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CHEMICAL COMPOSITION AND EVALUATION OF THE BIOACTIVITY OF *LAURUS NOBILIS* ESSENTIAL OIL FROM NORTH-WEST (MOROCCO)

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Abstract

The antibacterial and anti-Candida activity of *Laurus nobilis* essential oil is evaluated in vitro against ten bacterial strains resistant to conventional antibiotics and three yeasts. The extracted essential oil is analyzed chemically by gas chromatography and mass spectrometry (GC-MS). Twenty five components were identified, of which Eucalyptol represented 33.47%, the other predominant components were α-terpinyl acetate (17.39%), sabinene (9.18%), α -cadinol (7.31%), α-terpineol (6.07%), β-elemene (5.15%), β-selinene (4.54%), terpinene-4-ol (3.99%) and α -pinene (3.17%). The study of the antimicrobial effect showed that this oil has an inhibitory effect on *Acinetobacter baumanii, Salmonella sp* and *Citrobacter freundii* with a MIC of 1/500 v / v. The study of anti-Candida properties of *laurus nobilis* oil shows an inhibitory effect on *Candida albicans, Candida tropicalis*and *Saccharomyces cerevisiae* with a MIC of 1/250 v/v.

Keywords: *Laurus nobilis, essential oil, Chemical composition, antibacterial activity, anti-Candidaactivity.*

Introduction

Currently, the safety and effectiveness of chemical molecules used in medicine raise several questions. Indeed, it has been proven that the effectiveness of antibiotics has greatly decreased because of their misused; which led to the development of bacterial resistance [1-2]. Faced with the therapeutic limits of conventional antibiotics, new approaches are developed using oils , macerates and plants extracts as antibacterial and antifungal agents [3].

Laurus nobilis L. is an evergreen tree or shrub of the family Lauraceae that contains 50 genera and about 2500-3500 species [4]. *Laurus nobilis* also called in Arabic *"rakat Moussawa"*. *L. nobilis* originating from the Mediterranean regions (Turkey, Greece, Spain, Portugal, Morocco and Mexico) is characterized by leaves and aromatic flowers [5-6]. Dried leaves are widely used in cooking and the essential oil is generally used in the aroma industry [2]. *L. nobilis* has been used as a medicinal plant in several fields as an antibacterial [7], antifungal [8], antiproliferative, antioxidant and insecticide agent [9-10].

The present study aims to determine the chemical composition of *Laurus nobilis* essential oil (EO) leaves originating from north-western Morocco, and the evaluation of its antibacterial and anti-Candida capacity on antibiotic-resistant germs.

Methods

Plant material

The leaves of *Laurus nobilis* are recovered during the flowering period (March-April) in Kenitra region (N 34°_ 26' / W 06°_ 57'), north-west of Morocco. The botanical identification of species is carried out in the Botanical and Plant Protection Laboratory of Ibn Tofail University, Kenitra, Morocco.

Extraction of the essential oil

Extraction of the essential oil is performed by hydro-distillation in a Clevenger type apparatus [11]. The distillation is achieved by boiling 200 g of dried

and ground leaves in 1 L of water for 3 h. The essential oil is stored at 4 ° C in the dark in the presence of anhydrous sodium sulfate. The yield of the essential oil (%) is calculated as follow:

$$\text{Yield\%} = \frac{\text{HE (g)}}{\text{dry matter (g)}} \times 100$$

Chromatographic analysis

The chromatographic analyzes are carried out on a gas chromatograph (Perkin ElmerTM GC-680) coupled to mass spectrometry (Q-8 MS with ion trap). The fragmentation is performed by electronic impact under a field of 70 eV. The capillary column used is an MS Rxi®-5sil (1,4-bis (dimethylsiloxy) phenylene dimethyl polysiloxane (30m × 0.25mm), the thickness of the film is 0.25µm, the temperature of the column is programmed from 60 to 310 ° C at a rate of 4 ° C / min, the carrier gas is helium whose flow rate is set at 1 ml / min, the injection of the sample works in split mode. to a computer system managing a NIST 98 mass spectrum library.

