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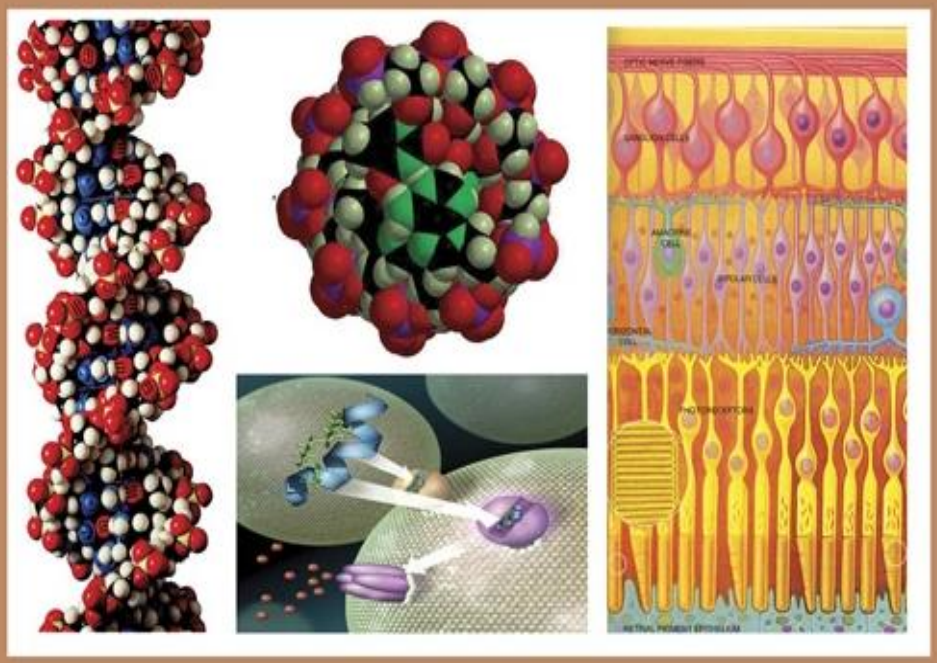

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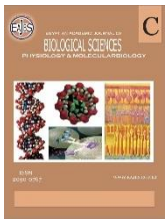

Chemical Composition; Antioxidant and Antibacterial Activity of *Lavandula officinalis* Flowers Essential Oil

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ABSTRACT

Several researches have been focused on aromatic plants extracted essential oils, indicating that they have many biological properties. In this context, we tried to evaluate the *in vitro* antibacterial and antioxidant activities of the *Lavandula officinalis* dried flowers essential oil. The extraction was carried out by hydrodistillation of the plant flowers, the yield was close to 1.45 %.The essential oil composition study by GC/MS ,identified 28 terpenes essentially composed by the acetate (17.85% , 89.980 min); 1.8-cineole (9.97 % ,53.512 min); γ-terpineol (10.23, % 52.425 min) and camphor (12.98 % ,24.410 min). The well and disk methods demonstrated a strong antibacterial activity of the *Lavandula officinalis* flowers essential oil. *Staphylococcus aureus* ATCC 9027, *Escherichia coli* ATCC 25922 and *Enterobacter aerugenes* ATCC 1710 were the most sensitive bacteria, with inhibition zone diameters speared from 30 to 40 mm. Also, this essential oil exhibited a slight antioxidant activity carried out by the DPPH and FRAP methods.

INTRODUCTION

Studies have been conducted on the development of new applications, and the exploitation of the essential oil natural properties in the food industry. Essential oils and their components are known to have antioxidant activities and could therefore be used as food preservatives agents, or approved as food additives (Sharififar *et al.*,2007). In addition, essential oils are starting to have a lot of interest as a potential source of *Natural Bioactive Molecules*. They are being investigated for their possible use as an alternative for protecting foods from oxidation (Sharififar *et al.*,2007). Undoubtedly, chemical or synthetic food preservatives are part of the range of techniques that ensure consumer safety, extend the food products life and limit their modification by oxidation (Sharififar *et al.*,2007); however, the search for new molecules has been necessary because these synthetics substances have shown a number of disadvantages and limitations in their use.

