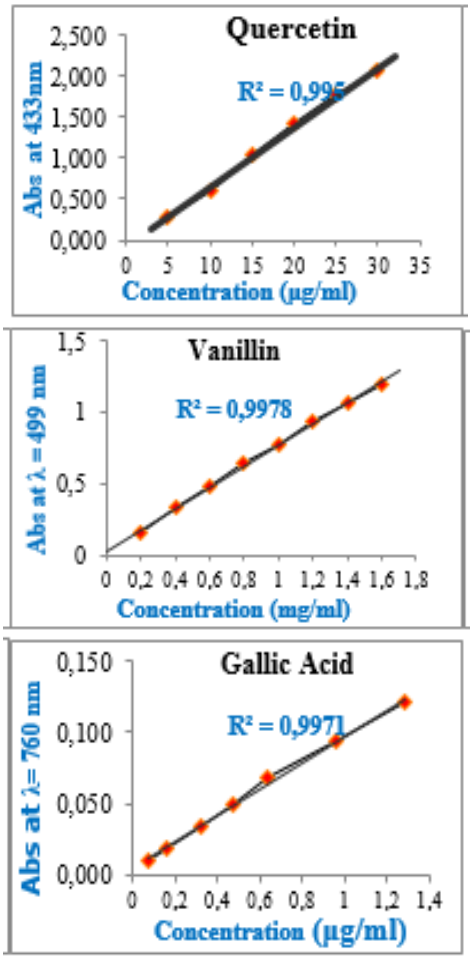

Figure 3: Calibration curves of Quercetin, Vanillin and Gallic acid

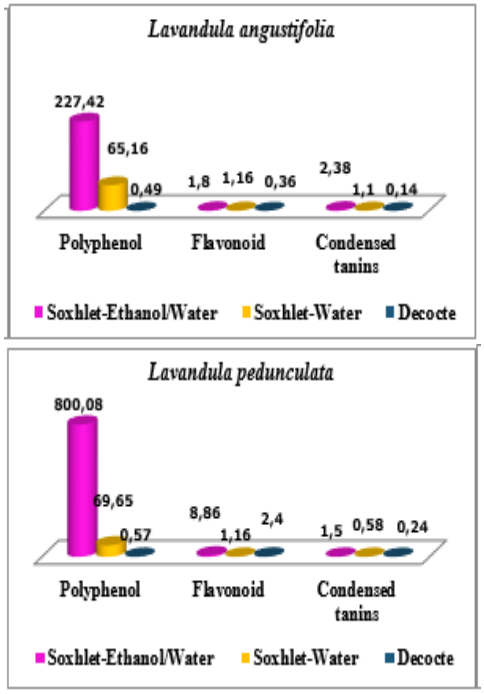

Figure 4: Condensed tannins, polyphenols, and flavonoids contents of *Lavandula* extracts

Concerning the flavonoid contents of the two plants, the different analyzed extracts follow the same order of classification between the two species and the three types of extracts as that described for the total polyphenols, but with lower levels. According to Figure 4,*L. pedunculata* is richer in these molecules for the three extracts (8.36 mg EC/gDM in the hydroethanolic extract) than *L. angustifolia* (1.36 mg EC/gDM in the hydroethanolic extract).These contents are lower than those of the ethanolic extract of the leaves of the stem and of the root of *L. dentata* obtained by Bettaieb[44]studies. Furthermore, for the condensed tannins, *L. angustifolia* is richer than *L. pedunculata* (2.38 mg CE/ g DM against 1.5 CE/ g DM).

Moreover, regardless of the type of solvent, the comparison between the two species shows that pedunculate lavender has higher polyphenol contents than *angustifolia* lavender, and this difference is more marked in the hydroethanolic extracts.

3.7. Evaluation of the antioxidant activities of the two lavenders

The EOs antioxidant activity and thatof the hydroethanolic and aqueous extracts, and decocted of *L. pedunculata* and *L. angustifolia* were evaluated using two in vitro tests:the DPPHfree radical scavenging activity and the reducing power (FRAP).

3.7.1. DPPH free radical scavenging activity

The antioxidant activity of the extracts and EOs of the two plants is determined by the DPPHfree radical reduction method. DPPHis a stable radical witha characteristic absorption at 515 nm; it is in the form of a purple solution. This color disappears when the DPPH is reduced by a free radical scavenger and becomes pale yellow inducing a change in optical absorption.

The results of this anti-free radical activity of the EOs and extracts showed the interspecific variability in the two species of lavender, as well as its dependence on the richness of terpene compounds. The results obtained during the test by the measurement of the percentage inhibition of the DPPH radical of the two EOs are presented in Figures 5 and 6.

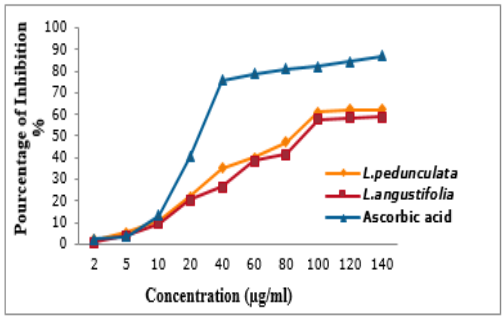

Figure 5: Inhibition Percentage of DPPH radical by *L. pedunculata* and *L. angustifolia* EOs and Ascorbic Acid

According to the obtained results, the percentage of free radical inhibition increases with increasing concentration of ascorbic acid or of the EOs and of the extracts of both lavenders. We also note that the percentage of maximum inhibition of the free radical for the two EOs is lower (58,75% for *L. angustifolia*, and 62,25% for *L. pedunculata*) than that of ascorbic acid (87,05 %).

