

DRYING METHODS AND THEIR IMPLICATION ON QUALITY, QUANTITY AND ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL OF *LAURUS NOBILIS* L. FROM MOROCCO

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ABSTRACT

The effects of various methods of drying on the chemical quality and antimicrobial activity of the essential oil of *Laurus nobilis* were studied. The most prominent component in the air-dried, fresh leaf and microwave-dried leaf oils is 1,8 Cineole (58,8, 35.62 and 42,9% respectively). The essential oil has undergone significant chemical transformation in its monoterpenoids when the leaves of plant in the question were dried by the three different methods. The oils have screened for antimicrobial activity against both Gram positive (*Staphylococcus aureus*, *Enterococcus hirae*) and Gram negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacteria and two fungal species (*Penicilium digitatum* and *Alternaria sp.*). The microbial strains tested have been found sensitive to all essential oils studied.

Keywords: *Laurus Nobilis*, *Essentials Oil*, GC/MS, *Antimicrobial Activity*

1. INTRODUCTION

The Lauraceae consisted of 52 genera and almost 3000 species. *Laurus nobilis* L., (bay) a member of the family named Apollo's Laurel in mythology, is a plant widely distributed in the Mediterranean countries (especially Italy and Greece and North America) and in Europe, has been used as spices in cookery and in USA as an ornamental plant (Riaz *et al.*, 1989; Barla *et al.*, 2007; Takaku *et al.*, 2007; Marino *et al.*, 2008; Derwich *et al.*, 2009). *Laurus nobilis* is an evergreen tree and shrub up to 2-15 m height. Leaves are about 8-4 cm long and 2.5-4.5 cm wide and dark green with wavy margin. This plant is basically cultivated in many temperate parts of the world, particularly in the Mediterranean countries (Turkey, Algeria, Greece, Morocco, Portugal, Spain, Belgium and Mexico) (Demir *et al.*, 2004; Belouahem-Abed *et al.*, 2011). In Morocco this plant is widely used in folk medicine for their Antiseptic, stimulating, stomachic

and sudorific properties (Ozcan and Chalchat, 2005). Many studies on the antimicrobial activity of the essential oils of *Laurus nobilis* species have been reported (Olivera *et al.* 2007; Derwich *et al.*, 2009; Keskin *et al.*, 2010; Sellami *et al.*, 2011; Jemaa *et al.*, 2012; Fethi *et al.*, 2013). To the best of our knowledge, there are not previous reports on the relation between the effects of drying methods and the antimicrobial activity of the essential oil of *Laurus nobilis*. In this study, we present the effects of various drying methods on the chemical compositions, the yield and antimicrobial activity of the essential oils leaves of *Laurus nobilis* from Morocco.

2. MATERIALS AND METHODS

2.1. Plant Materials

The leaves of the *Laurus nobilis* were gathered from the mountains of ouaouizerth, the area of Azilal

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(Morocco) in March 2008. They were identified by Prof. Ahmed OUHAMMOU from the Faculty of Sciences SEMLALIA of Marrakech (Cadi Ayyad University, Morocco). Voucher specimen was deposited at the Herbarium of the faculty of sciences SEMLALIA (Mark 7822). The plant material is divided into three portions of 500 g. The first portion is dried eight days in the laboratory, under normal air and at room temperature (25°C) (air-dried), the second portion is dried in the micro-wave (whirlpool, model AV M510/Wp/Wh at a MW frequency of 2450 MHz, input 1330W and supply 230 V-50 Hz) (MW-dried), which is maintained in 400W during 5 min, the third part is extracted immediately with the state fresh (Fresh).