Indeed, the Butyl hydroxyanisole (BHA) and Butylhydroxytoluene (BHT) would have carcinogenic effects (Kulevanova *et al.*,2000). These many natural properties of essential oils make them very promising preservatives for the food industry. The use of essential oils is a relevant choice in the face of a contamination-specific risk or the need to reduce or replace chemical or synthetics preservatives (Kulevanova *et al.*, 2000).This topic seemed to us very interesting since Algerian Flora is extremely rich in aromatic plants. Hence, our work aimed to promote the flowers *Lavandula* species, which grows spontaneously in the Collo region of Skikda (East Algeria). Essentially, the study was carried out to identify the chemical composition of the extracted *Lavender* flowers essential oil by the Gas chromatography-Mass spectrometry (GC/MS) and to determine the antioxidant and antibacterial power of this essential oil.

MATERIALS AND METHODS

Plant Material:

Lavandula officinalis: is a bushy tree that can reach up to 1 m high. The leaves are linear and green-grey, have a length ranging from 3 to 5 cm. The stem is woody and the flowers are a blue group in the arm of oval bracts, on the top of fertile branches forming a kind of a little loose, very aromatic.

- Domain: Eukaryota
- Kingdom: plant.
- Divison : vascular plants
- Phylum: Spermatophyta
- Subphylum: Angiospermae
- Class: Dicotyledonae
- Clade: Angiosperms
- Sub-Class: Dialypetales
- Order: Lamials (Labial)
- Family: Lamiaceae
- Genus: *Lavandula*
- Species: *officinalis L.*

Harvesting and Drying:

The *Lavandula officinalis* flowers come from the Collo region of Skikda (East Algeria). The Harvest was carried out manually, when the plant was in full bloom during the month of March 2019. The harvested flowers are dried away from the light and at room temperature for 15 days before being used for extraction.

The Tested Bacterial Strains:

The bacterial strains used in our study included *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 25922, *Baccilus anthracis* ATCC 6633, *Proteus mirabilis* ATCC 7002, *Enterobacter aerogenes* ATCC 1710, *Klebsiella pneumoniaie* ATCC 35218, *Acinetobacter baumannii* ATCC 7689, *Enterococcus cloacae* ATCC 27412 and *Listeria monocytogenes* ATCC 5849. All strains were provided by the Hygiene laboratory of Chlef 's Hospital.

The Essential Oil Extraction and GC/ MS Characterization:

The equipment used for hydrodistillation is the Clevenger type, it consists of a boiling flask, a Pyrex glass flask containing 10 g of dried flowers and 100 ml of distilled water, a cooler, and a collector. The determination of the essential oils chemical composition was made by gas chromatography coupled with mass spectrometry (GC/MS) of the type PerkinElmer 500. Samples are diluted in methanol (1/20) (v/v) and, the used gas was the helium with a 1 ml/min of flow rate. The temperature of the column was programmed from 60 to 275°C (Sing *et al.*,2006). The fragments were carried out by electronic impact under a field of 70 EV, with a balayage scan of 80-600 Uma, a quadruple analyzer with a solvent delay of 5.90 minutes. The components identification was based on the release time of each peak, the "retention time". The area limited by these peaks allowed the

concentration measure of each separated compound (Sing *et al.*,2006). Then, the essential oil was stored in the refrigerator in a hermetically sealed brown glass bottle at +4°C.

The Antioxidant Activity Evaluation: DPPH (1.1-Diphenyl-2-Picryhydrazyl) Method:

The free radical scavenging activity was determined *in vitro* according to the method described by (Burits and Bucar, 2000). The analysis was performed as 50 µl of the essential oils metanoic solutions tested at different concentrations (200 g/ml, 400g/ml, 600g/ml, 800g/ml and 1000g/ml) are mixed with 5ml of a DPPH metanoic solution (0.004%). The mixture was vigorously stirred and left to rest for 30 minutes in the dark at room temperature.

