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Study of the chemical composition, antibacterial and antioxidant activities of the essential oil extracted from the leaves of Algerian *Laurus nobilis Lauraceae*

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ABSTRACT

This work is part of the chemical composition's study, of the essential oil's antibacterial activity and antioxidant power. This oil is extracted, from an aromatic and medicinal plant of the Algerian flora, in order to find new metabolite products, which are characterized by a biological activity. The investigations and research on the essential oil; extracted from the dried leaves of Laurus nobilis Lauraceae, which was harvested in the region of Skikda (East of Algeria); and separated by gas chromatography coupled by a mass spectrometry (GC/MS); resulted in obtaining Twenty-two constituents, representing 99.7% of the essential oil of Laurus nobilis. The main compounds identified are 1,8-Cineole (45.36%), followed by bornylene (17.25%), linalool (8.13%), and sabinene (7.48%). The antimicrobial activity of the oil was tested using the agar disc diffusion method, by determining the inhibition zone and the minimum inhibitory concentration. The results have shown a great potential of the antimicrobial activity against the tested strains, with an enhanced sensitivity towards the Gram negative strains of Salmonella enterica and Klebsiella in comparison with the other tested bacteria. The tests results of the essential oil's antioxidant activity, obtained with the anti-radical method 2,2- diphenyl-1-1-picrylhydrazil (DPPH) and the iron-reducing power (FRAP), were compared with those of the ascorbic acid; the usual synthetic antioxidant. The comparison have demonstrated; firstly with the DPPH method a considerable level of antioxidant activity of the essential oil (IC_{50} = 72.78 ± 2.70 µg/ml), but still lower than that found for the synthetic antioxidant that is the ascorbic acid; while the opposite occurred with the iron reduction method, with an higher obtained value of (EC_{50} = 14.66 ± 0.96 µg/ml), for the essential oil's antioxidant activity.

Keywords: Antibacterial activity; Antioxidant activity; Bio-pesticides; Chemical composition; Essential oil, *Laurus nobilis*.

INTRODUCTION

The therapeutic use of plants is an integral part of the traditions of all the cultures. The medicinal valorization of these practices is done, especially, through the isolation and identification of new molecules. These compounds continue to offer new alternatives to modern medicine. Furthermore, they generally have the advantage of being less toxic than their counterparts of synthetic origin.

The history of aromatherapy was thus born and with the progress of science, new active ingredients and new pharmacological properties have helped in making the medicinal and aromatic plants (MAP) authentic drugs [1].

The study of essential oils is the current topic that intrigues research laboratories, where new perspectives are implemented for a more sophisticated conception of plant biotechnology. Essential oils are used in aromatherapy, pharmacy, perfumery and cosmetic products [2] for their richness in active ingredients that are charged with vital energy from natural origin. *Laurus nobilis (Lauraceae*) leaves and essential oils are used as precious spices, which are used as aromatizer for food in the culinary field[3].

In traditional medicine, *Laurus nobilis* is used for its antiseptic, anti-cancer, anti-spasmodic, anti-mutagenic properties, and as a treatment for digestive disorders [4-6].

Because of these ethno-botanical surveys, the most interesting of this study was to be able to determine the chemical composition of the essential oils extracted from the leaves of a plant, to explore the effect of these oils on some pathogenic bacterial strains and to use their antioxidant power as a natural alternative (Biopesticide).

EXPERIMENTAL SECTION

1.1. Vegetal material

The vegetal material used during the realization of this work consists of leaves of the *Laurus nobilis Lauraceae* species harvested in March 2013 from the city of Skikda (Algeria), the gps coordinates are (N 36 ° 52'18.011 'E 6 ° 53'14.786 '). The leaves are dried for 10 days in the open air, protected from sun's rays, moisture and at an ambient temperature.

The plant was identified by Pr. Chahma Abdelmajid, a botanist from the Department of Biology, University of Ouargla, Algeria, a specimen was deposited at the herbarium of the University under the number GO2013-2.

1.2. Extraction of the essential oil

The extraction of the essential oil is carried out by hydro-distillation in an apparatus type Clevenger wherein 100 g of dried leaves are immersed in a flask of 1000 ml of water for 3 hours. The essential oil obtained was dried with MgSO4 and kept in the refrigerator at 4°C.

