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Chemical Composition and Antibacterial Activity of Leaves Essential Oil of *Laurus nobilis* from Morocco

[1]Elhoussine Derwich, [2]Zineb Benziane and [3]Abdellatif Boukir

[1]Régional Center of Interface, University Sidi Mohamed Ben Abdellah, B. P.2626, Fez 30.000, Morocco
[2]Energy, Natural Resources and Modeling Laboratory, University Sidi Mohamed Ben Abdellah Faculty of Sciences, Fez, Morocco
[3]Bioactive Molecules Laboratory, University Sidi Mohamed Ben Abdellah , Faculty of Sciences and Technical, Fez, Morocco

Abstract: The extraction of essential oils of leaves of *Laurus nobilis* is obtained by hydrodistillation and analyzed by gas chromatography coupled with mass spectrometry (GC/MS) and gas chromatography with flame ionization detection (GC-FID) for determining their chemical composition and identification of their chemotypes. Their antibacterial activity was studied in vitro on tree bacterial strains: *Staphylococcus aureus*, *Staphylococcus intermedius* and *Klebsiella pneumonia.* The essential oil yields of the studies were 1.86%. The major component was 1,8-cineole (52.43%), other predominant components were α-terpinyl acetate (8.96%), sabinene (6.13%), Limonene (5.25%), α-pinene (3.72%), linalool (3.14%), terpinene-4-ol (2.56%), α-terpinene (2.12%), β-pinene (1.98%), α-terpineol (1.56%), bornyl acetate (1.89%), α-phellandrene (1.28%), myrcene (1.13%), camphene (1.05%), p-cymene (0.94%), σ-terpinene (0.98%) and eugenol (0.56%). The chemical compositions revealed that this leaves had compositions similar to those of other *Laurus nobilis* essential oils analyzed in other countries. 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.

Key words: *Laurus nobilis,* essential oil composition, 1.8-cineole, antibacterial activity, GC/MS, GC-FID.

INTRODUCTION

Medicinal plants have been used for centuries as remedies for human diseases because they contain chemical components of therapeutic value (Nostro *et al*., 2000). According to the World Health Organization (WHO) in 2008, more than 80% of the world's population relies on traditional medicine for their primary healthcare needs (Pierangeli *et al*., 2009).

Laurel (*Laurus nobilis*) is an evergreen tree cultivated in many warm regions of the world, particularly in the Mediterranean countries. The Lauraceae comprise 32 genera and about 2.000-2.500 species. *Laurus nobilis* a member of the family named Apollo's Laurel in mythology, is a plant native to the southern Mediterranean region and widely cultivated mainly in Europe and the USA as an ornamental plant (Barla *et al*., 2007). *Laurus nobilis*, an evergreen tree or shrub is cultivated in many temperate and warm parts of the world, particularly in the Mediterranean area (Turkey, Greece, Spain, Portugal, Morocco and Mexico). In Turkey, it is natively cultivated on the coastal up to an altitude of 600–800 m (Davies, 1990).

Essential oils are valuable natural products used as raw materials in many fields, including perfumes, cosmetics, aromatherapy, phytotherapy, spices and nutrition (Buchbauer, 2000).

The leaves of this plant have been used to treat epilepsy (Aqili khorasani, 1992; Zargari, 1990), neuralgia and Parkinsonism (Aqili khorasani, 1992). The essential oil obtained from the leaves of this plant has been used for relieving hemorrhoid and rheumatic pains (Zargari, 1990).

Laurus nobilis, is a native species to the Mediterranean region, which is cultivated in many countries with moderate and subtropical climate. The chemical composition of the volatile fraction as well as the composition

Corresponding Author: Elhoussine. derwich, Régional Center of Interface, University Sidi Mohamed Ben Abdellah, B. P.2626, Fez 30.000, Morocco
E-mail: elhoussinederwich@yahoo.fr + 00212.645.756.052

and bioactivities of the alcoholic and non-polar extracts have been studied extensively (Pino *et al*., 1993; Caredda *et al*., 2002; Conforti *et al*., 2006). Intensive research has been conducted on this species (Kilic, 2005; Akgül, 1986; Riaz *et al*., 1989; Ozek *et al*., 1995; Pino *et al*., 1999).