IC_{50} of the EOs is a parameter of the antioxidant activity which is summarized in Figure 6 for both plants. The IC_{50} results show that, ascorbic acid has a higher antioxidant power (4.78 mg/ ml) than that of the EOs of the two lavenders. On the other hand, the essential oil of *L. angustifolia* is slightly less effective (IC_{50}= 7,98 mg/ ml) than that of *L. pedunculata* (IC_{50}= 7,47 mg/ ml) (figure 7). This activity depends on the mobility of the hydrogen atom of the hydroxyl group of the phenolic compounds contained in the essential oil. In the presence of a free radical DPPH, the H atom is transferred to it; so as to transform it into a stable DPPH molecule, what causes a decrease in the free radical concentration and in the absorbance during the reaction from its start to the depletion of the antioxidant capacity of the hydrogen donor [45].

This activity may be due to camphor which is a major compound of the two studied EOs 41.15% (*L. pedunculata*) and 20.17% (*L. angustifolia*), which has a strong antioxidant activity [46]. It is not only the major EO compounds that are responsible for this antioxidant activity, but there may also be other minority compounds that can interact synergistically or antagonistically to create an effective system against free radicals [23]. The presence of carvacrol even at low concentration in the essential oil of *L. officinalis* (0,9%) may explain the DPPH radical scavenging activity [23].

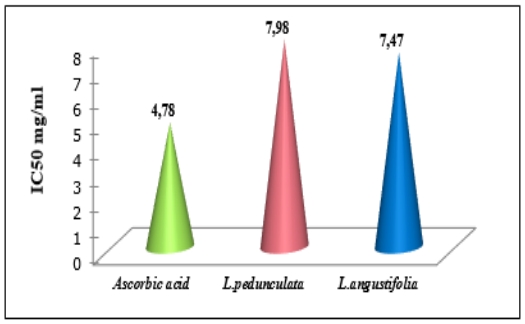

Figure 6: IC_{50} of *L. pedunculata* and *L. angustifolia* EOs and Ascorbic Acid.

The three extracts of the two studied lavenders, revealed significant antioxidant potential. The calculated inhibition percentages are shown in Figure 7. According to these results, the percentage of free radical inhibition increases with increasing concentrations of vitamin C (used as a positive control), or of the crude extracts.

The activity of vitamin C (ascorbic acid) clearly differs from the activity of the studied extracts. For all the tested concentrations, the percentage inhibition of vitamin C is greater than that of the crude extracts. Also, we note that the percentage inhibition of the hydroethanolic, aqueous and decocted extracts of *L. pedunculata* are greater than those of *L. angustifolia*. From the graph representing the antioxidant activities against the extract concentrations, we can determine the IC_{50} (figure 8), with IC_{50} values, of the order of 3,80 mg/ ml, 5,06 mg/ ml and 10.58 mg/ ml for the hydroethanolic, aqueous and decocted extracts respectively for *L. pedunculata*. For *L. angustifolia*, IC_{50} values vary between 4.10 mg/ ml (hydroethanolic extract), 9.06 mg/mL (aqueous extract) and 10,69 mg/ ml (decocted) (figure 8). In general, there is no great divergence concerning the IC_{50} values of the hydroethanolic and decocted extracts for the two species. As for the aqueous extract, the value recorded by *L. pedunculata* was slightly higher than that of *L. angustifolia*. By comparing the IC_{50} values of the EOs to those of the extracts, we notice that the hydroethanolic extracts of the two lavenders have an antioxidant power, which is greater than that of the EOs. Most of the antioxidant activities of the plants are due to the presence of phenolic compounds [47].

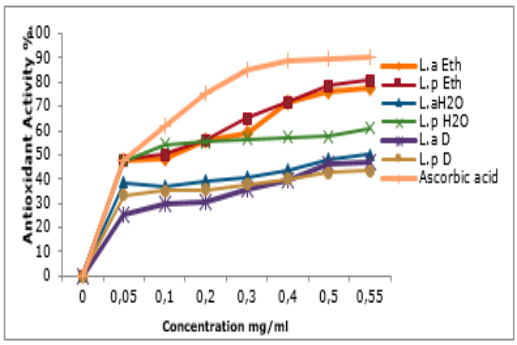

Figure 7: Inhibition Percentage of DPPH of different extracts of *L. pedunculata* and *L. angustifolia* and the ascorbic acid.

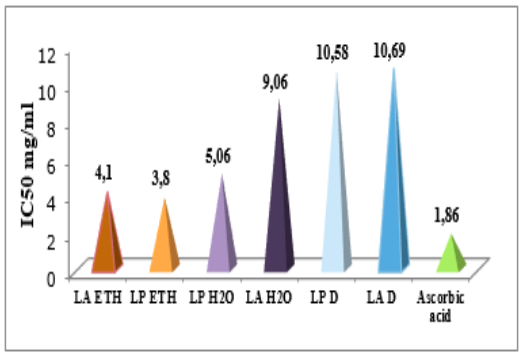

Figure 8: IC_{50} of different extracts of *L. pedunculata*, *L. angustifolia* and the ascorbic acid

These results confirm those of Catarina et al. [48]. In fact, the hydroethanolic and aqueous extracts of *L. pedunculata* revealed an antioxidant potential withIC_{50} values between 530–14 µg/ml and 1833–17 µg/ml for the aqueous and hydroethanolic extracts respectively. These results are also confirmed by the studies of Costa et al. [36], where the antioxidant potential of *L. pedunculata* was determined using the TBARS test and the highest tested concentration was 5 mg/ ml. In addition, Ferreira et al. [49] tested the ethanolic extracts and decoctions of *L. pedunculata* using the inhibition of DPPH and the β-carotene methods, in which these samples showed an antioxidant activity with values, which vary between 93 and 20 mg/ ml respectively.