2.2. Extraction of the Essential Oils

The essential oils of the fresh and dried Leaves of *Laurus nobilis* were obtained by hydrodistillation using a Clevenger-type apparatus in 4 h time. For this purpose we put 500 g the plant material in direct contact with water inside a flask over a heat source. The flask was connected to a condenser allowing the accumulation water vapor loaded with essential oil droplets. The next step would be collecting the essential oil in a graduated burette where the volume was read directly. The essential oil was dried over anhydrous sodium sulfate, filtered and stored at 4°C in amber glass vials until analysis and antimicrobial tests. The essential oils yields were estimated according to leaves by using the following Equation 1:

$$\text{RHE}(\%) = (\text{mHE} / \text{mS}) * 100 \quad (1)$$

Where:

mHE = Essential oil mass (g)

mS = Dry or fresh leaves mass (g)

RHE = Essential oil yield (%)

2.3. Gas Chromatography/mass Spectrometry Analysis (GC/MS).

Essential oils of dry leaves of *Laurus nobilis* were analyzed by GC-MS: Trace GC ULTRA, equipped with VB-5 fused silica capillary column (5% phenyl Methylpolysiloxane, 30 m, 0.25 mm; film thickness 0.25 µm), coupled to mass spectrometer (Polaris Q MS Ion Trap, ion source 200°C, 70 ev). The oven temperature was programmed from 40 to 80°C at a rate of 4°C/min and then programmed to 100°C at a rate of 1°C/min and

from 100 to 300°C at a rate of 20°C/min and then kept constant at 300°C for 2 min. The injector temperature was 220°C; split ratio was 1:10. Diluted sample (1/10, v/v, in Methanol) of 1 µL was injected; helium was used as the carrier gas at 1.4 mL min⁻¹.

2.4. Identification of the constituents

Through using a formula as described by Dool and Kratz (1963) on VB-5 column. The Retention Indices (RI) were calculated by comparing the retention times of the eluting peaks with those of C₈-C₂₀ n-alkanes. Identification of the essential oil components was made by comparing their retention indices and mass spectra with the NIST library (NIST/EPA/NIH MASS SPECTRAL LIBRARY Version 2.0 a, build Jul. 1 2002) as well as by comparing them with those reported in the literature. The components from the gas chromatography-mass spectral analysis are reported in **Table 1**.

2.5. Antimicrobial Tests

2.5.1. Microbial Strains

Gram (+): *Staphylococcus aureus* (CIP 53.154), *Enterococcus hirae* (CIP 58.55), Gram (-): *Pseudomonas aeruginosa* (CIP A22), *Escherichia coli* (CIP 54.8). Yeast: *Candida albicans* (ATCC 2091) and two fungal phytopathogenics species (*Penicillium digitatum* and *Alternaria* sp) isolated from specimens food (Bioprocesses engineering unit, Faculty of Sciences and Technology, Cadi Ayyad University of Marrakech, Morocco).

2.5.2. Preparation of Test Microorganisms

Bacterial strains were cultured at 35°C in Mueller-Hinton broth for 24 h, while the fungal strains cultured at 30°C using Sabouraud broth for 48 h. Before the bacterial experiments were carried out, 5 mL of broth (Mueller-Hinton and Sabouraud broth) was inoculated with newly harvested bacteria and incubated for 18h at the adequate temperature. These bacterial suspensions (approximately 10⁶cfu/mL) were used to inoculate the test medium containing the essential oil.

Table 1. Weight difference between fresh and dry cases of *Laurus nobilis*

Plant	Fresh	Air- dried	MW-dried
Weight (g)	500	275	150
	250	138	76
Weight decrease (%)	0	45	70

2.5.3. Antimicrobial Activity Test

The antimicrobial activity of the selected essential oils was determined by dilution of essential oils in solid medium (agar) method (Ruberto and Baratta, 2000; Pintore *et al.*, 2002; Dobre and Niculita, 2012). In each sterile Petri dishes (5 cm in diameter), were mixed aseptically 1 mL of the essential oil with a concentration determine dissolved in DMSO (dimethylesulfoxide 5%) and 10 mL of culture medium still in a state of superfusion (Mueller-Hinton for bacteria and Sabouraud yeast strains). The Petri dishes were carefully agitated and let cool during 30 min for the solidification of the medium. In each dishes, the mixture was inoculated by spot with 1 μ L of bacterial suspension. But for yeast, the discs (1 mm in diameter) of a 48 h culture were deposited, surface in bottom, in plates containing the mixture. Dilutions of the oils within a concentration range of 20 to 0,15 mg mL⁻¹ were also carried out.