There are many studies on chemical composition of the essential oil obtained from the leaves of Mediterranean and European *L. nobilis* (Riaz et al., 1989; Mohammadreza Verdian-rizi, 2008). In the study of Riaz *et al* (1989), the main components of the essential oil were cineol (44.12%), eugenol (15.16%), sabinene (6.20%), 4-terpineol (3.60%), α-pinene (2.74%), methyleugenol (2.48%), α-terpineol (2.19%) and β-pinene (2.05%).

Essential oils have been shown to possess antibacterial, antifungal, antiviral insecticidal and antioxidant properties (Burt, 2002). Some oils have been used in cancer treatment (Sylvestre *et al*., 2006). Some other oils have been used in food preservation (Faid *et al*., 1995) and aromatherapy (Buttner *et al*., 1996). Essential oils are a rich source of biologically active compounds. There has been an increased interest in looking at antimicrobial properties of extracts from aromatic plants particularly essential oils (Milhau *et al*., 1997). Therefore, it is reasonable to expect a variety of plant compounds in these oils with specific as well as general antimicrobial activity and antibiotic potential (Darokar *et al*., 1998).. The chemical composition and antimicrobial properties of essential oils extracted from diverse plant species have been demonstrated using a variety of experimental methods (Conner *et al*., 1984; Beuchat, 1994)

In the light of this work we have determined, the chemical composition, the yield and antibacterial activity of leaves essential oil of *Laurus nobilis.*

MATERIALS AND METHODS

Plant Material:

Laurus sp is a plant belongs to the family Lauraceae, which grows in the Mediterranean region. In this work, we studied the essential oils of plants collected in northern Morocco, a mountainous region where people frequently use this plant in traditional medicine. This plant has been used for many purposes since ancient times and the leaves and the bark are used in various food applications (Jirovetz *et al*., 1997).

Leaves from cultivated plants of *Laurus nobilis* have been collected during April 2009 and dried until constant weight. The apparent density of dried leaves material used for distillation was 100g/l.

Essential Oil Extraction:

Essential oils are products, generally, of rather complex composition comprising the volatile principles contained in the plants, and more or less modified during the preparation process (Bruneton, 1995). Fresh leaves (100g) of *laurus nobilis* were cut into small pieces, placed in a flask (2.5L) and hydrodistilled in a Clevenger-type apparatus for 2.5h. The oil samples were dried over anhydrous sodium sulphate and stored at 4°C in the dark.

Gas Chromatography (GC-FID) Analysis:

The GC (Trace GC ULTRA, Thermo Fischer) analysis were equipped with HP-5MS non polar capillary column (50m x 0.32mm, film thickness 0.20um), working with the following temperature program: 50∘C for 2 min, ramp of 5∘C/min up to 260∘C, and the final temperature kept for 20 min. The injector and detector temperatures were 240 ∘C and 260∘C respectively. The whole system operated at a constant flow of 1ml/min, nitrogen was used as gas carrier. The non polar column was connected to a FID, split ratio 1:30; injection of 1µl (10% cyclohexane solution). Retention indices were calculated relative to C_8-C_{29} alkanes, and compared with values reported in the literature (Davies, 1990; Adams R, 1995).

Gas Chromatography - Mass Spectrometry (GC/MS):

The GC-MS analysis using Trace GC ULTRA /Polaris Q (Thermo Fischer) was performed with a gas chromatograph ultra- Trace equipped with HP-5MS capillary column (50m x 0.32mm; coating thickness 0.20um) and ion trap mass detector. Temperatures of the transfer line and the ionic source were 300∘C and 200∘C, respectively; scan range, 40-650 amu; 3.9 scans/s. Oven temperature programmed from 50 to 260 ∘C ramp of 3∘C/min; injector temperature was 250°C; carrier gas helium at 1 ml/min; injection of 1µl (10% cyclohexane solution); split ratio 1:30.

Identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their linear retention indices relative to the series to C_8–C_{29} alkanes, and on computer matching against commercial (NIST-MS) and laboratory-developed library mass spectra built up from pure

substances and components of known oils and MS literature data (Stenhagen E *et al*., 1974; R.P. Adams, 1995).