Moreover, Economou et al. [50] revealed that the essential oils of the flowers of the genus *Lavandula* have an antioxidant activity against the oxidative deterioration of lard. Likewise, Hui et al. [51] and Mansouri et al. [47]analyzed the antioxidant capacity of the lavender's EO on the inhibition of linoleic acid peroxidation, and the inhibition of linoleic acid peroxidation by vitamin E at the same concentration for comparison. They found that lavender essential oil has stronger antioxidant activity than vitamin E, against lipid peroxidation. However, these findings do not agree with the results of Wiesenfeld [52], who showed that extracts of lavender flowers that were collected from different regions do not have any antioxidant activity. These contradictory results are probably related to the difference in the chemical composition between these essential oils [53].

3.7.2. Iron reduction: FRAP (iron reducing-antioxidant power)

The obtained results concerning the study of the antioxidant activity using the iron reduction method "FRAP" of the EOs and extracts are represented in Figures 9 and 10.

L. pedunculata EO has a higher maximum optical density (OD= 0,831) when compared to that of officinal lavender (OD = 0,761), while ascorbic acid recorded the highest absorbance value (OD= 1,904) (figure 9). In addition, the EC_{50} results of the two EOs confirm this conclusion indicating that the concentration, that is effective to reduce 50% of the Fe^{3+} ions of the two EOs is of the order of 4,99 mg/ ml and 5,75 mg/ml for *L. pedunculata* and *L. angustifolia* respectively, while that of ascorbic acid is equal to 2,72 mg/ ml.

Iron reduction by the crude extracts of the two plants depends on the concentration of the extracts (figure 10).

The hydroalcoholic crude extract of *L. pedunculata* has a maximum optical density that is greater (OD = 1,247) than that of *L. angustifolia* (OD =1,17) and less than that of ascorbic acid (OD= 1,758). Likewise, we notice that, the decocted has the lowest antioxidant activity for the two species. In addition, all the EC_{50} values of the extracts confirm this conclusion, the results of extraction by soxhlet and the ethanol/ water mixture show that the effective concentration to reduce 50% of the Fe^{3+} ions, of the two crude extracts of the two lavenders is of the order of 4,34 mg/ml, and 4,74 mg /ml for *L. pedunculata* and *L. angustifolia* respectively. While for aqueous extracts, they are around 5.33 mg/ml (*L. pedunculata*) and 6,05 mg / ml (*L. angustifolia*). Finally, those of the decocted show EC_{50} values of around 6,41 mg/ ml (*L. pedunculata*) and 7,48 mg/ml (*L. angustifolia*). From these results, it can be concluded that the hydroethanolic extracts have an antioxidant activity that is similar to that of the EOs, and rather exceed it. The reducing power of lavender species is probably due to the presence of hydroxyl group in phenolic compounds, which can serve as electron donor. Therefore, antioxidants are considered to be reducing and inactivating oxidants [54]. Some previous studies showed that the reducing power of a compound can serve as a significant indicator of its potential antioxidant activity [55,56].

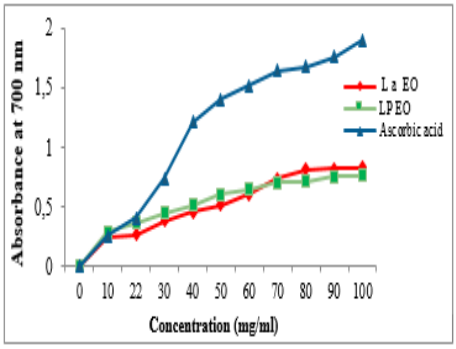

Figure 9: The Antioxidant activities of ascorbic acid and lavenders Eos using the FRAP method

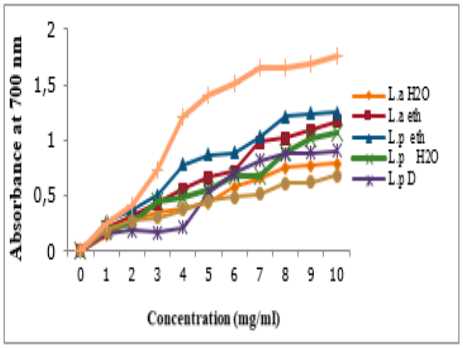

Figure 10: The antioxidant activities of different extracts of *L. pedunculata* and *L. Angustifolia* and ascorbic acid by the FRAP method

3.8. Antibacterial and antifungal activities of the two lavenders

3.8.1. Antibiotic sensitivity tests

The antibiotic sensitivity profiles of the strains indicated in tables 8 and 9 are carried out according to the recommendations of the French Society of Microbiology and EUCAST**.** The results of the sensitivity tests are presented in Tables 8 and 9.

Table 8: Antibiotic sensitivity tests for *acinetobacter baumanii* and *Enterobacter cloacae*

Tested ATB	*Acinetobacter baumanii*	Tested ATB	*Enterobacter cloacae*
TIC75	**R**	**TIC75**	**R**
CAZ30	**R**	**CAZ30**	**S**
MEM10	**R**	**OFX5**	**R**
TIM85	**R**	**AMC3**	**R**
IPM10	**R**	**IPM10**	**S**
CT50	**R**	**CT50**	**R**
TOB10	**R**	**FOX30**	**S**
CIP5	**R**	**AML10**	**R**
TE30	**R**	**CN15**	**S**
CN15	**S**	**AK30**	**I**
AK30	**R**		
PRL75	**R**		

S: Sensitive, I: Intermediate, R: Resistant b. Antibiotics (ATB), Ceftriaxone (CRO), Tobramycin (TOB), Ticarcillin (TIC), amoxicillin (AML), Cefoxitin (FOX), chloramphenicol (C), colistin(CT), Amoxicillin + clavulanic acid (AMC), ciprofloxacin (CIP), amikacin (AK), imipenem (IPM), ceftazidime (CAZ), Piperacillin (PRL), Trimethoprim + sulfomethoxazole (SXT), Tetracyclalexine (TE) (CN), Meropenem (MEM). Tticarcilin + Clavulanic acid (TIM), ofloxacin (OFX).c: Antibiotic disc load "in µg.

