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Chemical composition and Antimicrobial Activity of the Algerian *Laurus nobilis* Essential oil

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Abstract: The aim of this research is to determine the chemical composition and the antimicrobial activity of Algerian *Laurus nobilis* essential oil. The chromatographic analyses (CG/MS) have shown that the major components of *Laurus nobilis* essential oil are the 1.8 cineol (24,658%) and the linalol (18,563%). The *Laurus nobilis* essential oil has shown an antimicrobial activity against all the tested strains except the *Pseudomonas aeruginosa* which shown a strong resistance. The Minimum Inhibitory Concentrations (MIC), Minimum Bactericidal Concentrations (MBC) and Minimum Fungicidal Concentrations (MFC) have been determined by the agar dilution method, some micro-organisms were susceptible to the essential oils that have the MBC values between 1% and 0.03%. A fungicidal action has been obtained regarding the *Candida albicans*, a fungistatic one regarding the *Saccharomyces cerevisiae*, these results promise to get a scientific validation to the massive use of this species.

Keywords: *Lauris nobilis; Essential Oil; Antimicrobial Effect; MIC; Candida albicans; Gas Chromatography.*

I. Introduction

With the appearance of the pathogenic microorganisms that are resistant to the antibiotics, the antimicrobial essential oils have become a real alternative to the medicine of antibiotics against the infectious diseases [1].Many studies have shown the capacities of the essentials oils of getting rid of the most powerful microbes such as *Staphylococcus*, *Mycobacterium tuberculosis* or Typhoid *bacillus* [2]. The antimicrobial activity of the essential oils is due to their composition more specifically to the nature of their main components [3].

The essential oils do not gradually lose their effectiveness through the process of their use, and there is no need to multiply the doses in order to recover.

The wide variety of the essential oils components, prevent the microbes from organizing their resistance [4]. *Laurus nobilis* L, a member of the lauraceae family which contains 32 genera and about 2000-2500 species [5]. *Laurus*, derives its name from latin word of a celtic origin, it means evergreen which refers to the persistent green foliage of the plant all over the year [6].

Laurus *nobilis*, shrub or aromatic tree with 2 to 10m of height with a right stem, the lauraceae stems have a heterogeneous sclerous pericycle (fibers and sclerous cells) and cell essence [7]. *Laurus nobilis* grows in a shaded and a moist forest near the coasts often planted as an ornamental and aromatic plant [8]. It's common in ravins and humid forests such as those of Algiers and Constantine [9]. It is also cultivated for commercial reasons in turkey, Algeria, France, Greece, Morocco, Central America, Southern United States [10].
The leaves of the *Laurus* are considered as one of the most known flavors throughout the country, they are usually used as a valuable spice in cooking (in soups, stews, sauces) and as a flavor in the alimentary industry.
This plant plays a big role in the traditional medicine moreover it has recently become an interesting scientific research subject [11].
The aim behind this work is to show the characterization of the chemical composition of *Laurus nobilis* essential oil and to study its antimicrobial activity on the bacterial and fungal strains as well.

II. Experimental Section

II.1. Plant material

Lauris nobilis leaves were harvested in march 2014 from BEN ALI (a mountainous region in Blida) , the plants grow in the north west of the mountain slope ,at an altitude of around 750m, they grow in a shaded location they do not receive any special treatment. The identification of the plant was confirmed at the level of the laboratory of vegetal biology and botany of the department of Biology and the department of Agronomy (university of Saad Dahleb, Blida).

II.2. Extraction of essential oil

The isolation of the essential oil was performed by hydrodistillation using the Clevenger-type apparatus (Column alembic), the fresh leaves were collected than put into the alembic and due to the electric heating system , the water vapors which are loaded of the essential oils passed through the vegetal material and migrated to the refrigerant where it arrived to an alembic in which the cold water is continuously circulating due to a closed circuit system with a temperature around 12°c 13°c ,the water vapor which is loaded of the essential oils condenses into liquid [12], at this phase the essential oils separate from the water with a simple decantation. The yield of the essential oil is defined by being the connection between the essential oil mass we got after the extraction and the treated vegetal mass. The essential oil yield was estimated using the following equation:

$$\mathbf{R\ (\%) = (m\ /m_o)\ x\ 100}$$

Where m: essential oil mass (g), m_o: fresh leaves mass (g), R: essential oil yield (%)[13]. the essential oil is stored in amber glass bottles and kept away from light at a temperature of 4°C .