A coulometric evaluation was then carried out using a spectrophotometer at 517 nm. The inhibitory of the free radical DPPH by ascorbic acid was also tested with the same concentration for comparison.

The reaction kinetics and the calculating parameters of antioxidant activity for ascorbic acid and essential oil inhibition percentage %, IC_{50}, and antiradical efficiency were determined. All tests were performed in triplicate.

The Inhibition Percentage Determination:

The reduction of the free radical of DPPH as a percentage (I%) was calculated as follows: (Sharififar *et al.*, 2007).

$$I\ \% = ((CA - SA) / CA) \times 100$$

CA: The control absorbance, SA: The sample absorbance. The reaction kinetics of the essential oil and ascorbic acid, with DPPH is recorded at each studied concentration. The level of essential oil and ascorbic acid, depending on the percentage of the inhibitors, was determined at the end of the reaction to obtain the index. The extract concentration providing 50% inhibition was calculated. A lower IC_{50} value means a higher antioxidant activity.

The FRAP Iron Reduction Test (The Ferric Reducing Antioxidant Power):

The reducing power of iron (Fe^{3+}) in essential oils was determined according to the method described by (Oyaizu, 1986). Six different concentrations of the *methanolic aqueous* solutions (0.66-16.66 mg/ml), were mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of 1%(w/v) of $(K_3Fe\ (CN)_6$ solution. The mixture was incubated for 20 minutes in a water bath at 50°C. The incubated mixtures were allowed to cool at room temperature. After cooling, 2.5 ml of 10 % (w/v) TCA solution was added. The absorbance was measured at 700 nm. The positive control was represented by an antioxidant standard solution of ascorbic acid, in which absorbance was measured under the same conditions as the samples (Lis-Balchin and Deans,1997).

The Study of Essential Oil Antibacterial Effect (The Aromatogram):

The *Lavandula officinalis* essential oil antibacterial activity evaluation was made by the disk diffusion method and the good method test.

1-The Disk Diffusion Method:

The Mueller Hinton agar plates are flooding streaked by the bacterial inoculum. After drying of 30 min at 37°C, 6 mm diameter sterile paper discs were infused with *Lavandula officinalis* essential oil, with a volume ratio of 5 µl per disk, on the surface of the plate previously inoculated with the tested bacterial strains (Cermeli *et al.*, 2008; Belhadj *et al.*, 2006). After incubation, the absence of bacterial growth was occurred by a clear halo around the disk, which diameter was expressed in mm. Each test was repeated three times, the antibacterial activity can be determined by the sensitivity to essential oil as described by Joffin et Leyral (2005).

Resistance (not sensitive) (-): Diameter less than 8 mm

- ✓ Sensitive (+): Diameter between 9 and 14 mm
- ✓ Very Sensitive (++): Diameter between 15 and 19 mm
- ✓ Extremely sensitive (+++): Diameter greater than 20 mm

2-The Well Method Test :

The Well method is based on the essential oils migration power on a solid medium inside a Petri plate. This method was used to reveal the antibacterial effect of essential oil on bacteria, as well as the determination of bacteria resistance or sensitivity to essential oil. According to (Ponce *et al.*,2003), the essential oil sensitivity has been classified by the inhibitions diameters halos:

- Non-sensitive (-) for diameters less than 8 mm
- Sensitive (+) for diameters of 8 to 14 mm
- Very sensitive (++) for diameters of 15 to 19 mm
- Extremely sensitive (+++) for diameters more than 20 mm.

RESULTS

The essential oils are usually liquid at room temperature and volatile, which differentiates them from fixed oils. They are more or less colored and their density is generally lower than that of water. The *Lavandula officinalis* flower's essential oil color is light yellow.