Table 9: Antibiotic susceptibility tests for treated bacteria

Tested ATBs	*S. aureus*	Tested ATBs	*E. coli*	*S. typhi*	*Sh. dysenteria*
CIP5	S	CT50	S	S	S
VA30	S	MEM10	S	S	S
TE30	S	TIC75	R	S	S
CN15	S	AK30	S	S	S
MY15	S	C30	S	S	S
E15	S	PRL75	S	S	S
CAZ30	S	IPM10	S	S	S
TOB10	S	CIP5	S	S	S
SXT25	S	AMC30	S	S	S
FD10	S	CN15	R	S	S
FOX30	S	CAZ30	S	S	S
RD30	S	CRO30	S	S	S
OFX5	S	CTX30	S	S	S

S: Sensitive, I: Intermediate, R: Resistant b. Antibiotics (ATB), Ceftriaxone (CRO), Tobramycin (TOB), Ticarcillin (TIC), amoxicillin (AML), Cefoxitin (FOX), chloramphenicol (C), colistin(CT), Amoxicillin + clavulanic acid (AMC), ciprofloxacin (CIP), amikacin (AK), imipenem (IPM), ceftazidime (CAZ), Piperacillin (PRL), Trimethoprim + sulfomethoxazole (SXT), Tetracyclalexine (TE) (CN), Meropenem (MEM). Tticarciline + Clavulanic acid (TIM), chloramphenicol ©, ofloxacin (OFX), Vancanmycin (VA), Lincomycin (MY), Fusidic acid (FD), Refampicin (RD), Cefotaxime (CTX). c: Antibiotic disc load "in µg.

From the antibiogram, we can conclude that the *Acinetobacter* strain is multidrug resistant (Table 8) especially for: TIC75, CAZ30, MEM10, CT50, CIP5, and PRL75.Likewise, *Enterobacter cloacae* strain is resistant to TIC75, OFX5, AMC30, CT50 and AML10. As for the strains of *S. aureus*, *E. coli*, *S. typhi* and *Sh. dysenteria*, they are sensitive to all the antibiotics tested except *Escherichia coli*, which is resistant to TIC75 and CN15 (Table 9).

3.8.2. Determination of the antibacterial activity of the essential oils using the disk diffusion method (aromatogram)

The determination of the antibacterial activity of the essential oils consists of the determination of the inhibition diameters using the disk diffusion method. We tested the antibacterial effect of the EOs of the two lavender species on six bacterial strains.

Based on previous works, we considered that an EO or extract has a bactericidal action if their diameter of inhibition is greater than 12 mm [57,58].

The experimental results presented in Table 10 show that the EOs of the two lavenders act actively on Gram (-) bacteria (*E.coli*, *S. tiphy*, *A. baumani*, *E. cloacae*, *Sh. dysenteria*) and Gram (+) bacteria (*S. aureus*). The inhibitory effect of the tested EOs increases significantly with the volume. Moreover, their effectiveness is well proved by the obtained results on multidrug resistant *Acinetobacter*.

Moreover, the results indicate that the antibacterial activity of the essential oil of pedunculate *lavender* was found to be less active than that of *L. angustifolia*; this appears very clearly when they were tested against *E. coli* and *E. cloacae*. Also, according to the diameter of inhibition factor, the statistical study (ANOVA) confirms a highly significant variation of the two EOs of *L. pedunculata* (P = 1.12 x 10-4) and *L. angustifolia* (P = 0) between the calculated means, with P <0.05 (Table 10).

In addition, in comparison with the reference antibiotics, the effect of the tested EOs remains moderate for some used concentrations. But it should be noted that *L. angustifolia* EO, from the volume of "8 µl", exceeds the effect of reference antibiotics when tested against *E. coli* and *A. baumani*, these effects are probably due to the majority compound of the EOs. Our results corroborate with those found by other researchers: Bachiri et al.,[40] mentioned that the *L. pedunculata* EO (collected in Morocco) is active against Gram (-) bacteria (*E. coli, K. pneumoniae* and *P. mirabilis*) and Gram (+) (*S. aureus*). Mohammedi et al. [24], found that the EO of *L. stoechas* (a species very close to *L. pedunculata*) has a weak activity against *E. coli* and *K. pneumonia* (8,19 ± 1,49 mm and 5,88 ± 0,57 mm). Likewise, the antibacterial effect of 11 EOs of *L. stoechas latosensu* (*L. virdis*, *L. stoechas*; *L. pedunculata*, the subspecies and the hybrids) collected in Algeria was positive on Gram + bacteria "*Bacillus subtilis*", and Gram negative bacteria (*E. coli, K. pneumoniae, S. enteric* and *Ps. aeruginosa*) [59]. Finally, it was revealed that the volatile oil of *L. stoechas* (L.) from Tunisia, has an antibacterial activity against six bacterial species among which *S. aureus* is the most sensitive [60].

Table 10: Inhibition zone diameters of tested strains by studied *Lavandula* EOs using the disk diffusion method (aromatogram) (mm).