1.3. Chromatographic analysis (GC/MS)

The essential oil analysis of *Laurus nobilis* was performed at the INRAP (National Institute of Research and physico-chemical analysis) of Tunisia, The gas chromatograph used is an Agilent 6890, coupled to a mass spectrometer type Agilent 5975B with a quadrupole ionization voltage of 70 eV. The column used is a HP-5MS; 5% Phenyl Methyl Siloxane with a length of 30 m and an internal diameter equal to 0.25 mm. The wire thickness being 0.25 µm.

The operating conditions are :

- The temperature of the injector (1:50 split mode): 250°C.
- The temperature programming: from 50°C to 300°C at a rate of 2°C/min.
- The vector gas used is helium with a flow rate of 0.8 ml / min.

The temperatures of the quadrupole source are fixed, respectively, at 230°C and 150°C. The Linear retention indices (RI) for all the compounds were determined using n-alkanes as standards. Identification of individual compounds was performed by matching their mass spectral fragmentation patterns with corresponding data (NIST 05 and Wiley7 libraries), and by the laboratory database.

1.4. Antibacterial activity

1.4.1.Bacterial strains used

The microbiological material consists of eight bacterial pathogens strains, responsible of some serious infectious diseases. These bacteria are *Escherichia coli, Klebsiella pneumoniae, Leisteria monocytogenes, Proteus, Pseudomonas aeruginosa, salmonella, Staphyloccous aureus* and *Staphyloccous Sp.* They come from the microbiology laboratory of the hospital Mohamed BOUDIAF-Ouargla- and microbiology laboratory of the University Kasdi Merbah, Ouargla.

1.4.2.Disc diffusion method

The evaluation of the antibacterial effect of the essential oil was tested by the agar diffusion test according to the NCCLS guidelines [7].

The disc method is a product distribution method to test from a paper disc that can qualitatively measure the sensitivity of the strains towards the antimicrobial effects [8].

The disc method is chosen in this study for its reliability and simplicity. This method provides preliminary results on the strains susceptibility and the antibacterial activity of the product, thanks to the diameters of the inhibition zones appearing around the discs measured in millimeters. The bacterial strains were prepared in an appropriate medium culture and adapted to the standards. The disks impregnated in the essential oil are put on the surface of those medium and incubated at 37 ˚C for 24 hours. All tests are performed three times.

1.4.3.Determination of the minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of our oil was determined according to Benabderrahmane, Benali [9], for the microbial strains, by agar diffusion in Mueller-Hinton, with some modification; Because of the non-miscibility of essential oils in water, it was diluted diluted in DMSO (dimethyl sulfoxide) to achieve a concentration range of 1-0.01 mg/ml, and then incorporated into disks of 6.0 mm of diameter for each 0.01 ml of the product. The same volume of DMSO is used as a control product. The Microbial suspensions are prepared according to the standards (0.5 McFarland which is equivalent to (10^8 CFU/ml) [10]); 0.1 ml of inoculum is immediately seeded on agar using a sterile spreader.

The discs containing various concentrations of essential oil are placed directly on the surface of the agar. Under 37 °C during 24 hours. The MIC is the lowest concentration of the essential oil required to completely inhibit the growth of the microorganisms tested around the disc. All tests are repeated three times.

1.5. Antioxidant activity

1.5.1.Method of DPPH

The method used to evaluate the scavenger effect of the essential oil of the plant against DPPH radical, is that of Mighri, Hajlaoui, and al et Braca, Sortino, and al. [11, 12], with some modifications (essential oil diluted in absolute ethanol); 1 ml of essential oil at different concentrations diluted in ethanol are added to 1 ml of the DPPH solution prepared at 0.4 mM in ethanol. After 30 min of incubation in the dark, the absorbance reading at 517 nm; The mixture of 1 ml of the DPPH solution and 1 ml of ethanol is taken as control product.

The reduced level of these molecules by DPPH is expressed in percentage according to the following formula:

$$I\,\% = \left({}^{A_0 - A_e}/_{A_0}\right) \times 100$$

Where $\mathbf{A_0}$ is the absorbance of the control reaction and $\mathbf{A_e}$ is the absorbance of the Sample.

