Indian Journal of Natural Products and Resources
Vol. 16(3), September 2025, pp 399-409
DOI: 10.56042/ijnpr.v16i3.16174

# Efficacy of *Laurus nobilis* leaves essential oil for antimicrobial and antioxidant activities

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*Received 03 January 2025; revised received 08 June 2025; accepted 04 July 2025*

This study aims to characterize essential oils extracted (EOEs) from *Laurus nobilis* L. leaves via hydrodistillation (HD) using the Clevenger apparatus, microwave-assisted hydrodistillation (MWAHD), and water steam entrainment (WSE), and analyze their antimicrobial and antioxidant activity. The EOE yield obtained by MWAHD was 0.74% for a contact time of 40 min, whereas it reached 0.52 and 0.42% by HD and WSE for 200 min. Antioxidant activity results revealed that the extracted essential oils are excellent natural antioxidants and very effective oxidation inhibitors with a minimum concentration $IC_{50}$ of 0.254 (HD), 0.318 (MWAHD), and 0.025 mg/g (WSE). GC-MS analysis of the volatile compounds revealed the presence of 15 compounds in the Clevenger and MWAHD extracts, with 85.83 and 72.41% respectively, relative amounts, whereas 16 compounds were found in the WSE extract, representing 87.23% of the total compounds. All EOEs contained almost the same dominant components: 1,8 cineole (24.06% for HD; 12.04% for MWAHD and 29.94% for WSE), linalool acetate (10.86%, 11.74%, 12.72%), alpha-terpineol acetate (20.02%; 18.28%, 15.07 %), methyl isoeugenol (7.99%; 11.05% and 8.50%), iso eugenol (2.56%, 3.96% and 2.93%), limonene compounds (1.31%, 3.158% and 1.24%), espatulenol (1.25%, 1.94%, 1.25%). and cadinol (1.01%, 1.59%, 0.11%), respectively. EOE's microbiological study was carried out using the disk method for bacterial sensitivity strain determination. EOEs were found to be effective against both Gram-positive and Gram-negative bacteria in this study, but the WSE approach was more effective, with a minimum inhibition and inhibition zone values of 1.56 mg/mL and 23.5 mm respectively. Further studies on the effect of dominant compounds in these EOEs could help to highlight their antibacterial and therapeutic importance, and hence their use in medical formulations.

**Keywords:** Antimicrobial, Antioxidant activity, Essential oil, *Laurus nobilis* L, Microwave-assisted hydrodistillation, Water steam entrainment

**IPC code; Int. cl. (2021.01)**– A61K 36/00, A61K 36/54, A61K 127/00, A61P 31/00, A61P 39/00

## Introduction

Algeria has significant resources in aromatic and medicinal plants, which can be used in various fields, including pharmacy, cosmetics, perfumery, and the food industry. Medicinal plant extracts have been used for years in traditional medicine by different civilizations around the Mediterranean. Owing to the beneficial effects of *Laurus nobilis* L. essential oil, it is crucial to assess the quality of extracted oil by exploring different extraction methods to improve overall functionality. *Laurus nobilis* or laurel belongs to the Lauraceae family, native to the Mediterranean and Asia regions[1]. Laurel grows wild in the northern Tell region of Algeria. Natural products have a chemical composition rich in secondary metabolites, which play an important role in plant physiology. They exhibit various antioxidant, anti-inflammatory, anticancer, antibacterial, and antifungal activities. Additionally, there are currently hundreds of drugs based on active compounds isolated from plants. Essential oils are utilized in the food industry to enhance the flavour to a wide variety of products with exerted biological properties that can indirectly contribute positively to product preservation[2,3].

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Identification of bioactive chemical compounds by GC-MS revealed that laurel essential oil contains monoterpene hydrocarbons (α- and β-pinene, sabinene), oxygenated monoterpene hydrocarbons (1,8-cineole, α-terpinyl acetate, linalool, α-terpineol), and aromatic compounds (eugenol and methyleugenol, which can exhibit conservative properties through different mechanisms[4]

Additionally, antioxidant properties are associated with the presence of aromatic compounds[5,6,7], including eugenol, a known radical scavenger[8]. Despite the versatile roles of eugenol in health and disease, methyl eugenol, which is biosynthetically related[9] and generally present at higher levels than eugenol, has been shown to be toxic, thus limiting its direct application to foods and the cosmetics industry[10]. Eugenol is well known for its anti-inflammatory properties[11]. Many studies have demonstrated that eugenol can inhibit inflammation by modulating TNF-α, IL-1, IL-6, COX-2, PGE2, and NF-κB[11]. This compound also inhibited cell proliferation in gastric cancer in vivo by suppressing the NF-κB pathway. Eugenol was found to enhance the effectiveness of the anticancer drug gemcitabine and exert anti-inflammatory activity in human cervical cancer cells[12].

This study aims to compare the performance of essential oils obtained from Algerian *Laurus nobilis* leaves via different extraction techniques, carry out chemical identification, and assess their antioxidant and antimicrobial activities.