EO tested Concentration (µl/ml)	*S. aureus (mm)*		*E. coli (mm)*		*S. tiphy (mm)*		*A.baumanii (mm)*		*E. Cloacae (mm)*		*Sh. dysenteria (mm)*	
	L.a EO	*L.p EO*	*L.a EO*	*L.p EO*	*L.a EO*	*L.p EO*	*L.a EO*	*L.p EO*	*L.a EO*	*L.p EO*	*L.a EO*	*L.p EO*
2	**15±0.8**	**20±0.18**	**10±0.1**	**08±0.5**	**11±0.1**	**09±08**	**11±0.12**	**11±00**	**10±0.1**	**07±0.9**	**08±0**	**10±0**
4	**20±0.1**	**22±0.2**	**12±0.4**	**10±0.9**	**12±0.3**	**10±02**	**13±0.5**	**12±00**	**11±0**	**09±0.7**	**10±0.1**	**11±0.1**
6	**22±0.82**	**25±0.4**	**34±0.7**	**11±1.8**	**14±0.7**	**12±08**	**20±0.1**	**14±0.18**	**14±0.1**	**10±0**	**11±0.5**	**12±0.5**
8	**25±0.5**	**28±0.6**	**36±0.5**	**12±0.6**	**16±0.4**	**13±01**	**30±0.4**	**15±0.6**	**20±0.5**	**11±0**	**12±0.14**	**13±0.3**
10	**28±1.3**	**30±0.9**	**38±0.6**	**13±0.2**	**18±0.5**	**14±08**	**40±00**	**18±0.2**	**20±0.4**	**12±0.6**	**13±0.8**	**14±0.2**
12	**30±0**	**35±0.3**	**40±0.14**	**14±0.1**	**20±0.1**	**15±05**	**40±00**	**20±0.1**	**24±0.6**	**14±0.5**	**14±0.17**	**15±0.8**
6 (DMSO)	**06±00**	**06±00**	**06±00**	**06±00**	**06±00**	**06±00**	**06±00**	**06±00**	**06±00**	**06±00**	**06±00**	**06±00**
6 (DW)	**06±00**	**06±00**	**06±00**	**06±00**	**06±00**	**06±00**	**06±00**	**06±00**	**06±00**	**06±00**	**06±00**	**06±00**

EO : Essential oil ; *L.a : Lavandula angustifolia ; L.p : Lavandula pedunculata*

The antimicrobial activity of *L. pedunculata* EO is mainly due to its richness in the following constituents: fenchone, camphor, borneol, cubenol <1,10-di-epi>. Indeed, all these compounds are known for their antimicrobial properties. The antibacterial and antifungal properties of the EOs are linked to their contents of phenolic compounds, alcohols, ketones etc. these compounds are capable of inhibiting the development of microorganisms (bacteriostasis, fungistasis, virustase) or of having a lethal action (bactericidal, fungicidal, virucidal, sporicidal) according to different mechanisms of action: coagulation of intracellular organelles, alteration of membranes [61].

A study conducted by Vakilian et al. [62] proved the antiseptic effect of the essential oil of *L. angustifolia* on a group of 60 women, who have an episiotomy during their childbirth. They were treated with the essential oil of *L. angustifolia* that was freshly distilled, and diluted in olive oil to make a 1.5% solution. They took sitz baths with 5 to 7 drops of the prepared solution in 4 liters of water, twice a day for 10 days. On the 10th day, the group of women receiving treatment with the EO of *L. angustifolia* did not present complications of microbial origin compared to the control group. These results prove the effectiveness of L. *angustifolia* essential oil and confirm its antiseptic activity. Our results are also in agreement with those of Chahboun et al. [22] and Jianu et al. [63] who observed a significant antibacterial effect of *L. angustifolia* essential oil.

The experimental results presented by Oughendjioua et al. [31] showed that the essential oil of *L. angustifolia* from the Skikda region in Algeria has significant antibacterial activity when tested against three Gram+ cocci: β-hemolytic *streptococcus* of group A, *S. aureus* and *S. epidermidis*.

3.8.3. Determination of the antibacterial activity of lavenders EOs by (MIC) and (MBC) methods

Sensitive strains to both EOs were tested by the MIC and MBC methods, the results are shown in Table 11.

Determination of the MIC of the EOs, with a concentration range varying from 2 to 30 µl/ml, shows that the *S. aureus* strain is the most sensitive to the two EOs of *L. angustifolia* and *L. pedunculata*,

the MIC was manifested from the volume of 2µl/ ml. The MICs recorded for the other strains vary depending on the EO. Indeed, *A. baumanii* resisted up to10 µl /ml of *L. pedunculata* EO and to 6µl / ml of *L. angustifolia* EO. CMBs differ depending on the tested bacterial strains. *S. aureus* was the most sensitive to the two EOs, and its total inhibition (100%) was at a volume of 4 µl/ml. As for *A. baumanii, E. Cloacae* and *Sh. dysenteria,* CMB values remained identical to those of MIC (Table 11).

Table 11: Evaluation of the MIC and the MBC of *Lavenders* EOs against bacterial strains

Bacterial strains	*L. pedunculata* EO		*L. angustifolia* EO	
	MIC (µl/ml)	MBC (µl/ml)	MIC (µl/ml)	MBC (µl/ml)
E. coli	06±00	08±00	04±00	06±00
S. aureus	02±00	04±00	02±00	04±00
S.typhi	06±00	06±00	04±00	06±00
A.baumanii	10±00	10±00	06±00	06±00
E. Cloacae	06±00	06±00	04±00	04±00
Sh.dysenteria	06±00	06±00	06±00	06±00

3.8.4. Determination of the antibacterial activity of different extracts of the two lavenders using the disk diffusion method (aromatogram)

In this work, we studied the antibacterial power in vitro of the hydroethanolic (S1), aqueous (S2), and decocted (S3) extracts of both *L. pedunculata* and *L. angustifolia*. Using the disc diffusion method, the antibacterial activity of our extracts was determined by measuring the diameters of the inhibition zones. The results are summarized in Table 12.

