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## Phytochemical analysis, antibacterial and antifungal effect of *Lavandula dentata* L. essential oil and methanol extract

Noui Hendel<sup>a,b</sup>, Madani Sarri<sup>c</sup>, Djamel Sarri<sup>c</sup>, Soumia Seghiour<sup>a</sup>, Edoardo Napoli<sup>d</sup> , Mounir Selloum<sup>a</sup> and Giuseppe Ruberto<sup>d</sup> 

<sup>a</sup>Department of Microbiology and Biochemistry, Faculty of Sciences, University Mohamed Boudiaf of M'sila, M'sila, Algeria; <sup>b</sup>Laboratory of Biology: Applications in Health and Environment (LBAHE), University of M'sila, M'sila, Algeria; <sup>c</sup>Department of Nature and Life Sciences, Faculty of Sciences, University Mohamed Boudiaf of M'sila, M'sila, Algeria; <sup>d</sup>Istituto del CNR di Chimica Biomolecolare, Catania, Italy

### ABSTRACT

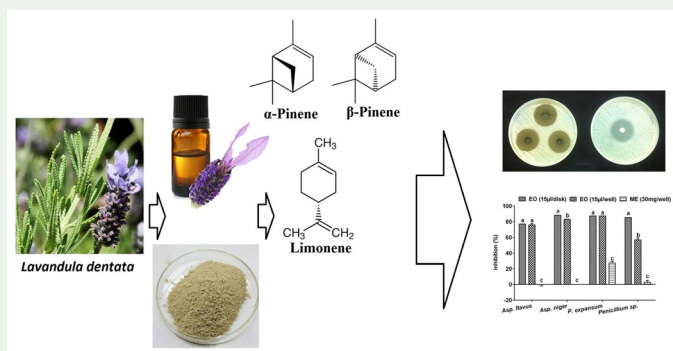
The aim of this study was to analyse the essential oil of *Lavandula dentata* from Algeria and to test the antioxidant and antimicrobial properties of this plant. The essential oil (EO) (57 constituents) included mainly  $\alpha$ -pinene,  $\beta$ -pinene, nopinone, linalool, cryptone, and limonene. The plant polyphenolic contents and the antioxidant activity were determined. The antimicrobial effect of the EO and the methanolic extract (ME) was assessed against referenced and clinical bacterial strains, and also foodborne fungal isolates. The EO minimal inhibitory concentration (MIC) values varied from 0.25 to 4 mg/mL and minimal bactericidal concentrations (MBCs) were less than 8 mg/mL except for *S. aureus*, clinical *Klebsiella*, *S. epidermidis*, and *B. subtilis*. The mould strains were significantly inhibited by the EO (87.50% to 88.33%). The MIC values were 3.60–15.62 mg/mL and 0.5–4 mg/mL for ME and EO, respectively. The minimal fungicidal concentration (MFC) values ranged from 31 to 125 mg/mL and from 2 to 8 mg/mL for ME and EO, respectively.

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**CONTACT** Noui Hendel  [noui.hendel@univ-msila.dz](mailto:noui.hendel@univ-msila.dz)  Department of Microbiology and Biochemistry, Faculty of Sciences, University Mohamed Boudiaf of M'sila, M'sila 28000, Algeria

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## 1. Introduction

People have employed medicinal plants for their therapeutic, cosmetic, nutritional, pharmaceutical, and industrial characteristics since the beginning of humankind. Nowadays, scientific research discovers the merits of the empirical prescriptions of medicinal plants (Lahsissene et al. 2009), after emergence to multi drug resistant bacteria and limitation of fully active antibiotics to treat bacterial infections (Aly et al. 2013). Drugs derived from medicinal plants are developed using botanical, phytochemical, biological, and molecular techniques (Marcy et al. 2005). Additionally, the food sector is increasingly turning to natural antioxidants as a substitute for synthetic ones as public concern about their safety rises (Brewer 2011; Al-Ansari et al. 2021).