The *Lavandula officinalis* Essential Oil Yield:

The obtained results indicate that the essential oil extraction performance by hydrodistillation was at 1,45 %. The extraction rate showed that all essential oils are almost extracted after the first 90 minutes.

The Essential Oil Analysis:

The essential oil composition studied by GC/MS identified 28 terpenes components, whose the principals were: the acetate (17.85% ,89.980 min); 1.8-cineole (9.97%,53.512 min); γ-terpineol (10.23 % ,52.425 min) and camphor (12.98 %,24.410 min) (Table.1).

Table 1: Chemical composition of *Lavandula officinalis* essential oil

No.	Chemical compounds	Index	%
01	Linalol	87.980	10.98
02	α-terpineol	73.205	0.5
03	χ-terpineol	52.425	10.23
04	Borneol	59.855	1,43
05	Iso-borneol	57.430	0.6
06	Terpinene-4-ol	61.751	0.7
07	Nerol	54.129	0,20
08	Lavandulol	60.235	0.7
09	Anthanilate acetate	89.980	17.85
10	Geranyl acetate	57.430	0,60
11	Neryl acetate	26.490	0,32
12	Oct-3-yl acetate	30.112	0.65
13	Acétate de lavandulyle	87.950	0.08
14	Myrcene	40.114	0.46
15	α-pinene	89.997	0.3
16	β-pinene	18.330	0.25
17	Camphene	24.410	12.98
18	E-β-ocimene	43.571	0.75
19	Z-β-ocimene	42.151	0.87
20	β-phellandrene	25.337	0.12
21	1,8-cineol	53.512	9,97
22	β-caryophylene	25.337	4.62
23	β-farnesene	29.990	2.73
24	Germacrene	54.129	0.27
25	α-humulene	43.622	0.95
26	Camphre	76.469	13,01
27	Octanone-3	13.201	1.7
28	Cryptone	55.624	0,35

The Antioxidant Activity Evaluation:

The antioxidant activity of essential oils was tested *in vitro* by two different tests: free radical reduction DPPH, and FRAP iron reduction.

The Free Radical DPPH Scavenging Test:

The anti-radical activity of *Lavandula officinalis* essential oil was tested *in vitro* by the DPPH test and compared to ascorbic acid (Fig. 1). The results underlined the antioxidant power of this essential oil increasing with its concentration. However, even with high concentrations, the antioxidant activity of this oil remains too low compared to that of ascorbic acid. The IC_{50} value of the *Lavender* essential oil and ascorbic acid are given in (Table.2).