According to table 11, the antibacterial effect of S1 and S2 extracts depends on the concentration of the extract; at 10 mg/ ml, both extracts had no effect on bacterial strains, while from 20 mg / ml the S1 extract of both plants had an antibacterial effect on *E.coli, S. aureus, A.baumanii* and *Sh. dysenteria*, and the S2 extract was only active on *E. coli* and *S. typhi*. As for *E. cloacae*, it was resistant to the three extracts. The ANOVA test confirmed a highly significant variation between the calculated means of the diameters of the inhibition zones of the three extracts, with $P<0.05$ (Table 11).

The difference in sensitivity of bacterial strains can be attributed to both the chemical nature of the tested extracts and the nature of the bacterial strains. According to the assay results, the hydroethanolic extract is the richest in polyphenols. At 40 µg/ ml, the hydroethanolic extract of *L. pedunculata* showed considerable inhibition zones on the tested strains, where a maximum of 23 ± 00 mm is noted for *E. coli*. The 40 µg/ml aqueous extract showed considerable inhibition zones on susceptible bacterial strains where the maximum of 20.5 ± 0.3mm is noted on *E. coli*. Therefore, we can say that the polyphenols contained in *L. pedunculata* are responsible for its considerable antibacterial effect compared to *L. angustifolia*, which contradicts our previous findings concerning the EOs effects, where *L. angustifolia* was the most powerful against some bacterial strains. The decocted (S3) had no effect on the studied strains; this can be explained by the nature of the extracts and their richness in polyphenols, while the decocted chemical composition, which is poor in phenolic compounds, is responsible for the absence of its antibacterial activity.

According to the study of Benyagoub et al. [39] on the effect of *L. angustifolia* methanolic macerate, this extract had a moderate antibacterial activity, that manifested only on Gram-positive bacteria: *S. aureus*, *B. cereus* and *Clostridium perfringens*, this result is different from ours, which indicated a considerable antibacterial effect on both Gram positive and Gram negative bacteria, while the aqueous macerate showed no antibacterial activity.

Table 12: Zones of inhibition diameters of tested strains by the three studied extracts using the disk diffusion method (aromatogram)

		Extracts of *L. pedunculata* (mm)			Extracts of *L. angustifolia* (mm)		
	Extracts [mg/ml]	S1	S2	S3	S1	S2	S3
S. aureus	10	06±00	06±00	06±00	06±00	06±00	06±00
	20	20±0.2	18±0.1	06±00	13 ±0.6	12±0.1	06±00
	30	21.1±1.2	19.2±1.5	06±00	13.5±00	12.3±0.1	06±00
	40	22.8±01	19.7±2.1	06±00	14.1±0.18	14.1±0.9	06±00
E. coli	10	06±00	06±00	06±00	06±00	06±00	06±00
	20	15.7±2.1	12.8±1.5	06±00	10±0.9	06±00	06±00
	30	22±0.2	20±0.1	06±00	14±1.3	06±00	06±00
	40	23±00	20.5±0.3	06±00	15±0.7	06±00	06±00
S. typhi	10	06±00	06±00	06±00	06±00	06±00	06±00
	20	06±00	06±00	06±00	06±00	06±00	06±00
	30	06±00	06±00	06±00	06±00	06±00	06±00
	40	06±00	06±00	06±00	06±00	06±00	06±00
A. baumanii	10	06±00	06±00	06±00	06±00	06±00	06±00
	20	15.5 ±0.5	06±00	06±00	13±0.6	06±00	06±00
	30	16 ±0.3	06±00	06±00	14.1±0.1	06±00	06±00
	40	17.5 ±0.1	06±00	06±00	14.2±0.3	06±00	06±00
E. cloacae	10	06±00	06±00	06±00	06±00	06±00	06±00
	20	06±00	06±00	06±00	06±00	06±00	06±00
	30	06±00	06±00	06±00	06±00	06±00	06±00
	40	06±00	06±00	06±00	06±00	06±00	06±00
Sh. dysenteria	10	06±00	06±00	06±00	06±00	06±00	06±00
	20	17.5±0.2	06±00	06±00	9±0.1	06±00	06±00
	30	18±0.1	06±00	06±00	10.1±0.3	06±00	06±00
	40	18.4±0.8	06±00	06±00	10.3±0.1	06±00	06±00
Distilled water	10	06±00	06±00	06±00	06±00	06±00	06±00
	20	06±00	06±00	06±00	06±00	06±00	06±00
	30	06±00	06±00	06±00	06±00	06±00	06±00
	40	06±00	06±00	06±00	06±00	06±00	06±00

3.8.5. Determination of the minimum inhibitory and bactericidal inhibitory concentrations of the tested extracts

The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (CMB) of the studied extracts, when tested against some sensitive bacterial strains are mentioned in Table 13. According to these results, there are differences between the studied extracts effects.

Table 13: Minimum and bactericidal inhibitory concentrations of the two hydroethanolic and aqueous extracts.

Concentration	*S. aureus*				*E. coli*			*A.baumanii*		*Sh. dysenteria*	
	S1 L.a	*S1 L.p*	*S2 L.a*	*S2 L.p*	*S1 L.p*	*S1 L.a*	*S2 L.p*	*S1 L.p*	*S1 L.a*	*S1 L.p*	*S1 L.a*
MIC (mg/ml)	40±00	20±00	40±00	30±00	30±00	40±00	30±00	30±00	40±00	40±00	40±00
MBC (mg/ml)	40±00	20±00	40±00	30±00	30±00	40±00	30±00	30±00	40±00	40±00	40±00

The minimal inhibitory activity of the S1 extract of *L. pedunculata* was revealed at a concentration of 20 µl/ml, 30 µl/ml, 30 µl/ml and 40 µl/ml respectively for *S. aureus*, *E. coli*, *A. baumanii* and *Sh. dysenteria*, while S1 extract of *L. angustifolia*, showed a MIC of 40 µl /ml for all the sensitive tested strains. The S2 extract of *L. pedunculata* inhibited the growth of *E. coli* and *S. aureus* at a concentration of 30 µl/ml, while S1 of *L. angustifolia* was active only on *S. aureus* at 40 µl/ ml. The weakest bactericidal activity was that of S1 extract of *L. pedunculata* when tested against *S. aureus*, which is more active than S2 of the same plant as well as those of *L. angustifolia*.