II.3. Chromatographic analysis

The chromatography analyses were performed using a Gas chromatography coupled with a mass spectrometry through Hewlett Packard 6890N machine coupled with mass spectrometry 5973N equipped with capillary column HP-5MS (30m ×0.25mm) with a coating thickness of 0.25µm, the volume of the injected samples is 1µl diluted in the dichloromethane. the fragmentation was performed by an electric impact of 70ev, the column temperature was programmed from 50°C to 250°C at a rate of 4°C mn^{-1}, using the helium as a carrier gas at a consonant flow rate of 1ml/mn .The identification of the components was based on the comparison of their spectrum mass with those of authentic standards found in the literature (database) (NIST 98).

II.4. Antimicrobial activity
II.4.1.Microbial strains

The strains which were tested in order to reveal the antimicrobial activity of *Laurus nobilis* essential oil are the followings: The strains of international collection ATCC (American Type Culture Collection). *Pseudomonas aeruginosa* ATCC 9027, *Bacillus subtilis* ATCC 9372, *Escherichia coli* ATCC 4157, *Enterococcus faecium* ATCC 6569, *Bordetella bronchiseptica* ATCC 4617, *Staphylococcus aureus* ATCC 6538, *Klebsiella pneumoniae* ATCC 4352, *Enterococcus faecalis* ATCC 29212 , *Enterobacter cloacae* ATCC 13047, *Proteus mirabilis* ATCC 49452, *Listeria monocytogenes* ATCC 15313, *Pseudomonas fluorescens* ATCC 13525, *Candida albicans* ATCC 24433, *Saccharomyces cerevisiae* ATCC 2601.
The clinical strains isolated from hospitalized patients at the HOSPIITAL OF MOSTAPPHA BASHA, ALGERIA **(***Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Acinetobacter baumannii, Lactobacillus* sp*, Citrobacter freundii, Enterobacter* sp*, Klebsielle oxytoca, Providencia alcalifaciens, Salmonella* sp*, Serratia marcescens, Shigella* sp*, Staphylococcus epidermidis, Salmonella Typhimurium*) were stored at 80°C in glycerol stocks .
The microbial strains tested were provided by the laboratory of microbiology center of research and Development of El-Harrach.

II.4.2. Culture media

In order to evaluate the antimicrobial activity of the essential oil two culture media were used: the agar Muller- Hinton for studying the sensitivity of the bacteria toward the essential oil.
The agar Sabouraud for studying the isolation of the yeasts and their sensitivity toward the essential oil.

II.4.3. Disk diffusion method

The antimicrobial activity is evaluated by the Disk diffusion method [14].which consists of using absorbent sterilized paper discs (9mm diameter)wet with essential oil ,the discs were placed on the surface of the agar .The bacteria were spreaded all over the discs, the spreading of the tested product throughout the disc determined the degree of concentration, the micro-organisms grow all over the surface of the agar except where they encounter a sufficient concentration of the product that would inhibit their growing ,after incubation around the discs, we observed a clear circular zone it is the inhibition zone, the effect of essential oil on microbial strains was estimated by the appearance of clear zones around the discs the more this diameter zone is big , the more the strains are sensitive to antibiotics the more is small, the more the bacteria is resistant**.** The diameter of the halo of growth inhibition was measured by vernier calipers (Mauser) and expressed in mm (including the diameter of the disc of 9 mm) [15]. All assays were performed in triplicate.
This method was taken from the principles of titling the antibiotics (European pharmacopoeia 2002) its application for the essential oils was approved by the microbiology department of CRD Saidal, it was also used by some authors [16-25].

II.4.4. Determination of minimum inhibitory concentration

The minimum inhibitory concentration was determined towards the bacterial strains and yeasts through the agar dilution method [26][27],by placing spots of 3 µl of standardized inoculum with 10^{-4} CFU/ml ,we also placed cellulose discs soaked with 3 µl of the microbial suspensions of the dilution 10^{-4} in order to determine the MBC, MFC .
The range of the concentration of the tested essential oil is the following: 0.007, 0.015, 0.03, 0.06, 0.125, 0.25, 0.5, 1.2 %. After incubation at 37° C during 24h for the bacteria and 25°C during 48h for the yeasts. MIC was defined as the lowest concentration of the essential oil that completely inhibited microbial growth after the incubation period [28].

