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Antibiotic Activity of the Essential Oil of Laurel (*Laurus nobilis* L.) on Eight Bacterial Strains

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Abstract: The recent studies unveil more and more therapeutic properties of the essential oil of Bay leaf (*Laurus nobilis* L.). The aim of this study is to determine the chromatographic profile of the essential oil of Bay leaf cultivated under the climatic conditions of the Algerian East and to test its antibiotic activity, against 8 bacterial strains (*Escherichia coli*, *Serratia* sp., *Proteus* sp., *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Streptococcus* D, *Pseudomonas aeruginosa* and *Acinetobacter baumanii*), by using different concentrations. The GC/MS analysis showed that the essential oil is rich in eucalyptol (35.31%), β linalool (22.52%), eugenol methyl ether (9.17%), camphene (7.37%) and 3 carene (5.39%). The antibiotic activity of the essential oil was determined by the diffusion on agar method. Measuring diameters of inhibition method of Vincent [1] indicated that bacterial strains which are very sensitive to even very diluted essential oil are *Pseudomonas aeruginosa*, *Streptococcus* D, *Serratia* sp. and *Klebsiella pneumoniae*. *Staphylococcus aureus*, *E. coli* and *Acinetobacter baumanii* exhibit less sensitivity and *Proteus* sp. is especially sensitive to the pure oil. *Laurus nobilis* L. is a Mediterranean endemic that presents an interesting antibacterial activity and its culture should be encouraged and expanded in Algeria.

Key words: *Laurus nobilis* L., Algerian east, essential oil, GC/MS, antibiotic activity.

1. Introduction

The Bay leaf is a shrub or tree able to attain 10 m of height, it has persistent and fragrant leaves, very frequently cultivated as tree of embellishment and as culinary plant [1, 2] called commonly Bay leaf.

It belongs to Lauraceae family; it is endemic in the Mediterranean region. The Bay leaf is rather common in the Algerian Tell and in the region of Constantine, and also in the fresh stations of the forests of the coastal Algerian one [3]. The Bay leaf (*Laurus nobilis* L) is the only Lauraceae that exists in Mediterranean region [4].

In Seraidi (extreme east of Algeria), the leaf of this specie has culinary and medicinal interest; it is used against certain gastrointestinal disturbances as the aerophagy for the babies, against the arterial hypertension and in the flu states.

The aim of this work is to characterize chemically (by GC/MS), the essential oil of the Bay leaf and to evaluate its antibiotic activity towards 8 bacterial strains (*Escherichia coli*, *Serratia* sp., *Proteus* sp., *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Streptococcus* D, *Pseudomonas aeruginosa* and *Acinetobacter baumanii*), by using different concentrations.

To reveal better the action of this oil on different bacterial strains, the effect of a number of antibiotics on each of the strains have first been established and the results of the antibiograms and the aromatograms were confronted.

2. Materials and Methods

2.1 Extraction of the Essential Oil

It was carried out on harvested leaves in the month of February 2009 and dried in the free air because this

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is the better procedure to safeguard the components of the essential oils [5]. The essential oil was extracted by the method of the training by the vapor thanks to the device of Likens Nickerson apparatus during two hours of time.

2.2 Chemical Analysis

It was carried out by GC/MS Shimadzu. Column type: QP 2010 S, of a length of 25 m and of an internal diameter of 0.25 mm. The gas vector used is helium, debit of 1.5 mL by min. The detector is flame ionization (FID). The temperature of the column was maintained at 60 ºC during 5 min then by increasing of 5 °C by minute until 220 °C.

2.3 Bacterial Strains

The essential oil of Bay leaf was tested on 8 bacterial strains: *Escherichia coli*, *Serratia* sp., *Proteus* sp., *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Streptococcus* D, *Pseudomonas aeruginosa* and *Acinetobacter baumanii*. Samplings were carried out from the University Hospital of Annaba, service of Microbiology.

2.4 Antibacterial Activity

The inhibition diameter was determined according to the method of Vincent, for the bacteria [1].

For every bacterium, were used 5 different concentrations: pure oil, diluted oil to the DMSO ($(H_3C)_2SO$) at concentrations: 1/2, 1/4, 1/8 and 1/16. Bacterial strains are maintained by transplanting on nourishing medium favorable to their growth from the preserved pure cultures and incubated during 24 hours at 37 ºC before being used at the tests of antibacterial activity. A bacterial suspension of each of studied strains is sown on a solid medium Muller-Hinton. *Streptococcus* D was sowed on cooked blood medium.