## Materials and Methods

### Chemicals

Chemicals of analytical grade were supplied by Merck, Aldrich: Methanol 96%, sodium carbonate, and nutrient agar for preservation of bacterial strains, nutrient broth for inoculum enrichment, Mueller–Hinton agar, ascorbic acid, gallic acid, Folin-Ciocalteu reagent, DDPH (2.2-diphenl-1-picrylhydrazyl).

### Preparation of plant material

*L. nobilis* leaves were harvested between September and October 2021 in the Sidi Ali wilaya of the Mostaganem region (Algeria). They were washed with distilled water to remove all impurities, then dried at 60°C for 24 to 48 hours to constant weight and stored in a moisture-free place.

### Extraction processes

Extraction of essential oils was carried out using three different methods: hydrodistillation using a Clevenger system (HD), water steam entrainment (WSE), and microwave-assisted hydrodistillation (MWAHD).

### Hydrodistillation

Exactly 100 g of chopped dried *L. nobilis* leaves mixed with 2 L of distilled water were subjected to HD for 3 hours. The distillate (essential oil and aromatic water) was separated by a simple decantation (separating funnel). The essential oil was collected and stored at 2°C in a stoppered opaque glass tube to protect it from air and light until further use.

### Water steam entrainment

The apparatus used was a Spring A 105 12 L Steam distiller. The same mass (100 g of chopped dried *L. nobilis* leaves) was placed on a perforated grid, not in direct contact with water. Water vapour from a boiler passed through the plant material and carried away the essential oil to form a mixture (aromatic water-essential oil). After 3 hours of extraction and condensation, the essential oil was collected after decantation, dried with anhydrous sodium sulfate, and stored in amber bottles at 2°C until further use.

### Microwave-assisted hydrodistillation

The *L. nobilis* leaves were extracted by MWAHD in a modified 2450 MHz microwave oven (MOD. MG-402T, UK) with a maximum power of 800 W. The dimensions of the cooking cavity were (L × W × H) 48 cm × 32 cm × 26 cm.

The same mass as above was mixed with 500 mL of distilled water in a 2 L Pyrex glass cylinder. The mixture was treated by microwave at 450 W and 100°C for 40 minutes. During the process, the steam passed through the condenser outside the microwave cavity, where it condensed. The essential oil was separated from water by decantation every 10 minutes, dried, and stored likewise at 2°C.

### Physicochemical analysis of essential oils

Physicochemical analysis was carried out on the essential oil from *L. nobilis* leaves: Yield and density were measured using a glass pycnometer, refractive index with a refractometer, colour and appearance were visually checked by three people.

### Extraction yield (R)

Essential oil yield R was estimated by the following equation:

$$R(\%) = \frac{mass\ of\ essential\ oil}{mass\ of\ dried\ plant\ leaves} \times 100$$

**Determination of humidity level**

Many medicinal plants are sometimes not intended for immediate use and have to be preserved for future use. Storage is possible only when certain procedures are carried out beforehand on the picked plants. One of the most common processes is drying. Most plants are high in water content, with around 60-80% water for fresh plants. Moisture content (or humidity level) of the plant was determined by oven drying 5g of fresh leaf powder at 105°C until constant mass.

Humidity level was determined using the following equation:

$$H(\%) = \frac{(mass\ of\ fresh\ leaves - mass\ of\ dried\ leaves)}{mass\ of\ fresh\ leaves} \times 100$$

**Relative density**

Equal volumes of each EO and distilled water were weighed using a pycnometer. First, an empty bottle was rinsed with distilled water, dried, and placed in a thermostatic bath at the pycnometer's calibration temperature (20°C). Once the equilibrium calibration temperature had been reached, the weight of the empty pycnometer was determined. The procedure was repeated with the distilled water and the EO. The relative density of the essential oil was calculated using the formula:

$$d = \frac{m_2 - m_0}{m_1 - m_0}$$

Where $m_0$ is the mass of the empty pycnometer (g), $m_1$ is the mass of the pycnometer filled with distilled water (g), and $m_2$ is the mass of the pycnometer filled with essential oil (g)[13,14].

**Refractive index**

The refractive index is a measure of the bending of a ray of light when passing from one medium to another; it is equal to the ratio of the sine of the angle of incidence to the sine of the angle of refraction, usually at the wavelength of 589 nm, at constant temperature. The refractive index was determined with a Hanna instrument refractometer at 21°C.

***GC-MS analysis***

GC-MS analysis was performed using a gas chromatograph coupled to a TQ8030 mass spectrometer (Shimadzu, Kyoto, Japan). The GC-MS spectra were obtained by setting the following conditions: The Ion source temperature was set to 200°C, and the interface temperature to 220°C. The oven was programmed from 50 to 220°C at a rate of 3°C/min. The injector and detector temperatures were set to 200 and 220°C, respectively. Helium gas was used as carrier gas with a flow rate of 3 mL/min. 0.8 µL of essential oil sample was injected in split mode with a split ratio of 1:100. Mass spectra were obtained by an electron impact (EI) of 70 eV, scanning a mass range from 45 to 500 units with a speed of 0.3 sec. The total and the average peak areas were used to determine the relative percentage amount of each component.