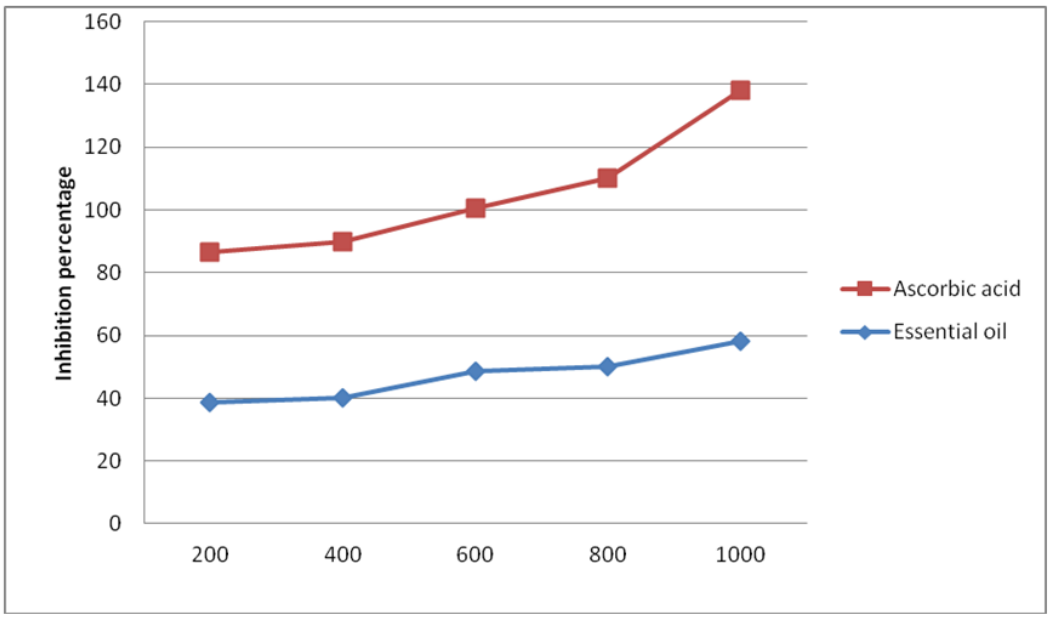

Fig 1: Inhibition percentage for essential oil and Ascorbic acid

Table 2: Antioxidant activity tested by; DPPH %, FRAP and IC_{50}.

	DPPH inhibition %	FRAP	IC_{50}
EO	9.18±0.97	0.25±0.00	38.08
Ascorbic acid	95.11±0.15	4.11±0.01	0.23
BHT	71.03±0.01	3.01±0.03	0.34

IC_{50}%: Signifies (indicates) the concentration (g/L) for a 50% inhibition / E.O: *Lavandula officinalis* essential oil.

The Determination of the Antimicrobial Activity:

The essential oil antibacterial activity was determined by using the well and the disk diffusion method. The result of well diffusion method underlines the antibacterial power of *Lavandula officinalis* flowers essential oil, against ten bacteria. The inhibitions zones diameters, ranging between 10 and 40 mm, indicating that all the tested strains were sensitive to the *Lavandula officinalis* flowers essential oil (Table.3 and Fig. .2).

Table 3: The inhibitions zones diameters of essential oil.

Tested pathogenic strains	Inhibitions zones diameters (mm)	Sensitivity and resistance
Pseudomonas aeruginosa ATCC 9027	30±0	+++
Escherichia coli ATCC 25922	40±0	+++
Proteus mirabilis ATCC 7002	15±0	+++
Enterobacter aerogenes ATCC 1710	30±0	+++
Klebsiella pneumoniaie ATCC 35218	00 ±00	_
Acinetobacter baumannii ATCC 7689	00 ±00	_
Enterococcus cloacae ATCC 27412	00 ±00	_
Listeria monocytogenes ATCC 5849	00 ±00	
Staphylococcus aureus ATCC 6538	20±0	+++
Bacillus anthracis ATCC 6633	25±0	+++