As an indication, the ascorbic acid as a standard known for its anti-radical effect was tested in parallel. As for the inhibitory concentrations (IC_{50}) they are calculated from the curves of linear regression. Tests were carried out in triplicate.

1.5.2.Ferric reducing power (FRAP)

The reductive activity of the Iron of our essential oil is determined according to the method described by Oyaizu [13]. A volume of 2.5 ml of phosphate buffer (0.2M, pH 6.6) was added to a 1 ml of plant extract at various concentrations, followed by 2.5 ml of potassium ferricyanide (1%); after stirring, the mixture was incubated at 50°C for 20 min. A volume of 2.5 ml of trichloroacetic acid at 10% is added to the mixture before being centrifuged at 3000 r/min. From these tubes, 2.5 ml are taken, to which are added 2.5 ml of distilled water and 0.5 ml of ferric chloride (0.1%). Reading the absorbance of the reaction mixture at 700 nm against a blank prepared similarly, while replacing the extract by ethanol that permit to calibrate the spectrophotometer (UV-Visible); a standard calibration curve is obtained from a standard antioxidant's solutions, which is the ascorbic acid at different concentrations, whose absorbance is measured in the same conditions as the samples. The EC_{50} value is the effective concentration that reduces the iron in an absorbance of 0.5. It is obtained by interpolation from the linear regression analysis [14]. However, an increase in absorbance corresponds to an increase in the reducing power of the extracts tested [15].

RESULTS AND DISCUSSION

2.1. Gas chromatography–mass spectrometry analysis of essential oil

The results of the analysis by gas chromatography-mass spectrometry of the chemical composition of the HE are presented in Table 1.

Table 1: Chemical composition of the essential oil of *Laurus nobilis* leaves from Algeria

Compounds	RI	Aera %
α-Thujene	739	0,24
1R-α-Pinene	767	3,18
Camphene	820	0,4
Sabinene	945	**7,48**
L-β-Pinene	953	2,32
β-Myrcene	1022	0,38
(+)-4-Carene	1147	0,27
1,8-Cineole	1253	**45,36**
γ-Terpinene	1373	0,57
Terpinolene	1535	0,2
Linalool	1655	**8,13**
Borneol	2008	0,19
4-Terpineneol	2090	2
α-Terpineol	2186	2,14
L-bornyl acetate	2766	0,64
Pseudolimonene	2970	0,6
Bornylene	3216	**17,25**
Methyleugenol	3574	6,84
Elemicine	4444	0,29
Nerolidol	4466	0,15
Spathulenol	4511	0,5
Caryophyllene oxide	4529	0,61
Total		99,74

RI : retention indices relative

A total of Twenty-two compounds were identified, corresponding to a percentage of 99.7% of all the isolated components. The 1,8-cineole appears to be the major constituent of the essential oil (45.36%), followed by bornylene (17.25%), linalool (8.13%), and sabinene (7.48%). The essential oil composition, showed similar tendencies to those published for other geographical regions: the monoterpene 1,8-cineole is reported to be the main component of the essential oil of Morocco [16, 17], Tunisia [18, 19], Egypt [20], of Türkiye [21, 22], Iran [23], Italy [24] and Argentina [25].

The essential oil's content showed some variations in the same plant, harvested from different geographical origins, and from different parts of the tree. While comparing between the oil's composition of the *Laurus nobilis*, collected in Tunisia and the one collected in Morocco, we noticed that there are some significant differences; which have given, for the first oil, concentration in 1,8-cineole of (56.31%), borneol (11.04%) and valencene (11.03%) [19], and for the second oil concentrations of: 1.8 -cineole (39.81%), 2-carene (13.03) and trans-ocimene (7.05) [17]. There is evidence that the chemical composition changes in the essential oils could be attributed to the geographical origin of the plant, the extraction technique, time of harvest and climate factors [26, 27].

2.2. Antibacterial activity

Regarding the bacterial sensitivity testing towards the essential oil, the results are summarized in Table 2. The values shown, are the results of three measurements. The inhibitory action results in the appearance of an inhibition zone around the paper disc impregnated with crude extract of the studied oil. The diameter of the inhibition zone varies from one bacterium to another. As reported in the literature, we considered that an extract has a bacteriostatic propriety if its inhibition diameter is larger than 8 mm [28].