**Determination of total polyphenols**

This test is based on the Folin-Ciocalteu method. The FC reagent consists of an acid yellow solution (Ac) containing a polymer complex of ions (heteropoly acids). In an alkaline environment, the Folin-Ciocalteu reagent oxidizes phenols to phenolate ions and partially reduces its heteropoly acids, resulting in the formation of a blue complex. The reduced reagent is detectable in the 690-710 nm range.

The total phenolic content of *L. nobilis* leaf extracts was determined by this method. A volume of 200 µL of ethanolic extract was mixed with 1 mL of Folin–Ciocalteu reagent diluted 1/10. 0.8 mL of 7.5% sodium carbonate buffer was added to the mixture after 1 minute of stirring. The mixture was stored away from light and at room temperature for 30 minutes. Absorbance was read at 765 nm using a Jenway UV-Vis spectrophotometer.

**Determination of antioxidant activity**

The antioxidant activity of essential oils was evaluated by measuring the scavenging power of 2,2-diphenyl-1-picryl hydrazyl (DPPH) radicals according to the method described by Ordoudi *et al.*[6]. A volume of 100 µL of each of the ethanolic essential oil solutions prepared at different concentrations was mixed with 2.9 mL of a 0.004% (w/v) DPPH ethanol solution. After 30 min of incubation in the dark and at room temperature, the absorbance was measured at 517 nm using a UVmini-1240 UV-Vis spectrophotometer. We proceeded in the same way for ascorbic acid, the reference antioxidant. A negative control of 100 µL of ethanol and 2.9 mL of the DPPH solution was also prepared.

The antiradical activity is expressed as a percentage reduction of the DPPH solution. The reduction power is determined by applying the following formula:

$$Antioxidant\ activity\ (\%) = \frac{(AC - AE)}{AC} \times 100$$

Table 1 — Physicochemical parameters of essential oils obtained via water steam entrainment, Clevenger hydrodistillation, and microwave-assisted hydrodistillation routes

| Parameter | WSE | Clevenger hydrodistillation | MWAHD |
|---|---|---|---|
| Yield R (%) | 0.42 | 0.50 | 0.74 |
| Extraction time (min) | 200 | 200 | 40 |
| Relative density | 0.915 | 0.922 | 0.918 |
| Refractive index | 1.484 | 1.490 | 1.487 |
| Odor | Strong spicy | Strong spicy | Strong spicy |
| Colour | Light yellow | Light yellow | Light yellow |

Where *AE* is the Absorbance of the DPPH° solution in the presence of essential oil, ascorbic acid, or absorbance of a test tube, and *AC* is the Absorption of the DPPH solution in the absence of essential oil and ascorbic acid, or absorbance of the control reaction.

**Determination of essential oil activity**

We tested the antimicrobial activity of the essential oil obtained from *L. nobilis* leaves via the different routes ($EO_{HD}$, $EO_{MWAHD}$, $EO_{WSE}$).

***Pathogenic bacteria***

Four strains from the international ATCC (American Type Culture Collection) collection were tested to detect the antimicrobial activity of *L. nobilis* essential oils: *Staphylococcus aureus* (ATCC 25923); *Pseudomonas aeruginosa* (ATCC 27853); *Bacillus cereus* (ATCC 10876); *Escherichia coli* (ATCC 25922).

***Yeast***

We used the yeast *Candida albicans* (ATCC 10231) supplied by the Microbiology Research Laboratory, Faculty of Exact Sciences and Computer Science, Abdelhamid Ibn Badis University – Mostaganem.

***Antimicrobial activity***

Two different methods were used to evaluate the antimicrobial effect of the essential oils obtained: the aromatogram method for the evaluation of the antimicrobial activity of the different essential oils and the micro-dilution method[15,16] for the determination of the minimum inhibitory concentration (MIC) from a range of extract concentrations in the culture medium. The diffusion method from a solid disk was used to highlight the antimicrobial activity of essential oils and antibiotics against pathogenic germs.

This method is based on the NCCLS guidelines for antimicrobial susceptibility testing[17]. Sterile disks of Whatman paper 6 mm in diameter, containing 15 µL of essential oil to be tested, were placed using sterile forceps on the surface of an agar medium appropriate for each strain, previously inoculated with 100 µL of microbial suspension whose turbidity was adjusted to 108 CFU/mL for bacteria, 106 CFU/mL for yeast[17]. Petri dishes were then closed and allowed to diffuse at room temperature for 30 min before being incubated at 37°C for 24 hours for the bacteria and at 25°C for 48 hours for the yeast.