The Petri dishes were incubated at 37°C for 24 h and 30°C for 48 h for bacterial strain and fungal strain, respectively. Amoxicillin (Amx) and Amphotericine B (Aph B) were used as the reference antibiotic control for bacteria and fungi respectively. After incubation Petri dishes were evaluated for the presence or the absence of colonies. For each treatment, the non-presence of colonies on all dishes tested was considered as an inhibitory effect. The lowest concentration of essential oil required to inhibit the growth of fungi and bacteria was designated as the Minimal Inhibitory Concentration (MIC). The experiments were repeated at least twice.

3. RESULTS

The **Table 1** shows that the drying by microwave allows diminution of 70% by weight of *Laurus nobilis* against air-dried plant does not exceed 45%.

The **Table 2** shows that the GC-MS analysis of the essential oil from different methods leaves drying of *Laurus nobilis* resulted in the identification of forty compounds. The essential oil yields of the fresh and leaves dried in normal air and by micro-wave were 1,64, 1,84 and 0,96% respectively. The reduction of the oil yield caused by microwave (0,96%) is clearly related to the energy absorption. In the different leaves essential oils of *Laurus nobilis* (Fresh, Air-dried and MW-dried) 25, 14 and 13 compound were identified, which made up 99; 98 and 80% of the total essential oil respectively.

The major compounds detected in the leaves fresh oils are 1,8 cineole (58,88%), endo-Fenchol (13,26%) and α -camphenal (9,27%). In the air-dried oils, the

most major components are: 1,8 cineole (35,62%), α -camphenal (9,65%), Myrtenal (8,86%), 3-carene (7,88%) and endo-Fenchol (6,35%). The major components of oil dried by micro-wave are 1,8Cineole (42,99%) and n-hexenal (12,24%).

Table 3 presents the MIC of different essential oils determined for two Gram positive and two gram negative bacteria and two fungal species using the dilution technique of essential oils on solid media (agar). The results show that all the essential oil tested have a substantial inhibitory effect on all bacterial and fungal strains but they are different.

4. DISCUSSION

4.1. Yield and Chemical Composition of Essential Oils

In **Table 2**, extraction results show a clear difference in yield which was turned down after drying the studied plants by micro-wave. This confirms many results in studies carried out in this area by mentioning the evident effect of drying (Jerkovic *et al.*, 2001; Okoh *et al.*, 2008; Sellami *et al.*, 2011; Fethi *et al.*, 2013), whether on yield decrease or on the change of essential oil chemical composition by concentration decrease of some constituents. Englund and Ralpa, (2000; Maroto *et al.*, 2004; Okoh *et al.*, 2008; Al-Jaber *et al.*, 2012). Our studies have shown a slight increase in yield of essential oil after air drying. Even if there is a decrease weight. (Combrinck *et al.*, 2006; Sellami *et al.*, 2011). On the other hand, the decrease in weight caused by MW-dried is expressed by the decrease in moisture to preserve the product for extended shelf life (Muller and Heind 2006; Rocha *et al.*, 2011). While drying plants contributes significantly to the loss of moisture which is expressed by the water content and the set of which is among other volatile fluids. So, the essential oil is one of the most important components of the moisture in plant especially when his constituents are very volatile. On balance, we can say that the microwave drying has a clear effect on reducing oil yield. It seems very important to make the extraction in the case of air-dried, several reasons to maintain the maximum amount of these essential oils. The optimization of this drying process contributes to physical, chemical and microbiological stability of the medicinal herbs.