The identification of the constituents is carried out by gas-chromatography-mass spectrometry (GC-MS) after calculation of their Kovats indices (IK).

Microorganisms studied

The germs used to determine the antimicrobial activity of *L. nobilis* EO are selected for their pathogenicity and antibiotic resistance: Two Gram-positive bacteria (*Staphylococcus aureus, Staphylococcus epidermidis*), eight Gram-negative bacteria (*Escherichia coli, Proteus mirabilis, Acinetobacter baumanii, Salmonella sp, Klebsiella pneumoniae, Citrobacter freundii, Enterobacter cloacae and Pseudomonas aeruginosa*) and three yeasts (*Candida albicans, Candida tropicalis, Saccharomyces cerevisiae*). The germs are maintained by subculture on nutrient agar favorable to their growth. The bacterial strains are cultured on Muller Hinton medium and yeasts on Sabouraud medium.

Microbiological procedure
Disk diffusion

The disk diffusion method is used for the determination of antimicrobial and anti-Candida activity [12]. A microbial suspension with an optical density of 1 McFarland is spread on the Petri dish of solid medium. A sterile Whatman paper disc 6 mm in diameter is impregnated with 15 µl of *L. nobilis* EO, and then deposited on the surface of the seeded agar; the whole is incubated for 24 hours at 37 °C for

bacteria and for 48 hours at 30 °C for the yeasts. Upon application of the impregnated disks, the essential oil uniformly diffuses, and after the incubation, the presence around the disks of a circular inhibition zone in which there is no growth of microorganisms denotes the sensitivity to the EO. The larger the inhibition zone, the more sensitive the microbe is. Amoxicillin (25µg) and ampicillin (10µg) are used individually as positive controls for bacteria. All tests are done in triplicate.

Minimal inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of the essential oil is carried out according to the method reported by Remmal et al., [13]. Cited by Nounah et al., [14]. The essential oil is emulsified with an agar solution at a rate of 0.2%. It makes it possible to obtain a homogeneous distribution of the EO in the medium. Dilutions are prepared at 1/10e, 1/25e, 1/50e, 1/100e, 1/200e, 1/300e and 1/500e in this agar solution. In test tubes each containing 13.5 ml of solid medium Muller Hinton (bacteria) or Sabouraud (yeast), sterilized by autoclaving for 20 min at 121 ° C, cooled to 45 ° C, 1.5 ml is added aseptically of each of the dilutions so as to obtain the final concentrations of 1/100, 1/250, 1/500, 1/1000, 1 /2000, 1/3000 and 1/5000 (v/v). The tubes are shaken well to homogenize the EO in the culture medium before pouring them into the Petri dishes. Controls containing the culture medium and the 0.2% agar solution alone are also prepared.

Plating is done by striations using a calibrated platinum loop to collect the same volume of inoculums. The latter is in the form of culture broth 24 hours for bacteria and 48h for yeasts. The incubation is at 37 °C for 24 hours for the bacteria and at 25 °C for 48 hours for the yeasts. Each test is repeated three times.

Results and discussion

Yield and chemical composition

The yield of *L. nobilis* EO leaves is 1.07% (v/w). This rate is much larger than reported values, 0.88% and 0.71% respectively [2-15]. Chromatographic analyzes of *L. nobilis* essential oil made it possible to identify 25 components, representing approximately 99.78% of the total composition **(Table 1)**. *L. nobilis* essential oil is characterized by a high content of monoterpenes: eucalyptol (33.47%), α-terpinyl acetate (17.39%), sabinene (9.18%), α -cadinol (7.31%), α-terpineol (6.07%), β-elemene (5.15%), β-selinene (4.54%), terpinene-4-ol (3.99%) and α- pinene (3.17%). These are the main components accounting for 90.27% of the EO, the 9.51% left represent secondary constituents. The composition of the *L. nobilis* essential oil leaves collected from the northern region of Morocco [16], is characterized by a higher concentration of eucalyptol (52.43%). Conversely, the chemical composition of *L. nobilis* from Tunisia [10] shows a lower level of eucalyptol (24.55%).