II.4.5. Determination of minimum bactericidal concentration and minimum fungicidal concentration:

After tracking the discs where no growing has taken a place at the time of the determination of MIC, the experimentation continues this time for the determination of MBC and MFC , it consists of determining the lowest concentration of the essential oil where no visible microbial high-growing is shown only after subculture in a unscathed medium of the essential oil at 37°C during 24h for the bacteria and 25°C during 48 h for the yeasts.

III. Results and Discussion

III.1. Essential oil of *Laurus nobilis* yield:

The *Laurus nobilis* fresh leaves yield of essential oil was 0.82%this yield was lower than the one of the same plant in turkey which was 0.86% and higher than the one of Egypt which was 0.68%, the difference of places, and seasons could be the reason behind these fluctuations [29].

III.2. Chemical composition of the essential oil:

The chemical composition of *Lauris nobilis* oil is listed in the table 1.

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

Compound	(%)
Sabinène	0.246
1.8cineol	24.658
γ- terpnène	0.196
Cis-linalolxide	0.198
Linalol	18.563
L-4-terpineol	9.382
α –terpineol	9.875
exo-2-Hydroxycineole acetate	0.253
Eugenol	9.478
Methyl eugenol	9.871
Elemicine	1.808
Diisoocty phthalate	0.867
Total	85.395

The results of the GC/MS analysis revealed the presence of 12 terepene compounds that represent 85.395% of the total isolated components ,1.8 cineol as the main component of the EO (24.66%) followed by linalol (18.56%) α -terpineol (9.815%) methyl eugenol (9,871) eugenol (9,478%) L-4- terpineol (9,382%).The concentrations of the other components are less than 1%. The essential oil content of 1.8 cineol is less than the ones observed in the plants from Egypt (54.9%) Turkey (28%) Italy (45%) [30].

III.3. Antimicrobial activity

The results obtained in the antimicrobial and antifungal activity of the EO of the *Laurus nobilis* are shown in Table 2.
The EO of *Laurus nobilis* has showed an antimicrobial activity on all the tested strains except for *Pseudomonas aeruginosa* that was not affected thanks to its intrinsic resistance to a wide range of antibiotics, this resistance is due to the nature of its outer membrane [31-34]. It was

reported that the responsible components of the antimicrobial activity of EO are linolol, terpineol, eugenol, caryophyllene, nerolidol, α- humulene, viridiflorol [35].

***Table 2**: Diameter of Inhibition zone, Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC)*

Microbial strains	Inhibition zone diameters (mm)	MIC (%)	MBC/MFC (%)
Pseudomonas aeruginosa ATCC 9027	0,000±,0000	-	-
Bacillus subtilis ATCC9372	32,233±0,9292	0.03	0.25
Escherichia coli ATCC4157	22,267±0,6429	0.25	0.25
Bordetella bronchiseptica ATCC4617	14,700±0,6245	1	2
Staphylococcus aureus ATCC6538	67,233±0,6807	0.125	0.25
Klebsiella pneumoniae ATCC4352	48,633±1,3650	0.125	0.125
Enterococcus faecalis ATCC 29212	15,500±0,6245	0.25	0.5
*Enterobacter cloacae*ATCC13047	18,100±0,2646	0.25	0.5
Proteus mirabilis ATCC 49452	16,200±0,4359	0.25	0.5
Listeria monocytogenes ATCC 15313	14,967±0,5033	0.06	0.25
Enterococcus faecium ATCC6569	22,300±0,7550	0.5	1
Staphylococcus aureus	33,500±1,3454	0.25	0. 5
Pseudomonas aeruginosa	0,000±,0000	-	-
Escherichia coli	20,100±0,8544	0.5	0.5
Acinetobacter baumannii	18,667±0,5859	0.5	1
Lactobacillus sp.	16,033±0,2517	0.25	0.5
Citrobacter freundii	17,700±1,0440	0.125	0.25
Enterobacter sp.	20,667±0,5774	0.125	0.5
Klebsiella oxytoca	17,600±0,6000	0.25	0.5
Providencia alcalifaciens	16,397±0,5514	0.25	0.5
Salmonella sp.	24,573±0,5605	0.5	1
Serratia marcescens	15,010±0,0361	0.5	1
Shigella sp.	23,273±0,4456	0.25	0.5
Staphylococcus epidermidis	16,470±0,6883	0.25	0.5
Salmonella Typhimurium	22,000±0,3606	0.5	1
Candida albicans ATCC2443	14,000±,5568	0.25	0.5
Saccharomyces cerevisiae ATCC2601	19,300±0,4359	0.125	> 2