The results reported in Table 13 are expressed as the mean ± standard deviation. These results are in agreement with previous studies, where the hydroethanolic and aqueous extracts of *L. pedunculata* have also shown significant antimicrobial activity at low doses of MIC and MBC.

3.8.6. Sensitivity of fungal strains

The antifungal sensitivity profiles of the strains indicated in Table 14 were carried out according to the recommendations of EUCAST.

Table 14: Sensitivity test to the selected antifungal strains

Fungal strains	Antifungal: Fluconazole (V=20 µl)
Candida glabrata (L13)	S
Candida albicans (L14)	S
Candida spp (L15)	R
Aspergillus fisheri G2	R
Fusarum solani H13	R

Both *C. glabrata* and *C. albicans*strains are susceptible to the antifungal, while *Candida spp, A. fisheri* and *F. solani* are resistant.

3.8.7. EOs antifungal activity

Several studies confirmed the Lavender's antibacterial and antifungal activities in vitro[64]. Our volatile oils showed a very good antifungal power. These results revealed a strong antifungal capacity of the two lavenders EOs. According to Meena et al. [65] and Ponce et al. [66] studies, the diameter of the inhibition zones is noted as: not sensitive for diameters that are less than 8 mm; sensitive for diameters between 9 and 14 mm; very sensitive for diameters between 15 and19 mm, and extremely sensitive for diameters greater than 20 mm. Molds and yeasts showed high sensitivity (extremely sensitive) to the concentration of 20 µl of the two tested EOs, with diameters of the inhibition zones of *L. Angustifolia* EO of: 52 mm for *Candida albican* (L13), 22 mm for *C. glabrata* (L14), 33 mm for *Candida ssp* (L15), 20 mm for *F. solani* (H13) and 40 mm for *A. fisheri* (G2). The P value of the ANOVA test confirmed a highly significant variation of the two EOs, with P <0.05 (Table 15).

Table 15: *L. pedunculata* and *L. angustifolia* Eos effect on the yeasts and molds growth

Species	EO(mm)/C = 20 µl/ml						
	L13	**L14**	**L15**	**H13**	**G2**	**Distilled water**	**DMSO**
L. angustifolia	52±0,1	22±0,2	33±1,2	20±1,3	40±2,1	06±00	06±00
L. pedunculata	23 ±0,5	25±2,1	18±0,1	23±0,2	06±00	06±00	06±00

As for *L. pedunculata*, its EO was less active against *candida ssp* (18 mm) and inactive against G2 (Table 15). This effect is attributed to the content of the Eos and their major compounds, mainly camphor, fenchone and linalool. By analyzing the chemical composition of the two EOs, it was found that they contain a high percentage of alcohols, ketones and minority families such as esters and aldehydes… (figure 2), which justifies their effectiveness against bacteria and fungi. Indeed, it has been reported by researchers that the inhibitory activity of an essential oil results from a complex interaction between its different constituents [67, 68, 69]. According to previous studies, *L. angustifolia* EO contains chemical compounds with high efficiency and a wider spectrum, which explains its strong antifungal activity. These compounds are phenols (1,8 cineole, carvacrol), alcohols (α-terpineol, terpinen-4-ol, linalool), aldehydes and ketones (camphor, etc.) [70]. A study testing *L. pedunculata* EO antifungal activity confirms its effectiveness against three molds of which *Rhizopus stolonifera* was the most sensitive and the inhibition of its growth was almost complete at a concentration of only 0,5 µl / ml [39].

3.8.8. Study of the MIC and MFC of the two EOs

The antifungal activity of the two lavenders EOs on the tested molds and yeasts is of a fungicidal nature, which we have proved experimentally (Table 16).

The MIC tests revealed that all molds and yeasts were 100% inhibited at low concentrations. However, the antifungal activity depends on both: the essential oil and the strain. *L. angustifolia* EO was very active against all fungal strains, which were completely inhibited at a concentration of 20 µl / ml. In contrast, *Aspergillus* (G2) (mold) showed some resistance to *L. pedunculata* EO. The MFC/ MIC ratios of the two EOs are equal to 1 for all the studied fungal strains. These EOs therefore seem to have a fungicidal action against all sensitive fungal strains.

Table 16: MIC and MFC values of *L. pedunculata* and *L. angustifolia* EOs

	Extracts (mm)/ V = 20 (mg/ml)				
	L13	**L14**	**L15**	**H13**	**G2**
L,a S1	13±00	23±00	17.1 ±0.2	90±00	06±00
L,p S1	11±00	18.5±00	10±00	90±00	06±00
L,a S2	10±0.1	15±0.2	9.5.5±0.1	84±0.1	06±00
L,p S2	9.4±0.1	12.5±0.3	8.9±0.1	80±0.2	06±00
L,a S3	06±00	06±00	06±00	06±00	06±00
L,p S3	06±00	06±00	06±00	06±00	06±00

3.8.9. Activity of hydroethanolic, aqueous, and decocted extracts

The antifungal potential of the extracts of the two lavenders was tested against the fungal strains. The H13 strain which gave great results against the reference antifungal, was completely inhibited by the hydroethanolic and aqueous extracts (table16). On the contrary, the G2 strain was resistant to the three extracts.