It is interesting to note that at different concentrations, eucalyptol remains predominant in *Laurus nobilis* EO originating from several countries around the Mediterranean (Tunisia and Algeria [17-12], Italy [18], Jordan [19], Portugal [20] and Turkey [21]).

The difference in yield and percentage of chemical compositions of essential oils can be explained by genetic factors, edaphic, climatic, geographical origin and conditions of harvesting, extraction and storage of the essential oil [22-23-24-25].

Antimicrobial activity

The results of the *L. nobilis* EO microbial sensitivity test are summarized in **Table 2**. EO inhibits the growth of all microbial strains tested, with the exception of two bacteria: *Proteus mirabilis* and *Pseudomonas aeruginosa*. The diameter of the inhibition zone varies from 8 to 13 mm for bacteria and from 8 to 10 mm for yeasts. *Escherichia coli, Citrobacter freundii, Salmonella sp, Acinetobacter baumanii* and *Enterobacter cloacae* strains which are resistant to the two antibiotics Amoxycillin and Ampicillin are inhibited by *L. nobilis* EO. *Staphylococcus aureus* and *Staphylococcus epidermidis* resistant to the action of ampicillin are inhibited by *L. nobilis* EO, the inhibition effect on *Staphylococcus epidermidis* being greater than the one of the amoxycillin. The growth of *Klebsiella pneumonia* is strongly inhibited by the action of the two antibiotics (amoxycillin, ampicillin) compared to *L. nobilis EO*. Similar results are reported [17-4-26-27].

All the germs studied are sensitive to *L. nobilis* EO. The bacteria show a different sensitivity toward *L. nobilis* EO: *Acinetobacter baumanii, citrobacter freundii* and *Salmonella spp* are inhibited at the

minimum concentration of 1 / 500 (v/v) **(Table 3)**, while *Staphylococcus aureus, Staphylococcus epidermidis*, *Escherichia coli* and *Klebsiella pneumoniae* are less sensitive to EO with a MIC of 1/250 v/v. On the other hand, *Enterobacter cloacae* are the only one that is more resistant to the action of oil since its MIC is 1/100 v/v.

These results show that Gram-negative bacteria (*Acinetobacter baumanii, Citrobacter freundii* and *Salmonella sp*) are more sensitive to *L. nobilis* EO than Gram positive bacteria, unlike other reported studies [22-28]. The three yeast strains studied are inhibited starting from the concentration of 1/250 v/v. Other studies [8-15] report similar effects.

Research has shown that essential oils [27-29-30] rich in eucalyptol and poor in phenolic derivatives do not exhibit antimicrobial activity. However, the results obtained demonstrate that *L. nobilis* EO has antibacterial and anti-Candida activity on the majority of strains studied despite its high eucalyptol content. This activity could be due, on one hand, to the interaction between various aromatic structures; these molecules act most often by a synergistic action or by the presence of minor compounds that can contribute significantly to the activity of the essential oil [2], and on the other hand to the presence of pinene-type monoterpene hydrocarbons (α- pinene and β-pinene) [27-31] and terpinen-4-ol [32] known for their antimicrobial potential.

Conclusion

The objective of this work was to study the chemical composition of *Laurus nobilis* essential oil leaves from north-westernMorocco and the evaluation of its bioactivity. GC-MS analysis revealed the presence of 25 compounds that accounted for 99.78% of the total essential oil; the majority component was eucalyptol (33.47%). EO exhibits an interesting antibacterial activity to the four gram-negative bacteria*: Esherichia coli, Acinetobacter baumanii, Citrobacter freundii* and *Salmonella sp*, compared to that observed by the use of amoxycillin and ampicillin respectively, and an anti-Candida activity on: *Candida albicans, Candida tropicalis* and*Saccharomyces cerevisiae.*

Given the results found, the use of *Laurus nobilis* essential oil as a broad-spectrum antibiotic is encouraging.

