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Proprieties of *Laurus nobilis from Mascara (Algeria)*

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Abstract

Laurus nobilis is an aromatic plant, common in Algeria and widely used by local people as a source of spice and for medicinal purposes. The essential oil of this plant is the subject of this work in a physicochemical and microbiological study. The extraction of the essential oil was carried by steam distillation, the yield obtained from the leaves (1.5%),pH (5,65), the specific gravity (0,917), miscibility in ethanol (1V/4V) ,the refractive index (1,3329) ,the optical rotation(+ 0,5°), the indices acid(2,3) , ester(21,74), peroxide(2960) . The organoleptic and physico-chemical characters are consistent with those obtained in the literature with some differences that can be attributed to certain factors. Evaluation of antibacterial activity showed a sensitivity of Salmonella spp. with an MIC of 2,5 mg.ml^{-1}, and other bacteria of the intestinal flora of Westar rats: E.. coli and Lactobacillus sp. have a high potential for resistance with MICs respectively equal to 10 and 20 mg.ml^{-1} .

Keywords*: Laurus nobilis, essential oil, physicochemical character, MIC, intestinal flora, antibacterial activity*

Introduction

Medicinal plants have been used for centuries as remedies for human diseases because they contain chemical components of therapeutic value. *Laurus nobilis* L. belongs. to the family Lauraceae, which comprises numerous aromatic and medicinal plants**[1]** . *Laurusnobilis* L. native to Mediterranean regions is also known as sweet bay, bay laurel, Grecian laurel, true bay, and bay. The dried leaves are used extensively in cooking, and the essential oil is generally used in the flavourings industry **[9]**.Laurel essential oil, also called laurel leaf oil or sweet bay essential oil, is also used for the preparation of hair lotion due to its antidandruff activity and for the external treatment of psoriasis **[7]** .

Materials and Methods

Plant materials: *Laurus nobilis* L. leaves was harvested in April and June 2014from Mascara (Algeria), this leaves were dried for 10-15 days in darkness and at room temperature.

Isolation of the essential oils: Essential oils of leaves of Laurus *nobilis* is obtained by steam distillation of water, for 2h 30mn. The essential oil yield was estimated according to dry leaves byusing the following equation (Boutekedjiret *et al*., 2003). R (%) = (m /mo) x 100 Where m: essential oil mass (g), mo: dry leaves and fruit matter mass (g), R: essential oil yield (%).

The physicochemical indices of essential oils

1. Chemical characteristics

1.1. Acid indices (AI) : The acid expresses the number of milligrams of potassium hydroxide (KOH) required to neutralize the free acids contained in one gram of essential oil. It weighs 2 grams of essential oil, and is introduced into a glass flask. 5 ml of 95% ethanol and 5 drops of phenolphthalein (PP) at 0.2%. Neutralized by adding a0020urette through the ethanol solution of KOH (0.1 mol / l) until a pink color. We denote the volume of the ethanolic solution of KOH added. The calculation of AI is given by the formula: AI = 5.61 xV / M
5.61: Corresponds to 0.1 mol / L KOH
M: mass in grams of the essential oil
V: Volume in milliliters of ethanol solution of KOH (0.1 mol / l) used for titration.

1.2. Ester indices (IE): The ester value is the number of milligrams of KOH needed to neutralize the free acids by hydrolysis of esters contained in one gram of essential oil. It weighs 2 grams of essential oil, and is introduced into a glass flask. Was added through a burette 25ml of ethanol solution of KOH (0.5 mol / l). It adapts thecondenser and placed the ball on the heating mantle and allowed to heat for one hour. Allowed to cool then add20ml of distilled water and 5 drops of 0.2% PP. Finally, as the excess of KOH solution with hydrochloric acid 0.5 mol / l. alongside the operation cited, it makes a blank under the

same conditions and with the same reagents. The calculation of EI is given by the formula:

IE = (28.05 x (V0-V1) / M)-IA
28.05 g / l: corresponding to 0.5 mol / L KOH.
M: mass in grams of the test.
V0: Volume in cl ml solution (0.5 mol / l) used for the blank.
V1: volume in ml of the solution cl (0.5 mol / l) used to determine the IE of the essential oil.