## Results and Discussion

**Physicochemical parameters**

The results of some physicochemical parameters are reported in Table 1. Yield values indicated in the literature for *L. nobilis* L. vary from 0.63 to 0.70%. The results of this study show that the yield of essential oil obtained by microwave-assisted hydrodistillation was 0.74% for an extraction time of 40 min, compared to the respective yields of 0.50 and 0.42% by Clevenger-type hydrodistillation and water steam entrainment for an extraction time of 200 min and greater energy and water consumption. The yield obtained in this work for the extraction by hydrodistillation was higher than that obtained by Reis *et al*.[18] (0.2%) for an extraction of 180 min and close to that found in the literature of 0.61%[19]. These varying results may be due to several parameters influencing the yield, including the extraction method chosen and the equipment used[19-21].

The refractive index of the essential oils obtained was between 1.484 and 1.490. This index depends on the chemical composition, which increases as a function of the length of the acid chain, the degree of unsaturation, and temperature. The result of the refractive index of essential oil extracted by hydrodistillation of 1.490 is the same as that obtained elsewhere[22]. Relative density is generally lower than that of water, which is normative according to French standards AFNOR for essential oils[23].

**Organoleptic characteristics**

The extracted EO is an oily, mobile, and clear liquid. Its colour is slightly light yellow, its smell is characteristic, harsh and spicy, and it is immiscible with water.

**Moisture content**

The moisture content of *L. nobilis* leaves was 54.20%, close to 56.80%, as reported elsewhere[24]. The difference in moisture content may be due to environmental factors such as climatic conditions and geographical distribution, which promote an increase in the concentration of essential oil on the surface of bay leaves[25].

**Extraction kinetics**

Extraction kinetics of *Laurus* leaves by the three methods are shown in Fig. 1. The slope of the curves for the microwave-assisted method is sharper than that of HD and WSE. The time for the formation of the first drops of essential oil was 40 min for the HD and WSE methods and 5 min for the MWAHD method. The total extraction time was 200 min for HD and WSE and 40 min for MWAHD, with a yield of 0.52, 0.42, and 0.74% respectively. This shows that the microwave-assisted extraction method was faster than the other methods because microwave energy is directly used to heat the molecules.

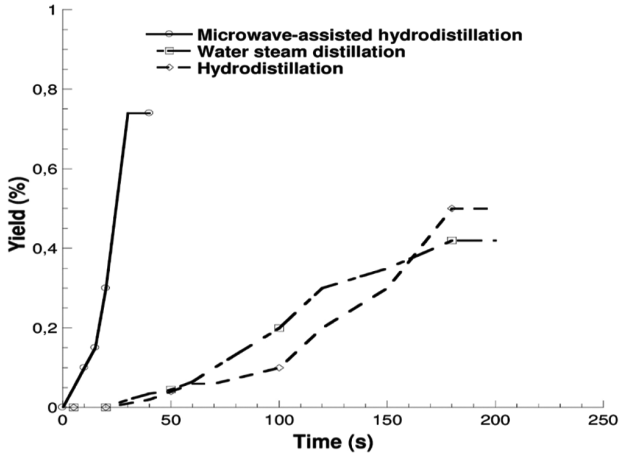


Fig. 1 — Essential oil yield from *L. nobilis* leaves as a function of time for water steam entrainment, Clevenger hydrodistillation, and microwave-assisted hydrodistillation extraction methods.

**GC-MS analysis of the essential oils**

Relative abundance of main volatile compounds of *L. nobilis* essential oils from references[26,27] is shown in Table 2. It can be noted that the main volatile compounds of the essential oils are 1,8-cineole, α-terpinyl acetate, linalool, methyleugenol, and eugenol. The results of the GC-MS analysis of *L. nobilis* essential oils investigated in this study are depicted in Fig. 2 and summarized in Table 3.

The major compounds identified via GC-MS analysis were: 1,8 cineole, an antifungal agent (HD: 24.06%; MWAHD: 12.04% and WSE: 29.94%), Linalool acetate, a perfuming agent (10.86%; 11.74%,12.72%, respectively), α-terpineol acetate, an antiseptic (20.02%; 18.28%,15.07%), methyl isoeugenol ($HE_{HD}$:7.99%; $HE_{HDM}$:11.05%) and methyl eugenol is a healing, anesthetic agent ($HE_{WSE}$: 8.50%), iso eugenol ($HE_{HD}$: 2.56% and $HE_{MWEHD}$ 3.96%) and eugenol ($HE_{WSE}$ 2.93%) as well as limonene, a bioinsecticide (1.31%, 3.158% and 1.24%) Espatulenol and Cadinol with a percentage ranging from (1.25% - 1.94%). Table 4 shows the chemical composition and percentages of compounds identified in *L. nobilis* EOs of