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

	Compound	IK	%
1	α-Pinène	941	3,17
2	Camphène	955	0,27
3	Sabinène	977	9,18
4	β-Pinène	981	1,24
5	α-Phellandrène	1006	0,31
6	α-Terpinène	1020	0,48
7	Eucalyptol	1035	33,47
8	(Z)-β-ocimène	1030	T
9	γ-Terpinène	1064	0,86
10	Cis-sabinène hydrate	1070	0,3
11	Terpinène-4-ol	1180	3,99
12	α-Terpinéol	1190	6,07
13	cis-Piperitol	1218	0,07
14	Nérol	1227	0,44
15	Linalyl acétate	1258	T
16	Bornyl acétate	1291	0,25
17	α-Terpinyl acétate	1351	17,39
18	β-Elémène	1390	5,15
19	Caryophyllène	1420	1,46
20	β-Copaene	1432	T
21	α-Guaiene	1443	0,72
22	α-Humulène	1449	1,17
23	β-Sélinène	1485	4,54
24	δ-Cadinène	1518	1,94
25	α-Cadinol	1657	7,31
	Total identified %		**99,78%**

IK : *Kovats index.*
Tr: Traces amounts (< 0.05%)

Table 2: The antibacterial and anti-Candida activity of *Laurus nobilis* EO by the diffusion method on disc

Microorganisms	Inhibition zone diameter (mm) [a]		
	Essential oil	Antibiotics	
	Laurus nobilis	Ampicillin	Amoxycillin
BACTERIA			
Acinetobacter baumanii	12 ± 00	NA	NA[b]
Staphylococcus aureus	11 ± 0.41	NA	12
Enterobacter cloacae	08 ± 0.45	NA	NA
Klebsiella pneumonia	10 ± 0.28	13	14
Staphylococcus epidermidis	11 ± 0.41	NA	07
Escherichia coli	09 ± 0.88	NA	NA
Citrobacter freundii	13 ± 0.10	NA	NA
Salmonella sp	12 ± 0.12	NA	NA
Proteus mirabilis	00 ± 0.0	NA	NA
Pseudomonas aeruginosa	00 ± 00	NA	NA
YEASTS			
candida albicans	09 ± 00	NT[c]	NT
candida tropicalis	09 ± 00	NT	NT
saccharomyces cerevisiae	10 ± 0.24	NT	NT

a : Diameter of the inhibition zone including the disc diameter of 6 mm, by the agar disc diffusion method at a concentration of 15 µl of oil / disc and a concentration of 10 and 25 µg / disc of ampicillin and amoxicillin, respectively.
bNA, non active.
c NT, non tested.

Table 3: Minimum inhibitory concentration of*Laurus nobilis* essential oil

	1/100 v/v	*1/250 v/v*	*1/500 v/v*	*1/1000 v/v*	*1/2000 v/v*	*1/3000 v/v*	*1/5000 v/v*	*control*
	LN	***LN***	***LN***	***LN***	***LN***	***LN***	***LN***	***LN***
BACTERIA								
Acinetobacter baumanii	-	-	-	+	+	+	+	+
Citrobacter freundii	-	-	-	+	+	+	+	+
Salmonella sp	-	-	-	+	+	+	+	+
Staphylococcus aureus	-	-	+	+	+	+	+	+
Staphylococcus epidermidis	-	-	+	+	+	+	+	+
Escherichia coli	-	-	+	+	+	+	+	+
Klebsiella pneumonia	-	-	+	+	+	+	+	+
Enterobacter cloacae	-	+	+	+	+	+	+	+
YEASTS								
Candida albicans	-	-	+	+	+	+	+	+
Candida tropicalis	-	-	+	+	+	+	+	+
Saccharomyces cerevisiae	-	-	+	+	+	+	+	+

(–): inhibition; (+):growth; LN: Laurus nobilis