Table 2: Antibacterial activity of essential oil of the leaves of *Laurus nobilis*
The Values are expressed as ±SD (n = 3)

Micro-organisms	Disc diffusion assay (inhibition zone mm)	MIC (mg/ml)
Gram negative		
Escherichia coli	13.73 ± 0.25	0.5
Salmonella enterica	20.47 ± 0.68	0.2
Proteus	12.13 ± 0.06	0.33
Klebsiella pneumoniae	21.93 ± 0.4	0.11
Pseudomonas aeruginosa	10.73 ± 0.25	-
Gram positive		
Staphylococcus aureus	14.50 ± 0.5	0.25
Listeria monocytogenes	11.33 ± 0.57	-
Staphylococcus Sp	13.03 ± 0.25	0.66

The essential oil of *Laurus nobilis* reacted positively to the microbial strains tested. Note also, large differences in the diameters of the inhibition zones obtained, ranging from 10 to 21 mm. The *Laurus nobilis* plant showed some inhibitory activity of the microbial growth, this justifies its use in traditional medicine as an antibacterial treatment

[4, 6]. Our results show a great variability in the bacteriostatic qualities of the oil towards the different strains. Only the strains Gram negative *Salmonella enterica* and *Klebsiella pneumoniae* are more sensitive than the other bacterial strains tested, with a minimum inhibitory concentration, respectively of (0.2 and 0.11) mg/ml. The essential oil is considered moderately active against gram-negative strains (*Escherichia coli, Proteus*), and those of gram-positive (*Staphylococcus aureus, Staphylococcus Sp*) with an inhibition diameters, respectively, of (13.73, 12.13, 14.50 and 13.03) mm and a minimal inhibitory concentration, respectively, of (0.5, 0.33, 0.26 and 0.66) mg/ml. The *Pseudomonas aeruginosa* strains are proving to be more resistant, this is related to their great ability to develop resistance to many antimicrobial agents, hence their frequent involvement in hospital infections [29]. According to Oussalah, Caillet [30], the biological activity of an essential oil is to be related to its chemical composition, the principals compounds's functional groups (alcohols, phenols, aldehydes) and the synergistic effects between components. Most of the works that had as subject of study the mechanism of action of the active essential oils compounds claim that their main area of action is the bacterial plasma membrane [31]. The cell membrane of the bacteria is disintegrated [32], it loses its structure and becomes more permeable to ions. The lesion of the cell membrane can also allow the dissipation of the pH gradient and the decrease of the membrane potential [33]. The antimicrobial action of our oil can easily be attributed to its high level with mono oxygenated terpenes, 1,8-cineole which is already known for its antibacterial power to fight against several bacterial strains tested [16, 34]. The combined action (synergy) of different compounds at the origin of this extract may explain the variation in results for the same species from different regions. According to Oussou, Kanko [35], these molecules act mostly by a synergistic action, either alone or in the essential oil. In addition, these minors' compounds can significantly contribute to the activity of the essential oils [36].

2.3. Antioxidant activity

Regarding the antioxidant activity of the essential oils, it is determined by two different methods as follows: the DPPH method and the iron reduction power method. The results are summarized in Table 3. With the first method, 1,1-diphenyl, l-2-picryl hydrazyl (DPPH), we obtained a stable radical, purple while in solution and has a maximum absorption characteristic at 517 nm. The routine protocol applied is based on the disappearance of the radical when the DPPH is reduced by a compound with a free-radical property, causing the transformation of the color from purple to yellow. The IC_{50} value (otherwise known as the inhibitory concentration at 50%) is determined by our oil and the standards used. It is defined as the concentration of the sample required to achieve a 50% decrease in the absorbance of the initial solution of DPPH. The IC_{50} values are inversely proportional to the scavenger effect whose low values reflect a significant anti-radical effect [37]. From Table 3, the IC_{50} values obtained show that the ascorbic acid has a low rate of $IC_{50,}$ with a value of (IC_{50} = 6.42 ± 0.36 µg/ml) in comparison with our essential oil (IC_{50} = 72.78 µg/ml), reflecting a significant anti-radical potential. It appears from these results that the vitamin E (ascorbic acid) is a more effective antioxidant than the essential oil studied. In several reports, the antioxidant activity of essential oils can be linked to the phenolics products. Indeed the comparative study on the possibility of reducing the DPPH radical by different chemotypes, proved that the phenolic chemotypes show, in vitro, a much stronger and higher expressed antioxidant capacity than those of a non-phenolic chemotypes [38]. In our case, despite the low content of phenolic compounds in the essential oil of *Laurus nobilis* (Table 1), this oil showed a high antioxidant activity, allowing it to be used as a therapeutic tool, in various applications and in many other areas. This result is probably due to a synergistic or antagonistic interaction between the different constituents of the essential oil that can create an effective system against free radicals, which could explain this antioxidant tendency. In addition, it is reported by several authors that synthetic antioxidants have a much bigger ability to trap the DPPH radical than the essential oils [39-41].