1.3. Peroxide indices (PI): The peroxide is the number of micrograms of active peroxide content in one gram of products and oxidizing potassium iodide to release iodine under the conditions of the method described. Weigh 1g of the oil in a microwave tube you put in an Erlenmeyer flask, add 10 ml of chloroform and shake. Add 15 ml of acetic acid CHCOOH, then 1 ml of saturated aqueous KI, stopper immediately, shake the bottle and leave for 5 min in the dark. 75 ml of distilled water. Titrate carefully in the presence of starch, iodine released with Na2S2O3 solution (0.01 N) until complete discoloration of the solution.

The calculation of PI is given by the formula:

IP = 8000 V / m.
m: is the mass of the test.
V :is the volume of N/100 thiosulphate solution.
Their antibacterial activity was studied in vitro on tree bacterial Strains The bacterial strains tested were found to be sensitive to essential oils studied and showed a very effective bactericidal activity with minimum inhibitory concentrations (MIC) ranging from 0.01 to 1 mg/ ml. ***Laurus nobilis***. L. is a tree and has been used for its astringent, healing and diuretic properties.

Antibacterial Activity

Bacterial strains *Salmonella*, *Escherichia coli et Lactobacillus*, tested were isolate respectively from waste water , colon and ileum of *Rattus norvegicus* ,these three bacterial strains are purified; Identified and stored in Bioconversion Laboratory of Microbial Engineering and Health Safety, Department of Biology ,University of Mascara.

Disk diffusion method

The antibacterial activity was tested using the disk diffusion method (Davis, 1994). Bacterial cultures were reactivated by sub culturingon nutrient agar and incubated for 24 h at 37°C. From these, pure cultures were prepared by releasing bacterial inoculum strains in physiological water. The homogeneous suspension was equivalent to 0.5 McFarland, so an OD of 0.08 to 0.10 was read at 625 nm. Each essential oil was used at different concentrations: pure oil, diluted oil in DMSO (Dimethyl sulfoxide) to ratio 1/2, 1/4 and 1/8.Discs of 6 mm in diameter, previously sterilized, were used. 10 µl of essential oils was put on each disc and placed on agar. A witness disc (soaked in DMSO) was incubated under the same conditions to ensure that DMSO was devoid of antibacterial activity. After incubation for 24 h in an oven at 37°C, reading was done. The effect of essential oils on bacteria was estimated by the appearance of clear zones around the discs. The diameter of the halo of growth inhibition was measured and expressed in mm (including the diameter of the disc of 6 mm).

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) is the smallest concentration of essential oil, in which no growth is visible compared to the control without extract. It was evaluated on twelve tested strains by disc diffusion test. We used the dilution method on solid medium (incorporation) [2] and [14].Serial dilutions of essential oils were performed with DMSO for 2 h. Each dilution was incorporated into Mueller-Hinton medium, maintained, super cooled and poured into Petri dishes. The concentrations (in percent), of essential oils used are respectively: 1, 0.5, 0.25, 0.01, 0.125, 0.06 and 0.03. Witness discs containing culture medium and only DMSO were also prepared. Seeding was done as a deposit of bacterial suspension. After incubation at 35°C for six days, the growth was compared to the control.

Results and Discussion

Organoleptic properties of essential oils extracted

- **Color:** Reddish yellow
- **Odor:** Aromatic, pungent
- **Taste:** Very spicy

The yield of essential oil of ***Laurusnobilis*** is 1.5%. This result is similar than that given by Elharas *et al*. 2013but greater than that given by Kahouli, 2010 (0, 95 %,) &Ouibrahim *et al*. 2013 (1, 3%).

The quality of essential oils depends on many causes, including the process for obtaining the state of maturation and storage of the substance, its origin.