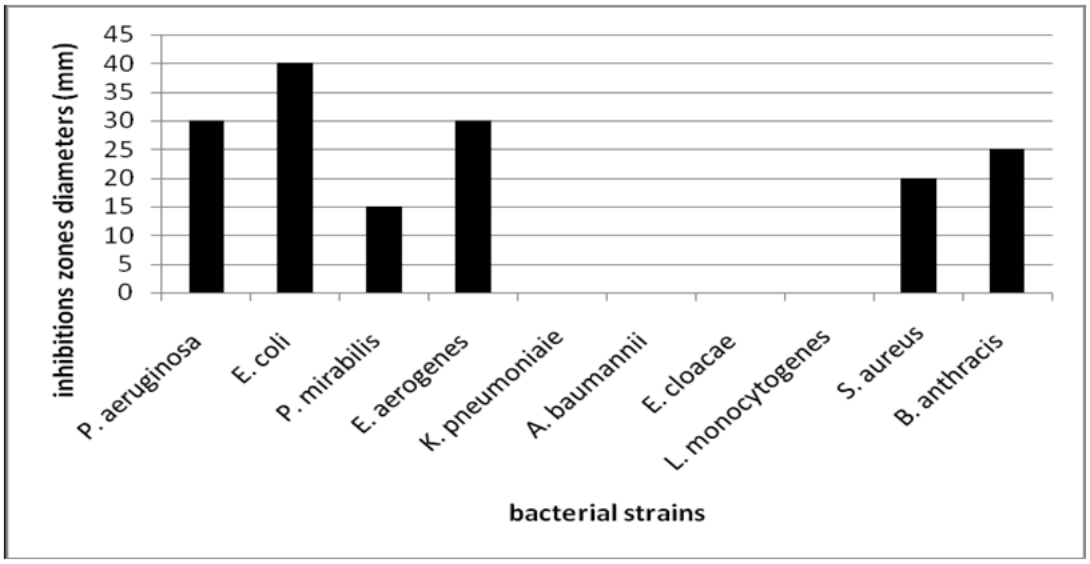

Fig .3: The inhibitions zones diameters comparison

Using the disk diffusion method in the MH (Mueller-Hinton) medium, all tested strains were sensitive to *Lavender* flowers essential oil with the exception of *Bacillus anthracis* ATCC and *Listeria monocytogenes* ATCC 5849 (Table.4 and Fig. .3).

Table 4: The *Lavandula* essential oil inhibitions zones diameters

Tested pathogenic strains	Inhibitions zones diameters (mm)	Sensitivity and resistance
Pseudomonas aeruginosa ATCC 9027	19±0	+++
Escherichia coli ATCC 25922	18±0	+++
Proteus mirabilis ATCC 7002	26±0	+++
Enterobacter aerogenes ATCC 1710	28±0	+++
Klebsiella pneumoniaie ATCC 35218	18±0	+++
Acinetobacter baumannii ATCC 7689	11±00	++
Enterococcus cloacae ATCC 27412	06±0	+
Listeria monocytogenes ATCC 5849	00±00	
Staphylococcus aureus ATCC 6538	24±0	+++
Bacillus anthracis ATCC 6633	00±00	_

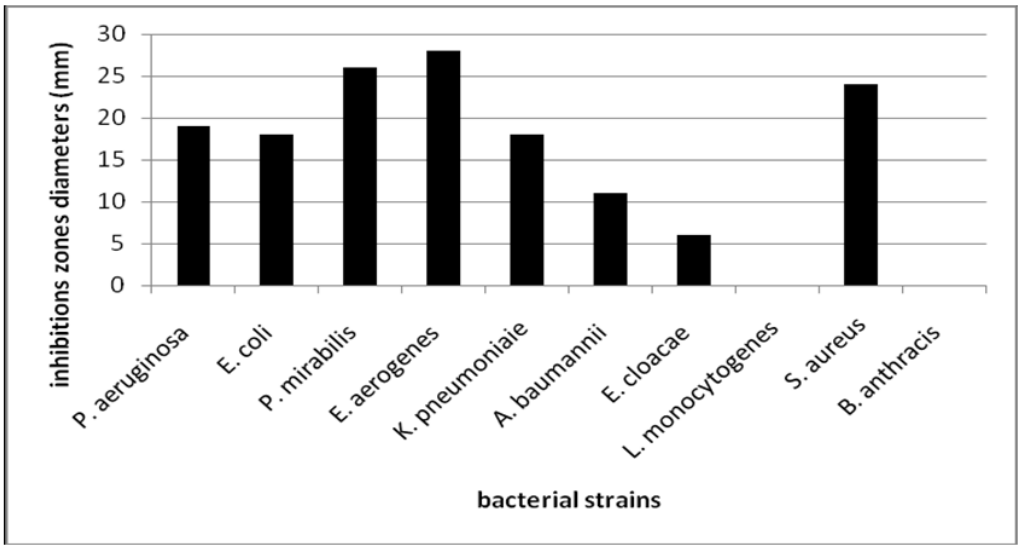

Fig. 3: The comparison of zones diameters inhibitions by disk method.