Table 3: The antioxidant activities of the essential oils from the leaves of *Laurus Nobilis*
Values are expressed as ±SD (n = 3)

	Scavenging activity	
	DPPH IC_{50} (µg/ml)	**FRAP EC_{50} (µg/ml)**
Laurus nobilis essential oil	72.78 ± 2.70	14.66 ± 0.96
Ascorbic acid	6.42 ± 0.36	66.73 ± 0.37

For the reducing power, the antioxidant activity of the sample is based on the reduction of ferric ion (Fe^{3+}), present in the complex [$K_3Fe(CN)_6$], into ferrous ion (Fe^{+2}). This causes the transformation of the ferricyanide of potassium yellow color to a blue one inside a reaction medium at 700 nm, whose intensity depends on the reducing power of the essential oil. The essential oil of *Laurus nobilis* showed an antioxidant activity with an EC_{50} value of 14.66 ± 0.96 µg / ml, while the ascorbic acid as a control product gave a value of 66.73 ± 0.37 µg/ml. Figure 1 (Fig .1.) shows that the reducing power increases with the concentration of the samples, which clearly demonstrates that the essential oil has a more effective antioxidant activity than that of the control product.

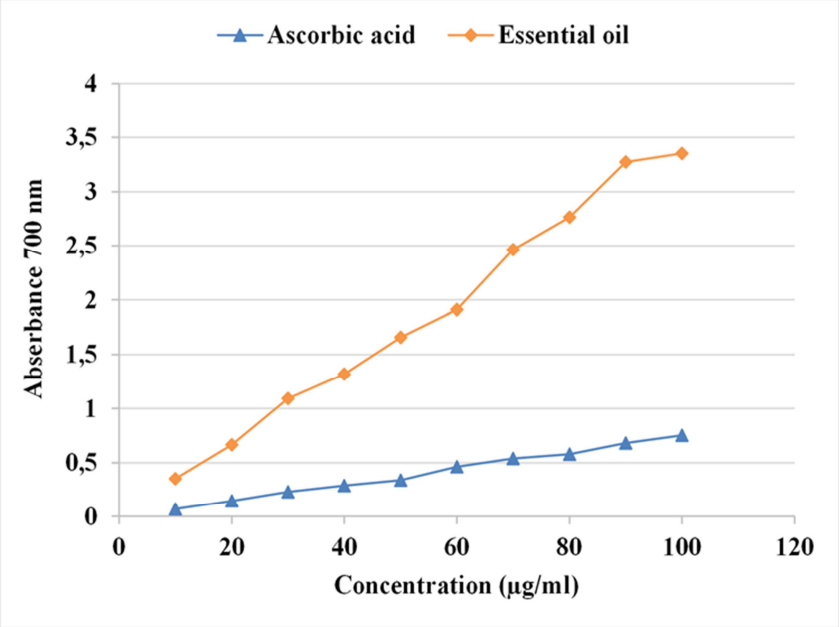

Fig .1. Reducing power of Laurus nobilis essential oil

CONCLUSION

This study aimed to characterize and valorize of the Algerian *Laurus nobilis* essential oils. The chemical profiles of the investigated oil highlighted by 1,8-Cineole, Bornylene, Linalool and Sabinene as major compound. A considerable degree of antibacterial and antioxidant activities was found in essential oil evaluated in this study. Our results clearly demonstrate that the essential oils of *Laurus nobilis* can well present an interesting alternative natural, which it can be useful for food preservation pharmaceutical treatment and aromatherapy products.

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