Asian Journal of Chemistry; Vol. 28, No. 9 (2016), 2069-2072

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2016.19903

Chemical Composition and Antimicrobial Activity of Essential oil of *Laurus nobilis* Leaves from Algeria

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Received: 10 February 2016; *Accepted*: 2 May 2016; *Published online*: 1 June 2016; AJC-17944

The extraction of essential oils of *Laurus nobilis* leaves is obtained by hydrodistillation and analyzed by gas chromatography coupled with mass spectrometry (GC/MS) for determining their chemical composition and identification of their chemotypes. The volatile extract was also subjected to screening for their potential antimicrobial activity *in vitro* against three pathogenic bacteria strains (*Staphylococcus aureus, Pseudomonas aeruginosa, Eschirichia coli*) and one yeast specie (*Candida albicans*) using the diffusion method from a solid disk. The essential oil yields of the studies were 0.79 %. The major components were 1,8-cineole (10.655 %), linalool (11.072 %) and terpenyl acetate (11.495 %), other predominant components were methyl eugenol (9.748 %), β-caryophyllene (5.874 %), eugenol (3.864 %) and α-terpineol (3.247 %). The chemical compositions revealed that this leaves had compositions similar to those of other *Laurus nobilis* essential oils analyzed in other countries but with a different percentage. The results of antimicrobial analysis showed that 0.25 % oil of laurel oil completely inhibits the growth of *Candida albicans*, whereas 0.5 % is fungicide. Regarding *Escherichia coli* and *Staphylococcus aureus*, *Laurus nobilis* oil, revealed bactericidal activity at the concentration of 2 % and 4 %, respectively.

Keywords: *Laurus nobilis*, Essential oil composition, GC/MS, Antimicrobial activity.

INTRODUCTION

Natural substances such as bioactive molecules from plants currently attracting particular interest by their multiple biological activities as appreciated in the field of human health and the food industry [1]. 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 [2]. Laurel (*Laurus nobilis L.*) is an evergreen tree up to 20 m high, native to the Mediterranean region [3]. It is the only European representative of the Lauraceae family [4]. It is also known as sweet bay, bay, bay laurel, Grecian laurel, true bay and Mediterranean bay. The dried leaves are used extensively in home cookery and the essential oil is used mainly in the flavoring industry. Laurel essential oil, laurel leaf oil or sweet bay essential oil were reported to be used in the preparation of hair lotion for its antidandruff activity and for the external treatment of psoriasis. This oil is generally obtained by hydro or steam distillation [3]. There are many studies on chemical composition of the essential oil obtained from the leaves of Mediterranean and European *Laurus nobilis* [5,6]. Riaz *et al.* [5] reported the main components of the essential oil were cineole (44.12 %), eugenol (15.16 %), sabinene (6.20 %), 4-terpineol (3.60 %), α-pinene (2.74 %), methyl-eugenol (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 [7]. 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. 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. The chemical composition and antimicrobial properties of essential oils extracted from diverse plant species have been demonstrated using a variety of experimental methods [8]. In the light of this work we have determined, the chemical composition, the yield and antibacterial activity of leaves essential oil of *Laurus nobilis* leaves from Algeria.

EXPERIMENTAL

Laurus nobilis 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 Algeria (Larabaa, Blida), 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 [8]. Leaves from cultivated plants of *Laurus nobilis* have been collected during April to June 2010 and dried until constant weight.

Essential oil extraction: Dried leaves (100 g) of *Laurus nobilis* were cut into small pieces, placed in a flask (2 L) and hydrodistilled in a Clevenger-type apparatus for 3 h. The oil samples were dried over anhydrous sodium sulphate and stored at 4 °C in dark [9].

Gas chromatography-mass spectrometry (GC/MS): Analysis of the chemical composition of our *Laurus nobilis* essential oils were carried out by gas chromatography coupled with mass spectrometry (GC/MS). The latter is carried out on a gas chromatograph Hewlett-Packard type (HP 6890 series) coupled with a mass spectrometer (HP 5973 series N MS, electron impact ionization). This study can simultaneously determine the number of components of gas, its concentration and their levels of outputs, which provide information on the volatility, their molecular masses and their polarities.

The operating conditions of gas chromatography (GC) are: Capillary column 5 % phenyl methyl siloxane has the following characteristics: Length: 30 m, internal diameter: 0.25 mm, film thickness: 0.25 µm

The operating conditions are: (1) splitless injector temperature: 250 °C; (2) temperature program: from 35 to 250 °C at 6 °C/min; (3) interface temperature: 280 °C; (4) carrier gas: helium at 1 mL/min (average linear velocity = 36 cm/sec); (5) The amount injected: 0.2 µL.

Mass spectrometer: (1) temperatures of the source and the quadrupole are set at 230 and 150 °C; (2) ionization energy 70 eV; (3) mass range: 35-400.