DISCUSSION

The yield level of flowers *Lavender* essential oil obtained in our experimentation was close to 1.45±0.25%. Similarly, **(**Laïb and Barkat,2011) and (Mohammadi and Atik ,2012) indicated that dry *Lavender* flowers from two Algerian regions have essential oil levels respectively at 1.36 % and 2.01 %. However, (Bouguerra and Zeghou, 2009) found that the *Lavandula officinalis* flowers collected from the same location gave a yield of 3.41 %. In addition, the results obtained by (Sidi Boulenouar and Ziane,2003), showed that dried *Lavender* flowers from the Ouchba and Zarifet regions yielded levels of essential oil, equivalent to 0.94 % and 0.70 % respectively. These changes in levels may be due to several factors, including the maturity degree of flowers *Lavandula*, the interaction with the environment (the climate type, the soil type), the time of harvest and the extraction method (Caillet and Lacroix ,2007).

The essential oil composition characterized by GC/MS identified 28 terpenes essentially composed by the acetate (17.85% , 89.980 min); 1.8-cineole (9.97 % ,53.512 min); γ-terpineol (10.23%,52.425min) and camphor (12.98 % ,24.410 min). However, (Kulevanova *et al.*,2000) in their study found 35 compounds with a dominant presence of Linalool (25.7%), Linalyl acetate (23.2%) and Lavandulyl acetate (12.4%) in flowers *Lavandula officinalis* essential oils, collected from the Kozjak Mountain of Macedonia. (Verma *et al.*,2009) analyzed the composition of *Lavandula officinalis* flowers, harvested from the Uttarakhand region (India), and identified 37 monoterpenes, which main compounds were: Linalyl acetate (47.56%), Linalool (28.06%), and α-terpineol (3.7%).

According to these results, we noticed that the chemical composition of *Lavandula officinalis* species essential oil harvested from Skikda (Eastern Algeria), was different from those in many works on the same species in different regions around the world; with a dominant number of monoterpenes in most cases, but in different proportions. This difference in composition is probably due to various conditions, including the environment, plant genotype, geographical origin, harvest period, drying place, temperature and drying time, and extraction method (Hui *et al* ., 2010).

The essential oil might provide a stable free radical 2.2 Diphenly-1-picrylhadrazyl (DPPH) Diphenyl-

picrylhydrazine yellow-colored, an IC_{50} % of 9.18±0.97 showing a weak antioxidant activity than that of ascorbic acid. It seems from these results that, ascorbic acid is the most effective antioxidant with an IC_{50}% of 95.11±0.15 compared to the studied essential oil.

These results can be compared to those reported by Mothana and co-workers (2012) showing a weak antioxidant activity by DPPH. However, several studies reported that the *Lavender* (*Lavendula*) species essential oil have a high antioxidant potential (Boumerfeg *et al*.,2009).

The slight antioxidant potential exhibited by *L.officinalis* can be explained by the fact that its essential oil contained many unsaturated compounds and few aromatic compounds, with more than one hydroxyl group. Nevertheless, the DPPH reduction test has a significant value related to an antioxidant activity compared to the standard (Economou *et al.*,1991; Caillet and Lacroix,2007). According to Haddadi *et al*,(2005), several factors seem able to change the inhibitory results; they reported that the hydrodistillation and the solvent extraction were operated at 100 and 70°C, respectively, which might induce thermal decomposition of certain active compounds.

Due to the complex reactive aspects of phytochemicals, the essential oils antioxidant activities cannot be evaluated by only a single method, but at least two test systems have been recommended for the determination of antioxidant activity to establish authenticity to potency (Bouhdid *et al.*,2006). There are many different methods for determining antioxidant functions each of which depends on a particular free radical producer which acting through different mechanisms (Bouhdid *et al.*,2006). For this reason, the *Lavandula officinalis* essential oil antioxidant activity was determined by two spectrophotometric methods, the DDPH and FRAP tests.