Antimicrobial Tests:

In recent years due to an upsurge in antibiotic-resistant infections, the search for new prototype drugs to combat infections is an absolute necessity and in this regard plant essential oils may offer great potential and hope. These products have frequently been reported to be antimicrobial agents (Mouhssen, 2004; Martinez N *et al*., 1973; Franchomme, 1981; Benjilali *et al*., 1984; Tantaoui *et al*., 1992; Panizzi *et al*., 1993; Remmal, 1994; Lacoste *et al*., 1996).

The selected essential oils were screened against four: *Staphylococcus aureus*, *Staphylococcus intermedius* and *Klebsiella pneumoniae*. The minimal inhibitory concentration (MIC) was determined only with microorganisms that displayed inhibitory zones. MIC was determined by dilution of the essential oils in dimethyl sulfoxide (DMSO) and pipetting 0.01 ml of each dilution into a filter paper disc. Dilutions of the oils within a concentration range of 0.01-1 mg/ml were also carried out. MIC was defined as the lowest concentration that inhibited the visible bacterial growth (NCCLS, 2005).

A negative control was also included in the test using a filter paper disc saturated with DMSO to check possible activity of this solvent against the bacteria assayed. The experiments were repeated at least twice.
In this study, antibacterial activity of *Laurus nobilis* oil was examined using different bacterial species. In addition, composition of volatile compounds, were also determined.

RESULTS AND DISCUSSION

Chemical Composition:

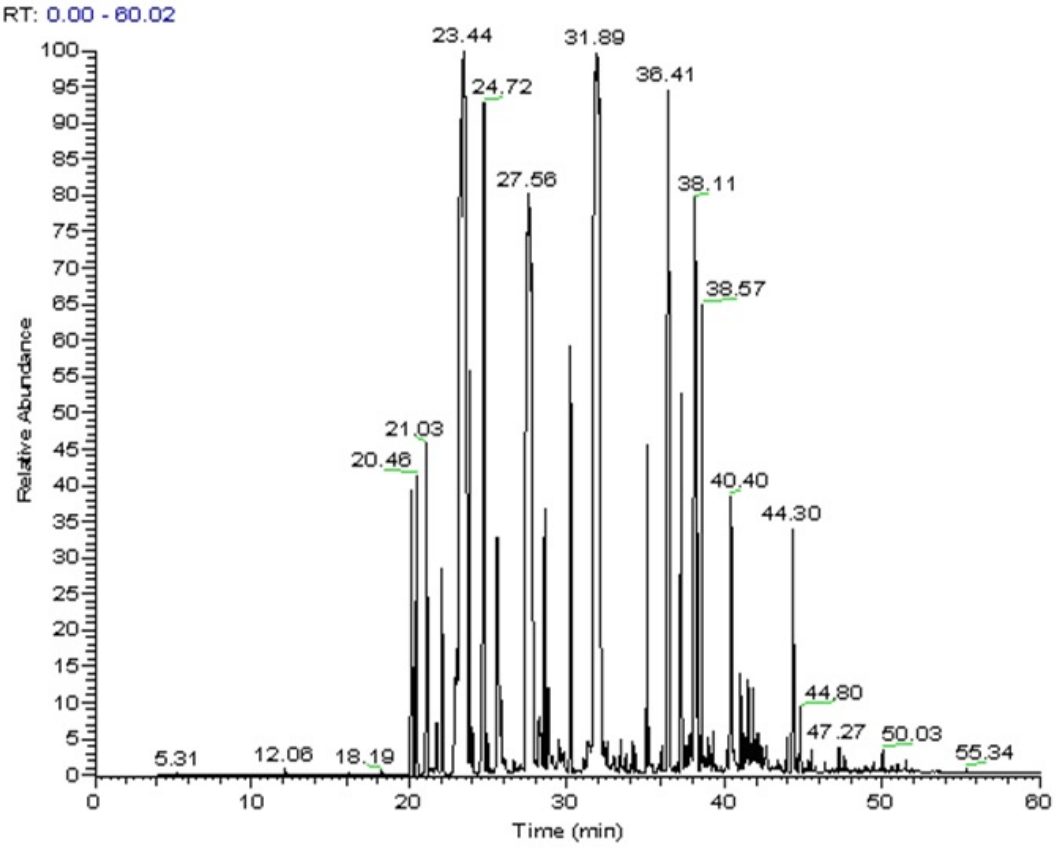

Fig. 1: Chromatogram of *Laurus nobilis*

The constituents of laurel leaf oil from Morocco are listed in order of their elution on the HP- 5MS column (Figure 1). The essential oil yields of the studies were 1.86%. In the leaf essential oil of *laurus nobilis* (Table 1), 26 compounds were identified, which made up 98.59% of the total essential oil. The most abundant

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

Peak	Compound	*RT (min)	**KI	Air (%)	***Mass range (m/z)
1	α-terpinene	20.46	1112	2.12	(154),71,111,93,43,86,41,69,55,68,154
2	terpinen-4-ol	21.03	1182	2.56	(136),68,93,39,67,41,27,53,79,94,92
3	linalool	22.14	1093	1.98	(136),93,41,69,39,91,77,79,27,92,53
4	1,8-cineole	23.44	1117	52.43	(154),43,93,81,71,69,84,68,108,41,55
5	α-terpineol	25.10	1177	1.56	(154),59,93,121,136,81,43,68,95,67,41
6	sabinene	27.56	973	6.13	(136),93,41,91,77,79,39,27,69,94,43
7	bornyl acetate	28.15	1261	1.89	(196),95,43,93,436,121,41,80,55,108,69
8	Terpinolene	29.41	1088	0.11	(136),93,121,91,136,79,77,105,39,41,107
9	methyl-eugenol	30.03	1376	1.70	(178),178, 136,147,103,91,107,179,151,41,77
10	α-terpinyl acetate	31.89	1335	8.96	(196),43,121,93,136,68,41,59,67,81,79
11	Germacrene D	32.03	1472	0.10	(204),161,105,91,41,119,79,81,93,77,27
12	Limonene	36.41	1020	5.25	(136),93,92,91,77,79,41,39,121,27,105
13	α-phellandrene	37.45	998	1.28	(136),93,77,91,136,79,94,41,80,92,39
14	α-pinene	38.11	928	3.72	(153),71,41,43,93,55,69,80,39,121,27
15	β- pinene	38.57	966	3.14	(136),93,91,136,121,77,92,79,43,41,105
16	myrcene	40.40	944	1.13	(136),41,93,69,39,27,53,79,77,67,91
17	(Z)-3-Hexenol	41.24	862	0.20	(100),67,41,39,55,82,31,69,53,54,27
18	camphene	42.07	949	0.05	(136),93,79,91,77,41,121,80,94,107,39
19	σ-terpinene	43.50	1062	0.98	(136),93,91,136,121,77,92,79,43,41,105
20	p-cymene	44.80	1034	0.94	(134),119,134,91,120,117,41,77,39,65,115
21	eugenol	47.27	1325	0.56	(164),164,103,77,149,131,91,55,104,137,133
22	Sabinene	48.04	973	0.58	(136),93,41,91,77,79,39,27,69,94,43
23	3-carene	49.13	1005	0.48	(136),93,91,79,77,92,121,80,136,94,105
24	Isobornyl acetate	50.26	1262	0.34	(196),95,43,121,93,136,41,108,110,55,82
25	α-thujene	51.25	973	0.21	(136),93,41,91,77,79,39,27,69,94,43
26	β-Elemol	55.34	1545	0.19	(208),208,193,209,91,65,133,79,77,177,105
Total				98.59	
Yields (%)				1.86	

*RT: Retention time obtained by chromatogram (Fig1).
**KI: Kovats Index was determined by GC-FID on a HP-5MS column.
***Mass range (m/z) was determined by mass spectrometry (PlarisQ).

constituents were 1.8-cineole (52.43%), α-terpinyl acetate (8.96%), sabinene (6.13%), Limonene (5.25%), α-pinene (3.72%), and β-pinene (3.14%). According to published data (Lawrence B, 1987; Pino J *et al*., 1993), 1, 8-cineole is within 27 to 60 %, so the analyzed leaf oil is lower in this oxide which is important for the characteristic flavor of this spice (Pino J *et al*., 1999).