Table 2 — Relative abundance of main volatile compounds of *L. nobilis* essential oil obtained elsewhere[25, 26]

| Component | Group | Relative abundance (%) |
|---|---|---|
| sabinene | Monoterpene hydrocarbons | 0.7–12.2 |
| α-pinene | | traces–7.7 |
| β-pinene | | traces–5.0 |
| α-terpinene | | traces–4.1 |
| γ-terpinene | | traces–6.1 |
| 1,8-cineole | Oxygenated hydrocarbons | 25.7–63.2 |
| linalool | | traces–18.5 |
| α-terpinyl acetate | | traces–27.0 |
| α-terpineol | | traces–9.3 |
| γ-terpineol | | traces–1.9 |
| terpinen-4-ol | | traces–6.0 |
| borneol | | traces–12.8 |
| β-caryophyllene | Sesquiterpene hydrocarbons | traces–1.8 |
| eugenol | Phenylpropanoids | traces–6.5 |
| methyleugenol | | traces–21.4 |

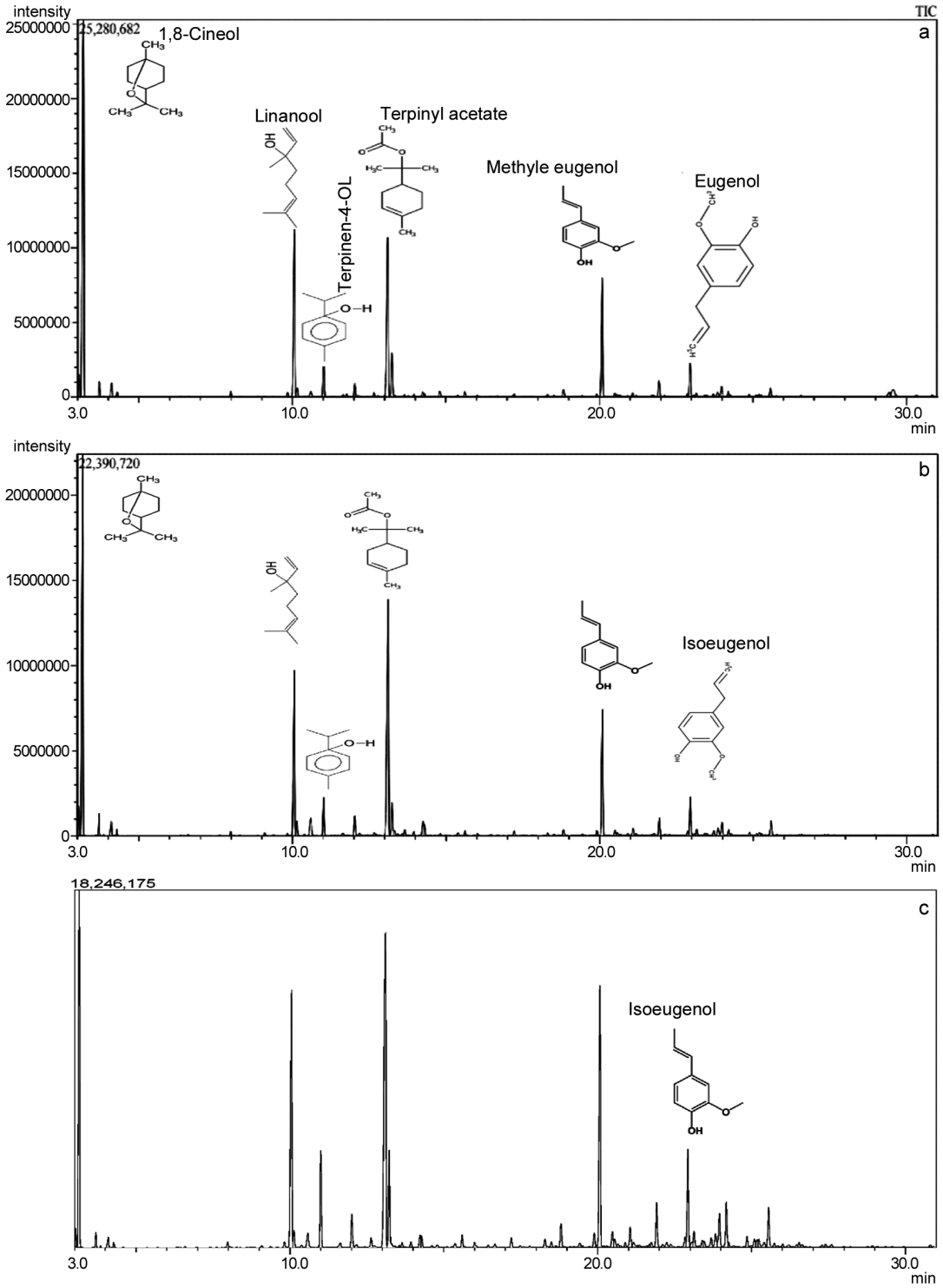


Fig. 2 — GC-MS chromatograms of *L. nobilis* essential oils via, a) water steam entrainment, b) clevenger hydrodistillation, and c) microwave-assisted hydrodistillation.

Mediterranean origin[28-31]. These results highlight a remarkable difference in chemical composition and extraction yields depending on the extraction method used. It should be noted, however, that the chemical composition of the essential oils of a plant depends on several factors such as geographical origin, harvest period, drying location, drying temperature, duration, and the extraction method.