The flora of Algeria includes 05 species of the Lamiaceae family's *Lavandula*, including *L. stoechas* L., *L. dentata*, *L. coronopifolia* Poiret (= *L. stricta* Del), *L. multifida* L., and *L. pubescens* Dec ssp. *antineae* (Maire) de Miré et Qz. It is an aromatic woody sub-shrub with inflorescence in dense spikes. The flowers are bracteole-shaped with a tubular calyx presenting 5 uneven short teeth, a corolla exerted with a dilated tube in the throat, with 2 lips, the upper with 2 lobes, the lower with 3 and stamens included (Quezel and Sante 1963). The essential oils from *Lavandula* species are primarily cultivated for use in 'aromatherapy' items, food processing, cosmetics, and perfumery (Harborne and Williams, 2002). Some studies have demonstrated biological activities of extracts from *L. dentata*, such as antioxidant (Bettaieb Rebey et al. 2017), anti-inflammatory (Algieri et al. 2016), antibacterial (Bachiri et al. 2016), antifungal (Asdadi et al. 2016), insecticidal (Wagner et al. 2021), larvicidal (Dris et al. 2017; El-Akhal et al. 2021), antibiofilm (Müller-Sepúlveda et al. 2020) and neuroprotective properties (Qneibi et al. 2019). In Algeria, *Lavandula dentata*, known as 'Djaida', grows commonly in the North-Center to North-West and extensively rare elsewhere. It is employed in traditional medicine to treat renal colic and the common cold (Bousmaha et al. 2005). It has been the subject of few studies that have been based on the composition of its essential oil (Bousmaha et al. 2005; Dob et al. 2005). Nevertheless, to our knowledge, there have been few investigations on the antibacterial, antifungal, and antioxidant properties of essential oil and methanolic extract from *L. dentata* of Algeria.

The objective of this study was to determine the polyphenol and flavonoid contents of the essential oil (EO) and methanolic extract (ME) of this plant, to determine the essential oil composition, and to assess its antioxidant activity, as well as its effect on the development of various pathogenic mould isolates, *C. albicans*, and reference and clinical bacteria.

## 2. Results and discussion

### 2.1. Composition of the essential oil

The hydrodistillation process has yielded a yellow-colored EO with a strong and persistent odour (yield 0.9% v/w). Fifty-seven compounds were identified (94.21% of the oil); with  $\beta$ -pinene, limonene, linalool, nopinone, cryptone,  $\alpha$ -pinene, myrtenal, fenchone, pinocarveol, trans-linalool oxide, pinocarvone and cuminaldehyde as the notable constituents comprising 70.29% of the oil. As shown in Table S1, the content

of the EO in monoterpenes (32.33%) and oxygenated hydrocarbons (55.34%) was very high compared to that in sesquiterpenes (4.32%). It should be noted the presence of a non-terpenoid compounds in a small amount (2.22%). The EO was reported to be composed mainly of 1,8 cineole, carvacrol,  $\beta$ -bisabolene, linalool, linalyl acetate and camphor from *L. dentata* growing in Morocco (Imelouane et al. 2009; Asdadi et al. 2016; EL Hassouni et al. 2017; Jilali et al. 2023); of 1,8 cineole,  $\alpha$ -terpinolene and  $\beta$ -pinene from that growing in Algeria (Dob et al. 2005; Bousmaha et al. 2005; Benbelaïd et al. 2014; Dris et al. 2017); of camphor and 1,8 cineole from that growing in Tunisia (Bettaieb Rebey et al. 2017; Dammak et al. 2019); of camphor and fenchone from that growing in Saudi-Arabia (Aly et al. 2013; Al-Sarar et al. 2014); and of camphor from that growing in Yemen (Mothana et al. 2012). Others studied EO of cultivated *L. dentata* reported *p*-Mentha-1,5-dien-8-ol (Nevein et al. 2014) and 1,8 cineole (Martins et al. 2019) as main component. From these data 1,8-cineole (eucalyptol) is mentioned in almost all *L. dentata* EOs. This component was not found in our *L. dentata* EO; it is composed mainly of  $\beta$ -pinene, limonene, linalool and nopinone; which make it different and similar to *L. dentata* EO studied by Imelouane et al. (2010) and Ouedrhiri et al. (2017) in terms of  $\beta$ -pinene as main component. The flowering tops EO studied by Dridi et al. (2021) gave the major components  $\beta$ -eudesmol, myrtenol, and sabinol. These differences can be attributed to a variety of reasons, including geographic variations, timing of plant collection, physiological parameters, ecological conditions, genetics, and the part of the plant under study.