Calibrated discs, of 6 mm of diameter, impregnated of pure and diluted essential oil are disposed on the solid medium; after 24 hours of incubation at 37 °C, the reading of the results was made by the measurement of the inhibition diameter around the disc. Also, a disc witness (sterile without oil) was deposited and incubated in the same conditions to assure that the discs are lacking in an antibacterial activity.

3. Results and Discussion

3.1 Results

3.1.1 Yield in Essential Oil

The structures of secretion of the essential oil in the leaf of *Laurus nobilis* L. are essence cells localized in the mesophyll, as shown in Fig. 1.

The average yield of the extraction of six samples of leaves of Bay leaf was 0.53 mL/50 g of dried leaves, which is in the norms (0.5-1.50 mL for 50 g). The

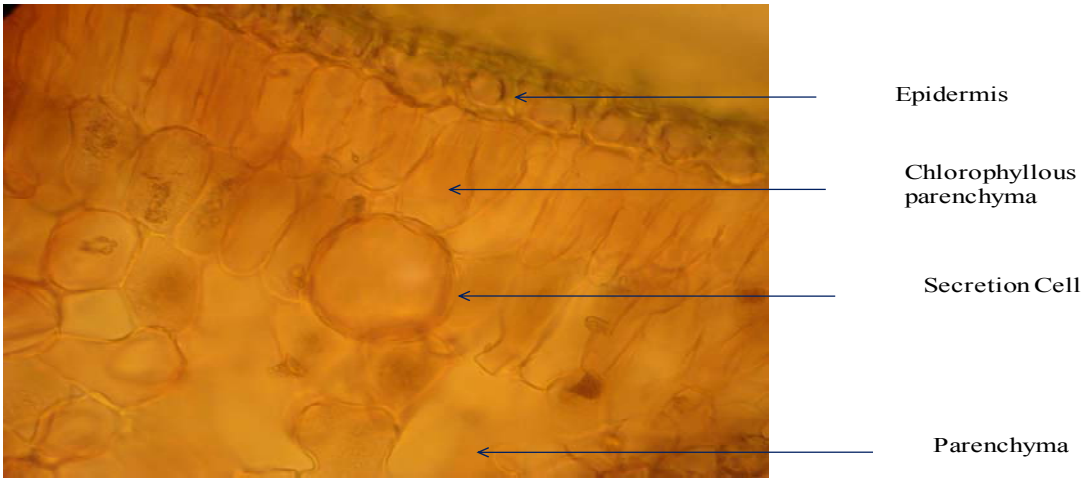

Fig. 1 Localization of the secretion cell in the mesophyll of the leaf of Laurel (seen under photonic microscopy Gr ×40).

yield, nevertheless, is considered as weak, this is explained by the fact that the region where was carried out the harvest is, at once, subject to topographic influence (mountainous stage) and the influence of the Mediterranean Sea. It results a low temperature and a strong atmospheric humidity. These conditions generate a decrease of the production of the essential oils especially that the harvest was carried out in the middle of the winter. In fact, the yield of essential oil is under the dependence of the geographic origin, phenologic stadium and environmental factors such as the temperature and the quality of the ground [6]. The essential oil of the Bay leaf has sticky aspect, yellow color blade, aromatic odor and it is less dense than water.

3.1.2 Chemical Composition of the Essential Oil of the Bay Leaf

The GC/MS analysis of the essential oil of Bay leaf reveals that the main components of this oil are: an ether-oxyde of terpenic nature: 1.8 cineole or eucalyptol (35.31%), which is the main component of the essential oil of Bay leaf, in all situations of geographic origin [7-9], considered drug [10] and phenologic stadium of the Bay leaf [11], as shown in Table 1.

For this reason the Bay leaf is considered as the essential source of this component in the industry of arums.

Diurnal and seasonal variations are other intrinsic factors affecting chemical accumulation in both wild and cultivated plants. Depending on the plant, the accumulation of chemical constituents can occur at any time during the various stages of growth. In the majority of cases, maximum chemical accumulation occurs at the time of flowering, followed by a decline beginning at the fruiting stage [12]. The property of every essential oil vary according to the harvest country, altitude, period of sunshine, conditions of harvests, quality of the distillation, storage and usage that some is done [13].