It is worth mentioning that, the hydroalcoholic extracts of the two lavenders gave inhibition diameters that were greater than those given by the aqueous extracts, on the other hand the decocted of the two species were inactive. Also, the S1 and S2 extracts of *L. angustifolia* were more active against sensitive strains compared to those of *L. pedunculata*. The ANOVA test confirms a highly significant variation between the calculated averages of the inhibition diameters of the three extracts, with $P < 0.05$ (Table 17).

These results are in agreement with those of Lopez et al. [71], who tested the antifungal activity of *L. pedunculata* extracts and found that the hydroethanolic extracts have significant inhibitory potentials.

Table 17: Effect of extracts of *L. pedunculata* and *L. angustifolia* on the growth of yeasts and molds

Fungal strains	*L. pedunculata* EO			*L. angustifolia* EO		
	MIC (µl/ml)	MFC (µl/ml)	MFC /CMI	MIC (µl/ml)	MFC (µl/ml)	MFC /MIC
L13	20±00	20±00	1	20±00	20±00	1
L14	20±00	20±00	1	30±00	30±00	1
L15	30±00	30±00	1	20±00	20±00	1
H13	20±00	20±00	1	20±00	20±00	1
G2	-	-	-	20±00	20±00	1

The minimum inhibitory concentrations (MIC) and the minimum fungicidal concentrations (CMF) of the studied extracts are mentioned in Table 18.

According to the obtained results, S1 and S2 extracts of *L. angustifolia* gave effective results against fungals and recorded the lowest MIC and CMF value (20 mg/ ml) for L13strain, similarly the inhibitory action of S1 extract of *L. angustifolia* recorded 30 mg/ ml of MIC and CMF.

Unlike the previous lavender, *L. pedunculata* was less active against all fungal species and recorded the highest values (40 mg/ml) of MIC and CMF for both S1 and S2extracts. The CMF/ MIC ratios of the two extracts (S1 and S2) were equal to 1 for all the fungal studied strains. These extracts therefore have a fungicidal action against all susceptible fungal strains.

Table 18: MIC and MFC values of *lavenders* extracts

Fungal strains	*L13*			*L14*			*L15*			*L13*		
	MIC (mg/ml)	MFC (mg/m)l	MFC /MIC	MIC (mg/ml)	MFC (mg/ml)	MFC /MIC	MIC (mg/ml)	MFC (mg/ml)	MFC /MIC	MIC (mg/ml)	MFC (mg/ml)	MFC /MIC
L.a S1	30±00	30±00	1	40±00	40±00	1	40±00	40±00	1	20±00	20±00	1
L.p S1	40±00	40±00	1	40±00	40±00	1	40±00	40±00	1	40±00	40±00	1
L.a S2	40±00	40±00	1	40±00	40±00	1	40±00	40±00	1	20±00	20±00	1
L.p S2	40±00	40±00	1	40±00	40±00	1	40±00	40±00	1	40±00	40±00	1

4. Conclusions

This work aims the promotion of Moroccan MAPs in Ifrane region, especially lavenders: wild (*L. pedunculata*) and cultivated (*L. angustifolia*); It shed the light on the interspecific comparative study of *L. pedunculata* and *L. angustifolia* at the level of chemical composition (from a qualitative and quantitative point of view), the yields and the antioxidant and antimicrobial activities against certain bacterial strains, and fungal pathogens.

The results of the phytochemical screening of the aerial parts of the two studied lavenders, revealed a similarity in terms of their qualitative composition, with a slight difference in the level of catechic and saponoside tannins.

Moreover, the results of the extracts analysis revealed that *L. pedunculata* contains higher contents of polyphenols and flavonoids than *L. angustifolia*, which is richer in tannins.

The determination of the yields of the crude aqueous extracts, made it possible to retain the most efficient extraction method for each species; which is the extraction by soxhlet, and by hydroethanolic solution, followed by the aqueous extract by soxhlet and decocted, with a significant yield noted in the hydroethanolic extract of *L. angustifolia*. Also, the efficiency of the EOs extraction by hydrodistillation showed high profitability in *L. angustifolia*.

The results of the EO analysis showed that the two lavenders species contain common compounds but with different concentrations. *L. angustifolia* contains camphor and linalool as majority compounds, while camphor and fenchone are the majority compounds of *L. pedunculata*.

The antioxidant activity tests using two methods (DPPH and FRAP), revealed that the essential oil of *L. angustifolia* is less effective than that of *L. pedunculata*. On the other hand, there is no great divergence concerning the IC_{50} values of the hydroethanolic extract and decocted of the two species. As for the aqueous extract, the IC_{50} value recorded by *L. pedunculata* was slightly higher than that of *L. angustifolia*.

Antimicrobial activity tests (antibacterial and antifungal) of essential oils and extracts of the two species indicated a moderate antibacterial effect of *L. pedunculata* essential oil, in comparison with that of *L. angustifolia*, while the hydroethanolic and aqueous extracts of *L. pedunculata* had greater antibacterial power. In addition to that, the hydroethanolic and aqueous extracts of *L. angustifolia* showed a remarkable antifungal power against the tested fungi in comparison with *L. pedunculata*. We can thus conclude that, the two lavenders EOs antibacterial power at low concentrations was confirmed, while it was manifested at high concentrations for the extracts. The essential oils of the two studied lavenders are therefore more effective than their hydroethanolic and aqueous extracts.

Finally, the essence of *L. pedunculata* showed a very marked antioxidant effect, while the EO of *L. angustifolia* had the most important antibacterial power. This difference is very probably related to their chemical compositions (underlined by gas chromatographic analysis of the essential oils), but further studies concerning the genetic sequencing of the two lavender species, should be conducted so as to investigate possible alternative explanations for this dissimilarity.

Conflicts of interest

There are no conflicts of interest.

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