The oils consisted of a mixed products basically known of monoterpene and sesquiterpene. The composition of essential oil of *L. nobilis* has also been reported in previous research (Fiorini *et al.*, 1997; Simic *et al.*, 2004; Dadalioglu and Evrendilek, 2004; Sangun *et al.*, 2007; Jemaa *et al.*, 2012; Fethi *et al.*, 2013).

Table 2. Chemical composition of the essential oil from *Laurus nobilis* leaves using deferent drying methods

RI ^a	Compounds ^b	%		
		Fresh	Air-dried	MW-dried
803	n-hexenal	—	—	12,24
826	(Z)-3-Hexenol	—	—	1,09
904	Heptanal	—	—	1,92
952	Myrcene	—	0,65	—
959	Camphene	0,49	4,87	—
973	Sabinene	—	0,67	—
1000	dehydro-1,8 Cineole	2,6	—	—
1001	3-carene	—	7,88	—
1003	α -phellandrene	—	4,41	—
1020	α -Terpinene	—	0,94	—
1032	Limonene	—	0,42	—
1045	<i>Cis</i> - β -ocimene	—	1,21	—
1052	γ -Terpinene	—	0,63	—
1060	1,8 Cineole	58,88	35,62	42,99
1088	α -Terpinolene	—	2,1	—
1105	<i>trans</i> -Sabinene hydrate	3,51	3,29	—
1106	linalool	—	—	2,28
1112	<i>endo</i> -Fenchol	13,26	6,35	3,6
1118	<i>cis</i> -p-Menth-2-en-1-ol	0,78	—	3,2
1132	α -camphenal	9,27	9,65	—
1134	α -campholenal	—	—	2,64
1147	Camphor	1,95	—	—
1157	<i>trans</i> -verbenol	0,74	1,14	—
1162	Borneol	0,53	0,89	—
1178	4-Terpineol	—	0,43	—
1194	thuj-3-en-10-al	3,29	—	—
1199	Myrtenal	1,68	8,86	—
1203	Verbenone	—	0,43	—
1234	Geraniol (Nerol)	—	0,93	—
1243	Linalyl acetate	1,91	3,22	—
1262	<i>trans</i> -Caryophyllene	—	0,33	—
1348	α -Terpinyl acetate	—	0,45	—
1500	Germacrene-D	—	2,08	—
1532	δ -cadinene	—	0,64	—
1885	Unknown	—	—	1,61
1895	Unknown	—	—	1,58
1980	Eremanthin (vanillosimin)	—	—	1,42
2056	Unknown	—	—	1,07
2080	α -Cadinol	—	—	2,1
2093	Eugenol	—	—	1,97
Total identified %		99	98	80
Yield (% w/w)		1,64	1,84	0,96

^aRetention indices (RI) on VB-5; ^bCompounds listed in order of elution from a non-polar VB-5 column; — not detected

And then, the essential oils composition show a similar pattern to those published for other geographical regions, 1.8-cineole was reported as the major component in the essential oil from Turkey (Dadalioglu and Evrendilek, 2004; Ozcan and Chalchat, 2005; Kilic *et al.*, 2005), China (Zheng-kui *et al.*, 1990), Tunisia (Bouzouita *et al.*, 2001), Mediterranean (Zola *et al.*, 1977), Argentina (Huergo and Retamar, 1978) and Italy (Flamini *et al.*, 2007). The following compounds: Camphene, *trans*-sabinene hydrate, *endo*-fenchol, α -

camphenal, linalyl acetate and myrtenale were only identified at the fresh and air-dried oils. These compounds were vaporized or transformed to other compounds in the micro-wave dried leaves. The results show that only micro-wave drying brought about significant losses of the major compounds (α -camphenal, myrtenal and 3-carene) in the essential oil when compared to the fresh and air-dried plant material. This might be due to some chemical transformations during the process of drying.

Table 3. Antibacterial and antifungal activities of *Laurus nobilis* essential oils from Morocco.