Table 3 — Composition of *L. nobilis* essential oils extracted by water steam entrainment (WSE), Clevenger hydrodistillation (HD), and microwave-assisted hydrodistillation (MWAHD) methods

| Compound | WSE | | HD | | MWAHD | |
|---|---|---|---|---|---|---|
| | $t_R$ (min) | % | $t_R$ (min) | % | $t_R$ (min) | % |
| Limonene | 3.050 | 1.24 | 3.047 | 1.31 | 3.039 | 0.46 |
| 1,8-Cineole | 3.186 | 29.94 | 3.175 | 24.06 | 3.158 | 12.04 |
| Linanool | 10.065 | 12.72 | 10.060 | 10.86 | 10.053 | 11.74 |
| Terpinen-4-ol | 11.018 | 3.09 | 11.015 | 2.45 | 11.0882 | 3.72 |
| α-terpinyl acetate | 12.030 | 15.07 | 13.117 | 20 | 13.105 | 18.28 |
| α-terpineol | 13.103 | 1.02 | 13.239 | 2.25 | 13.226 | 4.06 |
| β-Elemen | 14.246 | 0.39 | 14.251 | 1.09 | 14.228 | 0.57 |
| Caryophyllene oxide | 18.830 | 0.62 | 18.827 | 0.46 | 18.806 | 1.06 |
| Methyl eugenol | 20.089 | 8.50 | - | - | - | - |
| Methyl isoeugenol | - | - | 20.089 | 7.99 | 20.071 | 11.05 |
| Espatulenol | 22.940 | 1.25 | 21.940 | 1.25 | 21.914 | 1.94 |
| Eugenol | 22.956 | 2.93 | - | - | - | - |
| IsoeugenoL | - | - | 22.955 | 2.56 | 22.930 | 3.96 |
| Cadinol | 23.425 | 0.11 | 23.981 | 1.01 | 23.952 | 1.59 |
| Elemicin | 24.203 | 0.42 | 24.202 | 0.48 | 24.178 | 1.95 |
| Vanillosmin | 29.578 | 1.35 | - | - | - | - |
| Total | / | 87.23% | / | 85.83% | / | 72.41% |

(-) undetected

Table 4 — Comparison of chemical composition and percentage of compounds identified in *L. nobilis* essential oils of Mediterranean origin

| Content in identified compound (%) | Origin | Reference |
|---|---|---|
| α-pinene (3.4), sabinene (3.3), β-pinene (3.2 1,8-cineole (32.0), linalool (0.2), terpinen-4-ol (4.2), α-terpineol (1.4), terpinyl acetate (8.9), eugenol (1.5), methyleugenol (0.05), etc. | Italy | 28 |
| sabinene (4.5–10.6), β-pinene (1.8–4.0), limonene (1.3–1.9), 1,8-cineole (26.6–34.9), linalool (1.3–4.1), terpinen-4-ol (1.0–2.8), terpinyl acetate (15.3–31.7), eugenol (0.7–1.8), methyleugenol (1.8–6.4), bornyl acetate (0.7–1.5), spathulenol (1.9–5.3) etc. | Greece | 29 |
| α-pinene (4.3–6.5), β-pinene (2.0–4.2), sabinene (9.2–10.2), limonene (0.4–1.1), 1,8-cineole (45.1–53.0), linalool (1.4–3.7), terpinen-4-ol (1.1–2.1), α-terpineol (1.2–3.5), terpinyl acetate (11.4–13.1), eugenol (1.2–4.5), methyleugenol (2.3–4.6) etc | Turkey | 30 |
| 1,8-cineole (31.4-56), linalool (17.67), eugenylmethylether (12.40), isovaleraldehyde (9.65), and camphene (7.21), β-phellandrene (3.85), camphor (2.66), α-pinene (2.52), and eugenol (2.18). | Tunisia | 31 |
| 1,8-cineole (12.04-29.94), terpinyl acetate(15.07-20), linalool (10.89-12.72),methylisoeugenol (7.99-11.05), and eugenol (2.56-3.96),terpinen-4-ol(2.45-3.72),α-terpineol(1.04-4.06), limonene (0.46-1.31), β-elemen(0.31-1.09),espatulenol(1.25-1.94). | Algeria | This study |

Table 5 — Phytochemical analysis of *L. nobilis* leaves investigated

| Compound | Alcaloïds | Tannin | Flavonoïds | Saponins | Starch |
|---|---|---|---|---|---|
| Test | Mayer reagent | Reaction with $FeCl_3$ | + + | --- | --- |
| | + + + | + + + Gallic tannin | | | |
| | Wagner reagent | + + + | | | |
| | + + + | Catechic tannin | | | |
| | | --- | | | |

+ : present ; ++ : abundant ; +++ : very abundant ; --- : absent

**Phytochemical analysis**

Phytochemical analysis was carried out on *L. nobilis* leaves using standard tests described by Trease and Evans[32]. The results are summarized in Table 5. As shown in Table 5, *L. nobilis* leaves contain alkaloids, flavonoids, tannins, reducing compounds, and gallic tannins with variable intensities, while catechic tannins, saponins, and starch are absent.