## 2.2. Total phenolic and flavonoid contents

The evaluation of the total phenolic content in plant extracts is of a great importance as the phenolic compounds have direct action in the antioxidant activity because these metabolites are strong scavengers of radicals and reducing agents (Khadri et al. 2010). Flavonoids are polyphenolic compounds that play an important role as messengers and physiological regulators (Köksal et al. 2017). Based on the colorimetric analysis of total phenolics, the overall amount of polyphenols in the ME and the EO in comparison with the normal gallic acid solutions was assessed at  $308 \pm 11.78$  and  $7.69 \pm 0.25$   $\mu$ g GAE/mg, respectively. On the same basis for the total flavonoids, the amount in the ME and the EO in comparison with the normal quercetin solutions was assessed at  $27.01 \pm 2.41$  and  $24.51 \pm 2.02$   $\mu$ g QE/mg respectively. These data indicate a high level of phenolic content in the ME compared to the EO, but the contents of flavonoids are close to each other with no significant difference. Our plant ME and EO contain higher level of polyphenols compared to those of the same plant from Morocco (160.24 mg/g and 4.09 mg/g respectively) (Bachiri et al. 2016; Dammak et al. 2019).

## 2.3. Antioxidant activity

Table S2 displays the  $IC_{50}$  values. Our results showed the very strong DPPH free radical scavenging power of the *L. dentata* ME, Much greater than that of the EO of the same plant and less important to that of BHA. Similarly, the same order was observed in the  $\beta$ -carotene/linoleic acid bleaching test (BHA > ME > EO). the  $IC_{50}$

values determined in this study are different from others for the same plant. Imelouane et al. (2010) studied the antioxidant activity of the *L. dentata* EO, and their results showed a substantially lower anti-free radical capacity than that obtained in the current study ( $IC_{50}=4.91\pm 0.07\mu\text{L/mL}$ ), with  $IC_{50}$  values of around  $32.12\pm 0.57$  for the EO and  $41.29\pm 1.20\mu\text{L/mL}$  for the aerial parts. Likewise, Dridi et al. (2021) mentioned lower antioxidant activity of the *L. dentata* flowering tops EO ( $IC_{50}=113.29\pm 0.012\text{mg/mL}$ ). El Hassouni et al. (2017) also found lower free radical scavenging activity with an  $IC_{50}$  of around  $70\pm 1.03\mu\text{L/mL}$ . The same as El Abdali et al.'s (2022) finding with a free radical scavenging activity  $IC_{50}$  of  $12.95\pm 1.3\text{mg/mL}$ , and  $IC_{50}$  value of  $35.72\pm 1.21\text{mg/mL}$  in terms of  $\beta$ -carotene bleaching test of the *L. dentata* EO. However, a similar finding was noticed by Ghanimi et al. (2021) ( $4.75\text{mg/mL}$ ). Moreover, the work of Bettaieb Rebey et al. (2017) on the antioxidant activity of *L. dentata* extracts demonstrated statistically significant variations depending on the organ studied, with an  $IC_{50}$  not exceeding  $0.05\text{mg/mL}$  for the roots,  $0.20\text{mg/mL}$  for the leaves and  $0.17\text{mg/mL}$  for the stems. These findings show the difference in the antioxidant activity according to the origin of the plant and also according to the plant parts. The phenolic compounds of this species may be the major contributors to its antioxidant potential, due to the fact that polyphenols have been shown to be powerful antioxidants that may scavenge free radicals by giving them an electron or hydrogen atom (Mondal et al. 2020). Moreover, phenolics and flavonoids play an important role in the activation of antioxidant enzymes and hence induce the antioxidant defense system in the human body, leading to the prevention of cancer and other related diseases (Yumita et al. 2023).

#### 2.4. Antimicrobial activity

Our study of the *in vitro* antimicrobial activity of methanol extract (ME) and EO from *L. dentata* was performed, using the disc diffusion method for EO and the well diffusion method for ME, on MHA (for bacteria), PDA (for fungal spores) and SDA (for yeast) media. The antimicrobial activity was estimated in terms of the diameter of the inhibition zone (IZ) around the discs and the wells containing the extracts to be tested against 18 bacterial strains and 5 fungal strains. Table S3 displays the obtained results.

Based on the inhibition zone diameter, all the tested bacteria may be qualified as sensitive to the *L. dentata* EO since halo diameters are over 9 mm. With the exception of the clinical isolate *Kl. pneumoniae*, which was resistant to the ME, all the other bacterial strains were sensitive.