Among Monoterpenes, are present linalol and camphene. Sesquiterpenes represented by sesquiterpenic lactones (cadinene and caryophyllene) constitute 22% of this oil. Predominant alcohol is terpineol with a percentage of 3.18.

Table 1 Principal components of the essential oil of Bay leaf.

Components	TR	Area	Quantity in %
α-pinene	3.663	12748929	0.89640921
3-carene	4.497	76733841	5.39534904
α-limonene	5.891	43240398	3.04034096
Eucalyptol	6.285	502269866	35.3158555
γ-terpinene	7.562	14651194	1.03016224
β-linalol	10.412	320365784	22.5257227
4-terpineol	12.833	29790547	2.09464816
α-terpineol	14.196	45293431	3.18469486
α-terpineol acetate	17.929	4247002	0.29861737
Camphene	19.165	104923164	7.37741112
Isoeugenol	22.17	41769277	2.93690275
Eugenol methyl ether	23.434	130506761	9.17625807
Delta cadinene	24.007	4574595	0.32165126
α-caryophyllene	24.786	3206549	0.22546051
Isopulegol acetate	25.855	8603251	0.60491618
α-spathulenol	29.137	20397912	1.43422841
α-cadinol	31.79	12296625	0.86460658

This composition confers to this essential oil expectorating, neurotonic, antispasmodic, antirhumatismal and anti-infectious properties.

3.1.3 Antibacterial Activity

The antibacterial activity results are summarized in Table 2.

The confrontation of the reading results of the antibiograms with those of the aromatograms for the eight bacterial strains reveals that:

Escherichia coli is sensitive to a very range of antibiotics (Colistin, Cefazolin, Ceftriaxone, Cefoxitin, Imipenem, Gentamicin, Amikacin, Pefloxacin, Nalidixic Acid, Cotrimoxazol and Chloramphenicol) but it is resistant to Ampicillin. The application of the impregnated discs of essential oil allowed noting that this strain is very sensitive to the pure oil and diluted at 1/2, on the other hand, it is of a weak sensitiveness to the essential oil diluted at 1/4, 1/8, and 1/16.

Proteus sp. is resistant to Ampicillin, Ticarcillin,

Table 2 Results of the antibacterial activity.

Dilutions / Souches	Pure		1/2		1/4		1/8		1/16	
	D (mm)	S	D (mm)	S	D (mm)	S	D (mm)	S	D (mm)	S
Escherichia coli	9.5	+++	11.5	+++	7.15	+	7.7	+	7.1	+
Proteus sp.	11.9	+++	6.65	+	< 6	-	< 6	-	< 6	-
Serratia sp.	8.6	++	12.15	+++	12.3	+++	9.9	+++	11.45	+++
Klebsiella pneumoniae	8.75	++	7.55	+	11.05	++	8.55	++	11.05	+++
Staphylococcus aureus	18.75	+++	23.35	+++	9.65	+++	7.55	+	6.4	+
Streptococcus D	23.6	+++	12.25	+++	10	+++	11.5	+++	12.25	+++
Pseudomonas aeruginosa	23.6	+++	12.25	+++	10	+++	11.5	+++	12.25	+++
Acinetobacter baumanii	7.3	+	9.3	+++	9.9	+++	11.45	+++	8.1	++

D (mm): inhibition diameter; S: Signification.

Cotrimoxazol and Chloramphenicol. It is sensitive to Cefoxitin, Pefloxacin and Nalidixic Acid. This strain showed a very big sensitiveness towards the pure essential oil but it is resistant to the different used dilutions.

Among the bacterial strains, The *Serratia* are the most resistant to the antibiotics [14]. The strain tested is resistant to Ampicillin, Ticarcillin, Ofloxacine and Cotrimoxazol but it is sensitive to Ceftriaxone, Imipenem, Pefloxacin and Chloramphenicol. This strain is rather sensitive to both pure essential oil and different used dilutions.

Klebsiella pneumoniae presents a resistance towards Amoxicilline, Ceftriaxone, Cefazolin, Gentamicin, Amikacin and Cotrimoxazol. It is sensitive to Ticarcillin, Cefoxitin, Imipenem, Pefloxacin, Nalidixic Acid, Chloramphenicol, Colistin and Furane. It is rather sensitive to the pure essential oil, diluted at 1/4 and 1/8, and very sensitive to the one diluted at 1/16.