**Polyphenol content**

Polyphenol content of ethanolic extract from laurel leaves was evaluated using the spectrophotometric method. Standard solutions of gallic acid were prepared (0.06 to 0.5 mg/mL) using Folin-Ciocalteu reagent. The homogenized mixture with carbonate buffer was incubated for two hours at room temperature. Absorbance measurements were carried out at 765 nm. The calibration curve equation obtained is Abs = 1.589 *C* + 0.068. *C* is expressed in mg equivalent of gallic acid per gram of dry matter weight (mgGAE/g). Polyphenol content of *L. nobilis* ethanolic extract reached 42.20 mgGAE/g, which was higher than the value 10.23 mg GAE/g reported by Muniz-Marquez *et al.*,[33] and similar to the values 46.76 and 42.35 mg GAE/ g reported by Lu *et al*.,[34] and Dobroslavić *et al.*,[35] respectively.

**Antioxidant activity**

To assess the antioxidant activity of essential oil, the method based on the scavenging of DPPH radical was used. Ascorbic acid was used as a standard. The concentration of the antioxidant needed to decrease the initial DPPH concentration by 50% ($IC_{50}$) was the parameter used to measure the antioxidant activity and represent the effective concentration. Fig. 3 shows percent inhibition (PI) as a function of essential oil concentration. At the lowest concentration (0.0075 mg/mL), the essential oils HD, WSE, and MWAHD had respective PIs of 32.2, 36.84, and 30.0% while at the highest concentration (0.5 mg/mL), inhibition percent reached 68.95, 86.42, and 54.702%, respectively. This shows that the *L. nobilis* species has significant antioxidant potential, with the essential oil from water steam entrainment being the most effective. The effective concentration ($IC_{50}$) is inversely proportional to the antioxidant capacity of a compound. The lower the $IC5_0$ value, the greater the antioxidant activity.

From Table 6, it can be noted that the essential oil obtained via water steam entrainment had the largest antioxidant activity, which may be due to the better effectiveness and preservation of the active substances with respect to HD and MWAHD. Despite the fact that the electromagnetic waves of the microwave improve the efficiency of the extraction (time, energy, and production) by increasing the molecular interactions between the sample and the electromagnetic field, they can increase the decomposition of chemical compounds like polyphenols, phenolic compounds, which have the ability to neutralize free radicals.

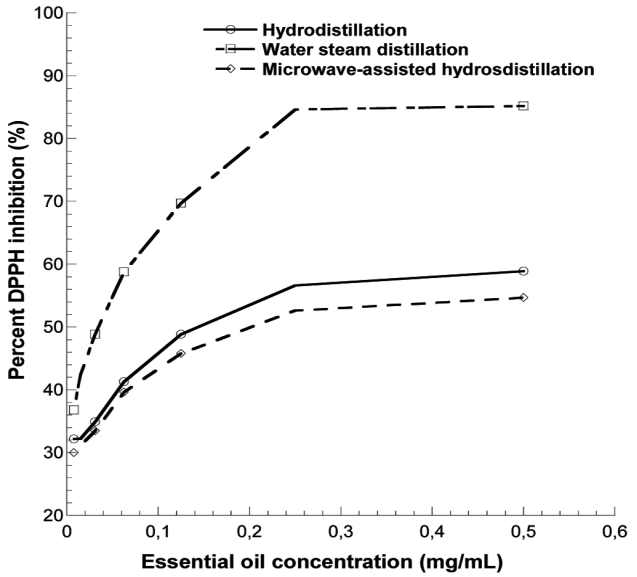


Fig. 3 — Percent inhibition of DPPH free radical versus concentration of essential oil obtained via hydrodistillation (HD), water steam entrainment (WSE), microwave-assisted hydrodistillation (MWAHD).

Table 6 — Essential oils inhibition zone in diameter on agar disc diffusion method and their $IC_{50}$ values obtained for water steam entrainment (WSE) hydrodistillation, (HD and microwave-assisted hydrodistillation (MWAHD) methods

| | Inhibition zone diameter (mm) and activity level | | |
|---|---|---|---|
| | WSE | HD | MWAHD |
| | Gram-postive bacteria (+) | | |
| *Bacillus cereus* (ATCC 10876) | 35 (+++) | 30 (+++) | 27.58 (+++) |
| *Staphylococcus aureus* (ATCC 25923) | 17 (++) | 14 (++) | 11 (+) |
| | Gram-negative bacteria (-) | | |
| *Escherichia coli (ATCC 25923)* | 26.33 (+++) | 18 (++) | 12(+) |
| *Pseudomonas aeruginosa*, (ATCC 27853) | 23.5 (+++) | 21 (+++) | 19.07 (++) |
| | Yeast (fungus) | | |
| *Candida albicans* (ATCC 10231) | 15 (++) | 15 (++) | 13.25 (++) |
| $IC_{50}$ (mg/mL) | 0.025 | 0.254 | 0.318 |