- The results of the physico-chemical analysis obtained are summarized in the following table

Table 1 The physicochemical indices of essential oil extracted from *Laurus nobilis*

Property		Values	Values of reference	Reference
Physical characteristics	pH	5,65	5,0 à 6,0	[10]
	Density	0,917	0,915 à 0,93	[10]
			0,91 à 0,944	[11]
	Miscibilitywithethanol	1V(HE)/ 4V(Ethanol)	1V(HE) / 0,5V(Ethanol)	[6]
	Refractive index at 20 °	1,3329	1,472	[10]
			0,46 à 1,477	[11]
	Rotatory power	+ 0,5°	-10° à +0,929°	[13]
			-19 ° à -10 °	[10]
Chemical Characteristics	Acid Indice	2,3	2,25	[11]
	Ester Indice	21,74	22,44	[11]
	Peroxyde Indice	2960	9600	[6]

The value of the density of our essential oil is 0.917. Referring to the following table 2, we note that our oils complex**.**

Table 2 Value of the density

	D < 0.9	0.9 < D < 1	D > 1
Essential oils	rich in terpenes	Have a complex composition	Products still contain the aromatic series, sulfides, nitrites

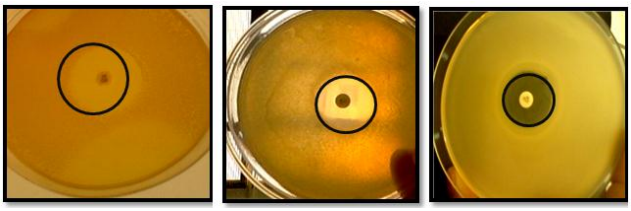
The organoleptic and physico-chemical characters are consistent with those obtained in the literature with some differences that can be attributed to certain factors.

Antibacterial study

Results obtained in the antibacterial study of the essential oilsof Laurus nobilis , are shown on Table 3;

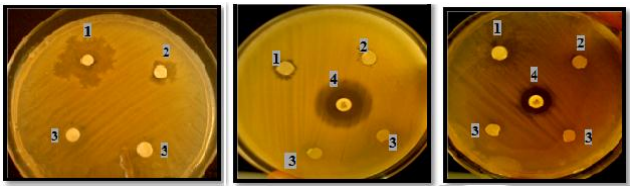
Table 3 Antibacterial Activity of leaves Essential oils of ***Laurus nobilis***

Essential oils	*Salmonella spp.*	*Escherichia coli*	*Lactobacillus sp.*
Disc diffusion assay(inyibition zone mm)	23mm	19,3	15
MIC(mg/ml)	2,5	10	20



Salmonella spp. (S) *Escherichia coli*(S) *Lactobacillus sp*. (R)

Photo 01 : zones d'inhibition exercées par la gentamicine sur les souches bactériennes testées



Salmonella spp. *Escherichia coli* *Lactobacillus sp.*

1: HE/DMSO (10%, v/v) | 2: HE | 3: Eau sterile/DMSO (10%, v/v) | 4: ATB

Photo 02 : Activité antibactérienne de l'HE évaluée par la méthode de diffusion par disque

The essential oil of *Laurus nobilis* has demonstrated a strong activity on the Salmonella, the highest sensitivity was in the Salmonella sp. that has an inhibition diameter of23mm (MIC of 2,5 $mg.ml^{-}$) , other bacteria of the intestinal flora of Westar rats: *E.. coli* and *Lactobacillus sp.* have a high potential for resistance with MICs respectively equal to 10 and 20 $mg.ml^{-1}$

Conclusion

The quality control of our essential oils with the physicochemical characteristics helped to highlight the quality of these oils have a complex composition, high acidity and physicochemical indices comparable to those obtained in the literature. Evaluation of antibacterial activity showed a sensitivity of *Salmonella spp.* with an MIC of 2,5 $mg.ml^{-1}$, and other bacteria of the intestinal flora of Westar rats: *E.. coli* and *Lactobacillus sp.* have a high potential for resistance with MICs respectively equal to 10 and 20 $mg.ml^{-1}$.

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