Staphylococcus aureus is resistant to Kanamycin and Penicillin. It is sensitive to Cefoxitin, Gentamicin, Amikacin, Pristinamycin, Erythromycin, Rifampicin, Vancomycin and Oxacillin. This strain is very sensitive to the pure essential oil and to the one diluted at 1/2 and 1/4; it is of a weak sensitiveness to the remaining of dilutions.

Streptococcus D presents a resistance to the Nalidixic Acid, Lincomycin and Penicillin but it is sensitive to Ampicillin, Erythromycin, Pristinamycin, Rifampicin, Vancomycin and Fosfomycin. This strain was not able to develop into any of the used concentrations. It presents a high sensitiveness to this essential oil.

Pseudomonas aeruginosa is resistant to Cefazolin, Imipenem, Gentamicin, Pefloxacin, Fosfomycin and Piperacillin but it is sensitive to Amikacin and Tobramycin. This strain presents a very big sensitiveness to the essential oil of Bay leaf with all concentrations.

The *Acinetobacter* are very resistant to the majority of the antibiotics [14]. The studied strain is resistant to the following antibiotics: Cefazolin, Cefoxitin, Gentamicin, Pefloxacin, Cotrimoxazol, Pristinamycin and Tobramycin. It is sensitive to Imipenem and Colistin. This strain is also sensitive to the diluted oil at 1/2, 1/4, 1/8 and 1/16 but it is, relatively, of a weak sensitiveness to the pure essential oil.

3.2 Discussion

It was found that the bacterial strains that are very sensitive to the essential oil of Laurel even very diluted are: *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Streptococcus* D and *Serratia* sp..

The result obtained with *Pseudomonas aeruginosa* and *Streptococcus* D can be explained by the presence

of sesquiterpene lactones that have a significant antimicrobial effect against both bacterial strains [15, 16].

Pseudomonas aeruginosa is recognized as least sensitive to the effects of the essential oils [17] because it displays an intrinsic resistance to wide variety of essential oils [18]. As it is known that the sensitivity of different *Pseudomonas* towards different antibiotics is sometimes paradoxical, it is essential to test the response of other species of *Pseudomonas* to essential oil of Laurel, to draw any conclusions.

The results obtained with *Klebsiella pneumoniae* are consistent with the result obtained by El Houssine [8] with the essential oil of Laurel of Morocco.

Staphylococcus aureus, *Acinetobacter* and *E. coli* are less sensitive to this oil. In effect, *E. coli* has a low sensitivity to diluted oil, which is consistent with the results obtained by Haddouche and Benmansour [18] but is not consistent with the results obtained by Bouzouita and his colleagues [15] with the essential oil of laurel oil of Tunisia. From the point of view of the chemical composition, both oils have similar major components [16]. The difference would probably be in the minor components of these two essential oils.

It is known that the biological activity of an essential oil depends on its chemical composition and synergy between the various components both majority and minority [19]. The synergy between the terpene (linalool), lactones, oxides (1.8 cineole) and monoterpenes (camphene, alpha pinene) seem to give to the essential oil of Laurel an interesting antibacterial activity. 1.8 cineole should have a role in this antibacterial activity because it is effective against several strains such as *E. coli*, *P. aeruginosa*, and *Staphylococcus aureus* [20] and it is the constant major component of this essential oil. Among the eight bacterial strains studied, *Proteus* sp. is the most resistant to this essential oil (except to its pure form).

4. Conclusions

The noble Laurel is endemic in the Mediterranean region. In Algeria, it has a culinary and medicinal value.

The essential oil extracted from Laurel grown in eastern Algeria and analyzed by GC/MS proved to be rich in 1.8 cineole (35.31%), β linalool (22.52%), Eugenol methyl ether (9.17 %), camphene (7.37%) and 3 carene (5.39%).

The composition of this oil gave an interesting antibacterial activity especially against *Streptococcus* D and *Pseudomonas aeruginosa* with a diameter of inhibition ranging from 10 mm to 23.6 mm. *Staphylococcus aureus* is especially sensitive to concentrated forms while *Serratia* sp., *Klebsiella pneumoniae*, *Acinetobacter baumanii* and *Escherichia coli* are sensitive to even very diluted oil (respectively, 11.45 mm, 11.05 mm, 8.1 mm and 7.1 mm). *Proteus* is the least sensitive strain.

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