Identification of individual compounds was made by comparison of their Mass spectra with those of the international reference Mass spectra library or with authentic compounds and confirmed by comparison of their retention indices with authentic compounds or with those of reported in the literatures [3,8,9].

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 [10,11]. The selected essential oil was screened against four species *i.e.*, *Staphylococcus aureus, Pseudomonas aeruginosa, Eschirichia coli* and *Candida albicans*. The minimal inhibitory concentration (MIC) was determined only with microorganisms that displayed inhibitory zones. The minimum inhibitory concentration, minimum bactericidal concentration (CMB) and minimum fungicide concentration (CMF) on microorganisms (bacteria and yeasts) subject to contact with *Laurus nobilis* essential oils and this by the solid medium dilution method using absorbent discs for bacteria and yeasts. The method used is that of the dilution in a solid medium. The essential oil solution must be (4 %) 0.4/10 (v/v). It was prepared in Mueller-Hinton agar (for bacteria) and Sabouraud agar (for yeast) supplemented with Tween 80 and kept super cooled. A dilution series of each was prepared at concentrations ranging from 4 to 0.03 % for the essential oil. The minimal inhibitory concentration (MIC) was defined as the lowest concentration that inhibited the visible bacterial growth. The experiments were repeated at least twice [12-15].

RESULTS AND DISCUSSION

The constituents of laurel leaf oil from Algeria are listed in order of their elution on the column (Fig. 1). The essential oil yields of the studies were 0.79 %. In the leaf essential oil of *Laurus nobilis* leaf (Table-1), 35 compounds were identified, which made up 83.86 % of the total essential oil.

TABLE-1
CHEMICAL COMPOSITION OF LEAVES ESSENTIAL OIL OF *Laurus nobilis*

Peak	Compound	Leaves essential oil of *Laurus nobilis L.*	
		RT* (min)	Air (%)
1	α-Thujene	11.712	0.045
2	α-Pinene	11.932	0.441
3	Camphene	12.424	0.090
4	Sabinene	13.316	1.259
5	β-Pinene	13.407	0.630
6	Myrcene	13.878	0.218
7	3-Carene	14.490	0.068
8	α-Terpinene	14.711	0.121
9	1,8-Cineole	15.302	**10.655**
10	*cis*-β-Terpineol	16.255	0.267
11	Linalool	17.449	**11.072**
12	Borneol	18.552	0.070
13	*iso*-Borneol	19.184	0.978
14	Terpen-4 ol	19.515	1.533
15	α-Terpineol	19.926	3.247
16	Nerol	20.729	0.357
17	Bornyl acetate	22.253	1.000
18	Terpenyl acetate	23.969	**11.495**
19	Eugenol	24.129	3.864
20	β-Elemene	24.962	1.992
21	Eugenol methyl ether	25.242	**9.748**
22	α-Gurjunene	25.413	0.599
23	β-Caryophyllene	25.764	**5.874**
24	α-Guaiene	26.025	0.955
25	α-Caryophyllene	26.466	0.846
26	Cedrene	27.098	1.693
27	β-Elinene	27.218	1.086
28	τ-Elemene	27.409	1.810
29	δ-Guaiene	27.559	1.434
30	β-Cadinene	27.910	1.478
31	Elemicine	28.422	1.691
32	Spathulenol	29.204	1.835
33	Caryophyllene oxide	29.335	2.218
34	β-Guaiene	30.248	1.004
35	α-Cadinol	30.739	2.190
Σ		-	83.863
Other unidentified compounds		-	15.881
Total		-	99.744
Yield (%)		-	0.79

*RT: Retention time obtained by chromatogram (Fig. 1)

The major components were 1,8-cineole (10.655 %), linalool (11.072 %) and terpenyl acetate (11.495 %), other predominant components were methyl eugenol (9.748 %),