Generally, *L. dentata* EO seems to have better antimicrobial activity: 7 bacteria (38.88%) were sensitive (IZ= 8–14 mm), 7 bacteria (38.88%) were very sensitive (IZ= 14–20 mm), and 4 bacteria (22.22%) were extremely sensitive (IZ >20 mm). *Bacillus cereus* was totally inhibited, *St. aureus* ATCC 43300 and the clinical strains *E. coli* and *S. typhi* were extremely sensitive; *Pr. mirabilis* ATCC 35659, *Ent. faecalis* ATCC 51299, *Ent. faecalis* ATCC 49452, *S. typhimurium* ATCC 13311, *Kl. Pneumoniae* ATCC 700603 and the clinical strains *Ps. aeruginosa* and *Kl. Pneumoniae* were very sensitive, whereas the other strains were sensitive.

The antimicrobial properties of *L. dentata* methanol extract were also between strong activity on *B. cereus* ATCC 10876 and *B. subtilis* ATCC 9372 (11.11% of the total strains); large activity on *St. aureus* ATCC 43300, *Ent. faecalis* ATCC 51299, *St. aureus* ATCC 25923 and *St. aureus* ATCC 6538 (22.22% of the total strains); and weak to slightly moderate activity, ranging from 10.33 mm to 14.33 mm inhibition zone diameter, on the other bacteria (61.11%). Notably, this extract was not active against the clinical isolate *Kl. Pneumoniae*.

These results corroborate those obtained by Bachiri et al. (2016), who found that crude aqueous extracts of *L. dentata* showed no effect on Gram-negative bacteria. Nevertheless, they found that these extracts show no efficacy against *S. aureus* (Gram-positive).

These results corroborate those mentioned by other researchers, including Imelouane et al. (2009), Mothana et al. (2012), Aly et al. (2013), Bachiri et al. (2016), and El Hassouni et al. (2017), who noted very effective activity of the *L. dentata* EO on Gram-positive bacteria and more or less moderate activity on Gram-negative bacteria. Nevertheless, Dridi et al. (2021) found very effective power on both Gram-type bacteria (IZ = 19.06–24.16 mm), with major sensitivity for *S. typhimurium* ATCC 14028 (Gram-negative).

The ME has moderate activity on the growth of *C. albicans* and the germination of *A. flavus* spores, but weak activity on the germination of the other strains, according to the results of the antifungal effect of *L. dentata*. Similarly, except for *A. niger*, which is more sensitive, and *C. albicans*, which is remarkably non-sensitive, the EO has a moderate effect on fungal spore germination (Table S3).

Our findings are consistent with those made by Mothana et al. (2012), who did not record any antimicrobial activity of *L. dentata* EO against *C. albicans*. In contrast, Asdadi et al. (2016) recorded a potential fungicidal effect of EOs from *L. dentata* against *C. albicans* isolated from a nosocomial infection. El Abdali et al. (2022) registered mycelial growth inhibition in *B. cinerea*, *A. alternata*, and *F. oxysporum*, reaching 100, 66, and 44%, respectively.

Figure S1 shows the percentage of mycelial growth inhibition caused by *L. dentata* on the seventh day of incubation. With inhibition percentage values ranging from 56.94% to 87.50% in the well diffusion method, the results clearly demonstrate the extremely considerable inhibition induced by the EO, applied by both well and disc diffusion methods, on all the tested fungal strains; and 77.17% to 88.33% in the disc diffusion method; this may be explained by the ease of EO diffusion on the discs compared to the wells.

The ME had no effect on the two *Aspergillus* strains, a moderate effect on *P. expansum* (27.38%), and a weak effect on *Penicillium* sp. (2.78%). It is noteworthy that the appearance of the colony and sporulation were moderately affected.