The essential oils composition showed 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 (Ozcan *et al*., 2005; Dadalioglu *et al*., 2004; Kilic *et al*., 2005), China (Zheng-kui *et al*., 1990), Tunisia (Bouzouita *et al*., 2001), Mediterranean (Zola *et al*., 1977), Croatia–Serbia (Politeo *et al*., 2007; Simic *et al*., 2004), Italy (Flamini *et al*., 2007), the Netherlands (Hokwerda *et al*.,1982) and Argentina (Huergo *et al*., 1978).

The essential oil content shows variations in plants of different geographical origin and also in different part of the tree. Recently, Yalçin H *et al* (2007); studied the composition of *Laurus. nobilis* oil collected from the Northern Cyprus Montains (Turkey), they reported that the essential oil of leaves is characterized by a high content of 1.8-cineole (58.59%), terpinen-4-ol (4.25%), α-pinene (3.39%), sabinene (3.32%) and β-pinene (3.25%). In our previous studies on the chemistry of Tunisian *Laurue nobilis* (Marzouki, Khaldi *et al*., 2008; Marzouki, Piras *et al*., 2008), considerable differences were observed in the essential oil composition between stems, leaves, buds and flowers.

Antibacterial Activity:

Results obtained in the antibacterial study of the essential oils are shown on Table 2. With the agar disc diffusion assay, oils were found to be active against *Staphylococcus aureus* and *Staphylococcus intermedius* at a minimal inhibitory concentration (MIC) of 0.35 and 0.56mg/ml respectively. *Klebsiella pneumoniae*, the oil from the leaves was found to be more active; the oils showed MIC values of 0.70mg/ml. The data indicated that *Staphylococcus aureus* was the most sensitive strain tested to the oil of *Laurus nobilis* with the strongest inhibition zone (13 mm). The *Staphylococcus intermedius* was, in general, found to be more sensitive among bacteria with inhibition zone of 10 mm. modest activities were observed against *Klebsiella*, with inhibition zones of 7 mm.

In general, the antimicrobial activities have been mainly explained through C_{10} and C_{15} 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

Table 2: Antibacterial activity of leaves essential oils of *Laurus nobilis*.

Essential oils	Microorganisms		
	Staphylococcus aureus	*Staphylococcus intermedius*	*Klebsiella pneumoniae*
Disc diffusion assay (inhibition zone mm)	13	10	7
MIC (mg/l)	0.35	0.56	0.70

Disc diameter 6 mm average of two consecutive trials
MIC: Minimal Inhibitory Concentration, concentration range: 0.01-1 mg/ml

antimicrobial effect of essential oils (Belletti *et al*., 2004). On the other hand, enantiomers of α-pinene, β-pinene, limonene and linalool have a strong antibacterial activity (Gina *et al*., 2007**;** Magiatis *et al*., 1999; Filipowicz *et al*., 2003; Koji *et al*., 2004). Pinene-type monoterpene hydrocarbons (α-pinene and β-pinene) are wellknown chemicals having antimicrobial potentials (Dorman *et al*., 2000). The major components of this oil, 1.8- cineole, has been known to exhibit antimicrobial activity against the bacterial strains (*E. coli, P. aeruginosa, S. typhi, Staphylococcus aureus, Staphylococcus intermedius, Bacillus subtilis*) (Sivropoulou *et al*., 1997).

Conclusion:

This study revealed a high level of chemical composition of the essential oils of *Laurus nobilis* originated from localities in northern Morocco. The leaf oil obtained from laurel grown in Morocco was characterized by GC-MS, GC-FID and 26 volatile compounds were identified which made up 98.59% of the total essential oil. The essential oil yields of the studies were 1.86%. The major component was 1.8-cineole (52.43%). Chemical composition of this essentials oil had a similar to that published for *Laurus nobilis* oils from different countries. As a result, the inhibitory effect of both oils on the growth of certain bacteria (*Staphylococcus aureus, Staphylococcus* and *intermedius Klebsiella pneumoniae),* is an interesting finding in view of their eventual application as natural antimicrobial compounds taking into account the increasing alarm on the use of traditional antibiotics.

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