Taking the inhibition zone diameters into consideration, the EO is extremely active on *S. aureus, K. pneumonia, B. subtillis, E. faecium, Salmonella* sp*, E. coli, Enterobacter* sp*, S.Typhimurium* **,** *Shigella* sp*.* On the contrary it is moderetly active towards *B. bronchiseptica, E. faecalis*, *E. cloacae* , *P. mirabilis*, *L. monocytogenes*, *A. baumannii , Lactobacillus* sp *, C. freundii, K. oxytoca, P. alcalifaciens, S. marcescens , S. epidermidis* ,But totally inactive on *P.aeruginosa.*

The hypersensivity of the *Staphylococcus aureus* strain could be explained by the probability of sensivity of bacteria : gram (+) to the external environmental changes in fact; like the temperature, ph. ,the natural extracts due to the absence of the outer membrance [17] [36] , antibacterial substance can easily destroy the bacterial cell wall and cytoplasm membrance , as a result a leakage and coagulation of the cytoplasm occurs[37].

We observe that different studied bacterial strains react differently to the tested EO even in the case of two strains of the same species .for example: *S aureus* ATCC6538 and *S aureus clinical*.

Th EO practiced a moderate antifungal activity towards *Saccharomyces cerevisiae* and *Candida albicans* with inhibition diameters of 18.33 mm and 13.33mm, our results accord

with those found by Quibrahim and al [38] .that reported the effectiveness of the oil on the tested microorganisms giving a lower inhibition diameters than ours : it was 13.5mm for *E coli* ATCC25922, 11.1mm for *P. mirabilis ,* 12mm for *K. pneumoniae,*11mm for *K. oxytoca,* 12.7mm for *Enterobacter* sp,11.4mm for *P. alcalifaciens ,*16.4mm for *Shigella* sp,12.5mm for *Salmonella* sp , 11.5mm for *S. marcescens ,*8.7mm for *C. freundii* , 9.2mm for *A. baumannii* ,11.3mm for *E. faecalis,*14mm for *S. aureus* ATCC25923,12.7mm for *S. epidermidis* [38].
on the other hand Elharas and al[39].have revealed that the Moroccan *Laurus nobilis* essential Oil practices a big inhibitory activity towards *Staphylococcus aureus* ,*Pseudomonas aeruginosa* with the diameters inhibition zone of 8.5cm and 2.12cm.
The tested bacterial strains and the method used for the antibacterial activity evaluation may be the reasons behind the different results [40].
The values of MIC accord with the ones of inhibition diameters ,the extracts that have caused a significant inhibition zone represent the smallest MIC between the corresponding strains ,this is not the case for *Staphylococcus aureus*, *klebsiella pneumonia* That have showed great zones of inhibition although their MIC is 0.215% .So apparently the *bacillus subtilis* have shown the lowest CIM with a value of 0.03%.The highest MIC was observed in *B. bronchiseptica*.by 1%, a high concentrations are required to inhibit the growing of *Bordedetta bronchiseptica* .
The essential oil of *Laurus* has practiced a bacteriostatic activity on *Escherichia coli* and *klebsiella pneumonia* with MIC values respectively from 0.25% and 0.5%, and practiced fungistatic activity on *S. scerevisiae* and *C. albicans* with MIC values respectively from 0.125% and 0.25%.

IV. Conclusion

This work is devoted to determine the chemical composition, the yield and the antibacterial, antifungal activity of the *Laurus nobilis* essential oil so to give a more attention and valorization to the Algerian flora. The average yield of the essential oil is 82%, the quantitative and the qualitative analyses of the essential oil by GC/MS have identified 12 components: the terpene oxide (1.8 cineole) as a major component with the indicative content of 24.66%.The evaluation of the antimicrobial activity has showed that the essential oil of *Laurus nobilis* has antimicrobial capacities against all the tested microbial strains except the *Pseudomonas aeruginosa ATCC 9027* and *Pseudomonas aeruginosa.*
The essential oil has showed a bactericidal action towards the studied strains) those bacteria were unequally sensitive towards EO), a fungicidal action towards *Candida albicans* and a fungistatic action towards *Saccharomyces cerevisiae.*
This work approved that the essential oils have a significant antimicrobial activities and could be a successful alternative to the traditional antibiotics that become powerless in front of the resistant microorganisms.

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