	MIC ^a mg/ml					
	Bacteria (Gram-)		Bacteria (Gram +)		Fungal	
	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>E. hirae</i>	<i>P. digitatum</i>	<i>Alternaria sp</i>
Fresh	3,5	4,25	2	2,5	2	2,25
Air-dried	3	3,5	1,5	1,75	1,75	1,5
MW-dried	3,25	3,25	1,75	2,25	2	1,5
Amx	0,0035	0,00025	0,00025	0,00052	-	-
Aph B	-	-	-	-	0,00156	0,002

^aMinimal inhibitory concentration of essential oil in mg/ml.

Effect of drying on the release or retention of volatiles depends on the type of composition (Fathi and Sefidkon, 2012). In general, the best results were observed with air-drying. Also, our result is in agreement with results of Ghasemi *et al.*, 2013; Pirbalouti *et al.*, 2013.

4.2. Antimicrobial Activity

The aim of this experiment has been basically to evaluate the antimicrobial activity of laurel essential oil. The results in this study indicate that the MIC of essential oil obtained from fresh leaves is higher than the essential oil from MW-dried leaves followed by the essential oil from Air-dried which has the best antimicrobial activity. This difference may be due to the difference in the composition of essential oils caused by various methods of drying of leaves of *Laurus nobilis*. Gram-positives (*S. aureus* and *E. Herea*) have been the most sensitive strain tested to the all essential oils of *L. nobilis*. Modest activities are observed against Gram-negatives (*E. coli* and *P. Aeruginosa*) with MIC of 3- 4,25 mg mL⁻¹. Same results found by Ghazi (2013) and Verma *et al.* (2013) opposite of those found by (Imelouane *et al.*, 2009a). The synergy between terpenes (linalool), oxides (1,8 cineole) and monoterpenes (camphene, α -pinene) gives to the essential oil of Laurel a good antibacterial activity (Ouibrahim *et al.*, 2013). The major components of this oil, 1,8- cineole, have been known to exhibit antimicrobial activity against the bacterial strains (*E. Coli*, *P. Aeruginosa*, *S. Typhi*, *S. Aurus*, *Rhizobium leguminosarum* and *Bacillus subtilis*) (Sivropoulou *et al.*, 1997). In general, the antimicrobial activities of essential oils are difficult to correlate a specific compound due to their complexity and variability (Imelouane *et al.*, (2009b). Belletti *et al.* (2004), showed that the antimicrobial activities have been mainly explained through C₁₀ and C₁₅ terpenes with aromatic rings and phenolic hydroxyl groups able to form hydrogen bonds with active sites of the target enzymes, although other active terpenes, as well as alcohols, aldehydes and esters can contribute to the overall antimicrobial effect of essential oils. On the other hand, enantiomers of α -pinene,

limonene and linalool have a strong antibacterial activity (Filipowicz *et al.*, 2003; Koji *et al.*, 2004; Tampieri *et al.*, 2005). Pinene-type monoterpene hydrocarbons (α -pinene and β -pinene) are well known chemicals having antimicrobial potentials (Dorman and deans, 2000; Rios and Recio, 2005; Kazemi *et al.*, 2012).

5. CONCLUSION

This study has revealed a high level of chemical composition of the essential oils of *Laurus nobilis* originated from azilal (Morocco). The effects of various drying methods on the chemical compositions, yield and antimicrobial activity of the essential oils leaves of *Laurus nobilis* from Morocco have been studied. Our data shown that Air-drying is the best method of drying the leaves of *Laurus nobilis* because its low MIC is against all strains tested bacteria and fungi. Drying of aromatic plants affects significantly in the quantity and the quality of essential oils. Therefore, the extraction from air-dried plants not only economically increases the yield for industries, but also, saves the pharmaceutical quality of essential oils. Finally, we can say that the oil can be proposed as a valuable source in the foods as a natural antimicrobial agent. This study enables us for further attention to identify the active compounds responsible for the biological activity of this plant and explained the exact mechanism of action by which essential oils exert their antimicrobial effect. More studies conducted to determine the antioxidant activity of these oils.

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