Table 7 — Minimum inhibitory concentration (MIC) of *L. nobilis* essential oils obtained for water steam entrainment (WSE), hydrodistillation (HD), and microwave-assisted hydrodistillation (MWAHD) methods

| | MIC (mg/mL) | | |
|---|---|---|---|
| Bacteria | WSE | HD | MWAD |
| *Bacillus cereus* | 6.25 | 25 | 25 |
| *Staphylococcus aureus* | 3.12 | 6.25 | 25 |
| *Escherichia coli* | 3.12 | 6.25 | 50 |
| *Pseudomonas aeruginosa* | 1.56 | 6.25 | 25 |
| *Candida albicans* | 3.12 | 3.12 | 12.5 |

**Antimicrobial activity**

Extracts have several modes of action on different bacterial strains. They are effective against a wide spectrum of pathogenic and non-pathogenic microorganisms but generally speaking, their action takes place in three phases: attack of the bacterial wall by the plant extract, causing an increase in permeability, followed by the loss of cellular constituents, then acidification of the interior of the cell blocks the production of cellular energy and the synthesis of structural components, and finally, destruction of genetic material, leading to the death of the bacteria. Antimicrobial activity of the essential oil was determined by measuring the diameter of the inhibition zone formed around each disk. The size of the diameter of the zones reflects the impact of the essential oil on the strains tested. The results summarized in Table 6 can also be expressed according to four activity levels:

(-) resistant strain ($D < 8$ mm); (+) sensitive strain ($9 \text{ mm} \leq D \leq 14$ mm); (+ +) very sensitive strain ($15 \text{ mm} \leq D \leq 19$ mm); (+ + +) extremely sensitive ($D > 20$ mm).

The results shown in Table 6 indicate that the bacterial strains tested showed different degrees of sensitivity. The diameters of the inhibition zones in all positively interacting extracts ranged from 11 to 35 mm, confirming that *L. nobilis* has a significant antimicrobial property, corroborating similar results published by Tomar *et al*.,[36] for *S. aureus* (23.1 mm), *E. coli* (27.1 mm), and *Bacillus cereus* (17.2 mm)[37]. The essential oil obtained via the water steam entrainment method resulted in the largest inhibition zone, which indicates that the variation in the antimicrobial activity of the essential oils may be due to different chemical compositions, functional groups (alcohols, phenols, terpene, and ketone compounds), and their synergistic effects.

**Determination of minimum inhibitory concentrations (MIC)**

The minimum inhibitory concentration (MIC) of *L. nobilis* essential oils was carried out only on germs that showed sensitivity by Aromatogram. We note from the results summarized in Table 7 that the five strains tested showed significant sensitivity to three essential oils of *L. nobilis* with MIC values ranging from 1.56 to 6.25 mg/mL for WSE, 3.12 to 25 mg/mL for HD, and 12.5 to 50 mg/mL for MWAD. These results confirm the qualitative analysis results (zones of inhibition of the different microbial strains). Although all three essential oils were effective against the microorganisms tested, we note that the MIC value varied according to the method of extraction of the essential oil, WSE being the most effective with an MIC value of 1.56 mg/mL for *Pseudomonas aeruginosa* and 3.12 mg/L for *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. Hence, the method of extraction is of paramount importance in terms of the preservation of active ingredients. Ouled Taarabt *et al.*,[38] showed that the antimicrobial activity of laurel EO was significant against *Pseudomonas aeruginosa* with an inhibition zone of 3 mm and a MIC of 50 mg/mL. Our results show better effectiveness of WSE with an inhibition zone of 23.5 mm and a MIC of 1.25 mg/mL.

## Conclusion

*L. nobilis* is a medicinal plant commonly used throughout the Mediterranean. Essential oils obtained by three different routes show that the extraction method strongly influences yield and antimicrobial activity. GC-MS analysis of the essential oils revealed that the majority and common compounds present were: 1,8-cineole, an antifungal agent; linanool, an antibacterial agent; limonene, a bioinsecticide; α-terpinyl acetate; eugenol; and methyleugenol in different proportions. The three essential oils obtained via hydrodistillation, steam entrainment, and microwave-assisted distillation were effective against the Gram-positive and Gram-negative bacteria and the yeast studied. However, the water steam entrainment method was the most effective with a minimum inhibition value of 1.56 mg/mL and an inhibition zone

of 23.5 mm. However, microwave-assisted distillation was rapid and yielded a high amount. The polyphenol content of the ethanolic extract of laurel leaves reached 42.20 mg GAE/g. The essential oil obtained by water steam entrainment distillation showed a significant free radical scavenging activity of 0.025 mg/mL. The pharmacological properties and toxicological evaluation of *L. nobilis* essential oils require further study for medical applications.

## Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgment

The authors wish to thank the General Directorate of Scientific Research and Technological Development and the General Direction of Higher Education and Training (DGRSDT of the Ministry of Higher Education and Scientific Research, Algeria) for their financial support – National PRFU project (Mostaganem University) # A16N01UN2701202 20002: 01/01/2022.

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