Antioxidants may act in various ways such as radical scavenging, the decomposition of the peroxide and chelating (Lu and Foo ,2001). All of these activities may be related to the compounds present in essential oil, including phenolic acids, terpenes and sesquiterpenes.

The action mechanism of the antioxidant activity of these compounds is not yet clearly understood. (Miguel,2010) reported that these compounds are known for their properties to free radical scavenging and to inhibit lipid oxidation. These compounds exhibit *in vitro* and *in vivo* antioxidant activity, inhibiting lipid peroxidation by acting as peroxyl radical scavengers and chain-breakers. In addition, phenols directly scavenge reaction oxygen species (hydroxyl radicals, peroxynitrite and hypochlorous acid). According to (Amensour *et al.*,2009) the essential oils antioxidant is believed to be mainly due to their redox properties, which play an important role in adsorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides.

The *Lavandula officinalis* essential oil showed an interesting antibacterial activity. This efficiency is probably due to essential oil volatiles during storage and/or extraction. In this study, we tested only pure essential oil, because, according to (Manou *et al.*,1998) and (Bagamboula *et al.*,2004), there is a relationship between the essential oil or the active ingredient and the inhibition zone; it seems to depend on the ability of essential oils to spread on the Petri dish. The well method is usually used as a preliminary assay to investigate the antibacterial activity. In this method, the parameters such as the volume of essential oil inserted in the wells, the thickness of the plaque layer vary greatly between studies (Lis-

Balchin *et al.*,1998); this means that this method is useful for the choice of active essential oils and for the determination of their antibacterial activity.

Escherichia coli ATCC 25922, *Enterobacter aerogenes* ATCC 1710 and *Pseudomonas aeruginosa* ATCC 9027, were the most sensitive bacteria, with inhibition zone diameters respectively 40, 30 and 30 mm. This could be explained by the fact that Gram-negative bacteria have structural devices more susceptible to essential oils.

Abdul Rahman *et al.* (2010) confirmed the great resistance of Gram-negative bacteria compared to Gram-positive bacteria. It's maybe due to the action of certain volatiles compounds of the studied essential oil and the presence of a lipopolysaccharide (LPS) layer in Gram-negative bacteria that could function as an effective barrier against any entering biomolecule (Bouhdid *et al* .,2006; Lis-Balchin *et al* .,1998). It seems that the resistance mechanism of these bacteria is at the level of their release pump. (Villaño *et al.*,2007). The outer membrane of Gram-positive bacteria is highly charged; it acts as a barrier to essential oils; it has been suggested that the active essential oils against the Gram-negative bacteria include secondary metabolite compounds, that are small sufficient to pass through the proteins in the outer membrane and thus to be able to access the cytoplasmic membrane (Caillet and Lacroix ,2007).

Escherichia coli ATCC25922 was more sensitive, despite being Gram-negative bacteria. It's suggested that the different components of essential oils show different levels of activity against Gram-negative and Gram-positive bacteria (Lu and Foo ,2001), and that the chemical composition of essential oils may vary according to several extrinsic factors (Lu and Foo ,2001).

It is also known that bacterial species do not also have the same sensitivity to an antibacterial agent. Therefore antibacterial action is sometimes partial and even after a decrease in the number of bacteria, there is a resumption of bacterial growth (Lis-Balchin *et al* .,1998 ; Lu and Foo ,2001). The antibacterial activity may also depend on the composition of the culture medium (Burits and Bucar, 2000). According to (Cosentino *et al.*,1999) and (Gulfraz *et al.*, 2008), the antibacterial activity of all essential oils is attributed to terpenoids and phenolic compounds. Tiwari *et al*., (2009) interpreted the antibacterial activity of the phenolic in terms of alkylics replacement in the phenol nucleus.

CONCLUSION

The natural properties, the strong antibacterial power and the antioxidant activity exibed by the *Lavandula officinalis* flowers essential oil make it a very promising preservative for the food industry.

Conflicts of Interest

The authors declare that they have no conflicts of interest with respect to the publication of this document.

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