According to the results obtained in Table S4, the ME seems to have antimicrobial activity against all the microorganisms tested in the MHB medium. The MIC values were between 31.25 and 3.60 mg/mL. The bacteria *B. subtilis* ATCC 9372, *E. faecalis* ATCC 51299, the clinical isolate *Ps. aeruginosa*, and the fungi *C. albicans* and *A. niger* were the most sensitive to the action of the ME with a MIC value of 3.60 mg/mL, whereas higher concentration (31.25 mg/mL) is required for *E. coli* ATCC 25922 and *S. typhimurium* ATCC 13311. The highest MBC value was recorded for *K. pneumoniae* ATCC 700603 (500 mg/mL), while the lowest value was recorded for *C. albicans* ATCC

10231 (3.60 mg/mL). The other values were between 62.50 and 15.62 mg/mL for the rest of the microorganisms, with the exception of *A. niger* and *S. typhi*, for which the MFC/MBC was 120 and 250 mg/mL, respectively.

With MIC values ranging from 0.25 to 4 mg/mL (0.25 mg/mL for *C. albicans* ATCC 10231) and CMB values below 8 mg/mL with the exception of *S. aureus* ATCC 6538, *S. aureus* ATCC 43300, *S. epidermidis* ATCC 12228, *B. subtilis* ATCC 9372, and the clinical *Kl. pneumonia*, the EO of *L. dentata* appears to have greater antimicrobial power.

These findings concur with those attained by Bachiri et al. (2016), who recorded MBC values close to those obtained in the present study for *E. coli*, *P. mirabilis*, and *S. aureus* (40.5 mg/mL), but nevertheless higher MICs than those of our study (32 mg/mL). Nevertheless, Dridi et al. (2021) registered very low MIC values varying between 0.1 and 0.6 µg/mL in their study on the inhibitory effect of the EO from *L. dentata* flowering tops on six bacterial strains. Furthermore, El Abdali et al. (2023) registered MIC values between 0.33 and 1.23 mg/mL of *L. dentata* EO and close values of the linalool component (0.41–1.44 mg/mL), tested against five bacterial strains.

Most studies relating to the action of EOs on the deterioration of organisms and pathogens have concluded that, generally, EOs are slightly more active against Gram-positive bacteria than Gram-negative bacteria. Gram-negative germs are less sensitive to the action of antibacterial agents. This would seem predictable because these germs have an outer membrane surrounding the cell wall, which limits the diffusion of hydrophobic compounds through its lipopolysaccharide layer. However, some studies have failed to conclude that Gram-positive germs are more sensitive (Imelouane et al. 2009). Furthermore, others noted that the sensitivity to EO from *L. dentata* flowering tops is not related to the structure of the bacterial membrane (Gram-positive or Gram negative) (Dridi et al. 2021). Moreover, other studies have shown the ability of the EOs to disrupt the membrane permeability of different microorganisms, such as *E. coli*, *S. aureus*, and *C. albicans* (Cox et al. 2000). Due to their hydrophobicity, the EOs have the ability to interact with the fungal plasma membrane; nevertheless, the hydrophilic nature of their functional groups and the lipophilic nature of their hydrocarbon skeleton play a major role in expressing their antifungal actions (hendel et al. 2019). The alkaloids, flavonoids, carbohydrates, and other phenolic compounds of the plant extracts may contribute to the antimicrobial action (Musyimi et al. 2007; Álvarez-Ordóñez et al. 2013); such as affecting the plasma membrane H<sup>+</sup>-ATPase in fungi (Trigui et al. 2013). The presence of monoterpenes such as α-pinene, β-pinene, limonene and linalool in the essential oil play an important role in the antimicrobial activity (Han et al. 2021; He et al. 2022; Singh et al. 2023), and linalool rich EO induce hypha growth suppression and spore germination prohibition (Lin et al. 2022).

### 3. Experimental

See [supplementary material](#).

### 4. Conclusion

This study assessed the EO composition, antioxidant, and antibacterial properties of wild *L. dentata* against significant foodborne fungal isolates, clinically relevant bacterial strains, and reference strains. High antioxidant activity was revealed in the ME

compared to the EO. The EO presented strong antimicrobial activity, especially on clinical bacterial isolates and all foodborne fungal strains. The ME affected the tested microorganisms moderately. In order to consolidate the antimicrobial effect of the extract, it is obvious to isolate and characterise bioactive compounds and separate antimicrobials from the methanolic extract. Our study has added an interesting finding to the research on the virtues of this medicinal plant, particularly as an alternative source of synthesised antimicrobials, and it could form an opening to discover its biological richness.

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## ORCID

Edoardo Napoli  <http://orcid.org/0000-0003-4281-3256>

Giuseppe Ruberto  <http://orcid.org/0000-0002-6610-6110>

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