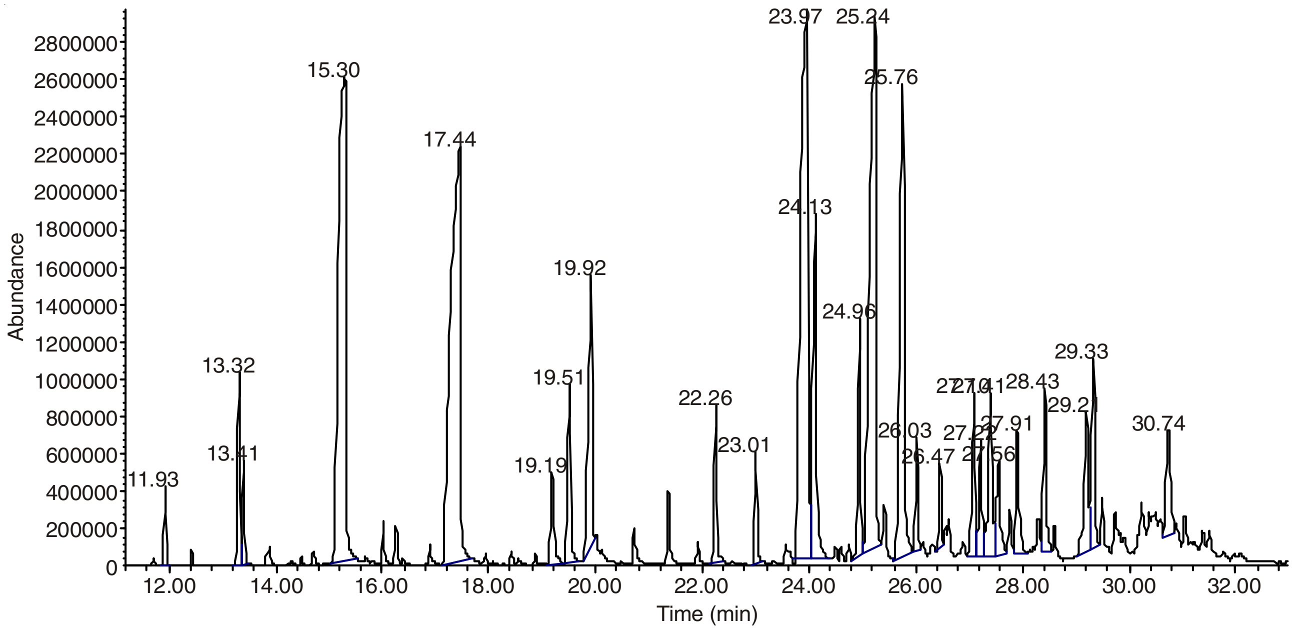

Fig. 1. Chromatogram of leaves essential oil of *Laurus nobilis*

β-caryophyllene (5.874 %), eugenol (3.864 %) and α-terpineol (3.247 %). The chemical compositions revealed that this leaves had compositions similar to those of other *Laurus nobilis* essential oils analyzed in other countries but with a different percentage. 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 Algeria and Tunisia [3].

The essential oil content shows variations in plants of different geographical origin and also in different part of the tree. Yalçin *et al.* [16] 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 of Tunisian *Laurue nobilis*, considerable differences were observed in the essential oil composition between stems, leaves, buds and flowers [17].

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 *Escherichia coli* at a minimal inhibitory concentration (MIC) of 2 % and 1 %, respectively. In case of *Candida albicans*, the oil from the leaves was found to be more active. The oils showed MIC values of 0.25 %. The data indicated that *Staphylococcus aureus* was the most sensitive strain tested to the oil of *Laurus nobilis* with the strongest inhibition zone (18.11 mm). The *Escherichia coli* was, in general found to be more sensitive among bacteria with inhibition zone of 16.79 mm. modest activities were observed against *Pseudomonas aeruginosa*, with inhibition zones of 12 mm. while the greatest inhibition diameter with oils obtained from the leaves of *Laurus nobilis* against *Candida albicans* yeast by 21 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 antimicrobial effect of essential oils [18]. On the other hand, enantiomers of α-pinene, β-pinene, limonene and linalool have a strong antibacterial activity [19]. Pinene-type monoterpene hydrocarbons (α-pinene and β-pinene) are well known chemicals having antimicrobial potentials [20]. 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*) [8].

Conclusion

This study revealed a high level of chemical composition of the leaves essential oils of *Laurus nobilis* originated from localities in northern Algeria. The leaf oil obtained from laurel grown in Algeria was characterized by GC-MS and 35 volatile

TABLE-2
ANTIBACTERIAL ACTIVITY OF LEAVES ESSENTIAL OILS OF *Laurus nobilis*

Essential oils	Microorganisms			
	Bacteria			Yeast
	Escherichia coli	*Pseudomonas aeruginosa*	*Staphylococcus aureus*	*Candida albicans*
Disc diffusion assay (inhibition zone mm)	16.79	12	18.11	21
Minimal inhibitory concentration (%)	1	–	2	0.25
Minimum bactericidal concentration (%)	2	–	4	–
Minimum fungicide concentration (%)	–	–	–	0.5

compounds were identified which made up 83.86 % of the total essential oil. The essential oil yields of the studies were 0.79 %. The major components were 1,8-cineole (10.655 %), linalool (11.072 %) and terpenyl acetate (11.495 %). The chemical composition of this essential oil had a similar composition to that published data for *Laurus nobilis* oils from different countries. As a result, the inhibitory effect of both oils on the growth of certain bacteria (*Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa*) and certain yeast (*Candida albicans*), 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.

ACKNOWLEDGEMENTS

The authors thank the Regional Center of Sonatrach, Boumerdes, Algeria for GC/MS analysis.

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