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Chemical characterization of Eucalyptus (*Eucalyptus globulus*) leaf essential oil and evaluation of its antifungal, antibacterial and antioxidant activities

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Abstract

This study investigates the chemical composition of the essential oil (EO) extracted by hydrodistillation from dry Eucalyptus leaves (*Eucalyptus globulus*) and its antifungal, antibacterial and antioxidant potential. The Eucalyptus leaves were harvested in the commune of Seraïdi (north-eastern Algeria). Chemical analysis carried out by chromatography coupled with mass spectrophotometry (GC-SM) revealed the presence of 20 molecules representing approximately 100% of the overall component, with a yield of 1.58%. This oil is composed mainly of linalool (30.09%), followed by b-Linalyl oxide (13.93%), Camphor (12.09%), 1,8-Cineole=eucalyptol (10.95%) and Bergamol (10.03%). Other constituents were identified at relatively medium (Epoxylinalol - 8.82%, Borneol - 5.71%) and low (alpha-Terpinol - 1.11) levels. This result shows that this EO differs from those usually extracted from eucalyptus leaves because it is of linalool chemotype and not eucalyptol. The determination of the minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) was carried out to evaluate the antifungal activity of Eucalyptus EO on the growth of *Fusarium roseum* mycelium. The values recorded are 2500µg/ml for the MIC and 4000µg/ml for the MFC. The results obtained revealed an antifungal activity of this oil for practically all doses applied against Fusarium mycelial proliferation despite the low reported levels of 1,8-cineole compared to the other components. The antibacterial activity against the *Pseudomonas savastanoi* strain was also examined which revealed effectiveness of this oil. In parallel, the DPPH test revealed a moderate antioxidant activity of the studied EO compared to Vit C with an IC50 17mg/ml probably due to its components' antagonistic or synergistic effect.

Keywords: *Eucalyptus globulus* essential oil, Chemical composition, Antifungal activity, Antibacterial activity, Antioxidant activity

1. Introduction

Industrialization, population growth, agricultural development and the requirement to improve global agricultural production by taking all necessary measures to ensure food security are all related to excessive amounts of pesticides and, consequently, the increase in pollution [1]. Pesticides are among the most dangerous environmental pollutants due to their stabilities, mobilities, and long-term effects on living organisms, and they cause many disorders [2]. The amount of pesticide in direct contact with target organisms is extremely small compared to the amount applied, as only 0.1% of pesticides sprayed in the fields reach their target. The rest disperses into the environment contaminating air, soil and water. Thus, undesirable side effects may affect the entire ecosystem [3]. Chen et al. [4] reported that pesticide poisonings caused by insecticides, rodenticides and herbicides accounted for 7,16%, 6,47%, and 3,42% of the global total [5]. They can also lead to biodiversity loss in many species [6].

Despite the advantages of using pesticides to improve yields, their massive use and, in some cases, the non-respect of doses cause serious damage to fauna and flora by disturbing the ecosystem. Therefore, searching for an alternative to chemical products by using natural substances becomes an obvious solution to limit the damage caused by various pests. Among them, essential oils seem to be very promising. EOs are characterized by a set of biological activities proven by the scientific community, among which we can briefly mention the antimicrobial [7], antioxidant [8], anti-inflammatory [9,10], analgesic [11] and anticancer [12]. However, there is a disparity in the antimicrobial activity of EOs, as it is more directed toward fungi than bacteria and much more active against Gram-positive bacteria than Gram-negative bacteria. Several researchers have already highlighted the effectiveness of using EOs as bio-pesticides and have shown that their antimicrobial activity depends on the nature of their chemical compounds. For example, the Mountain Savory EO is toxic to the apple scab pathogen [13], which is related to the major phenolic compounds (Carvacrol and thymol) present in this oil [14].

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Similarly, the *Thymus vulgaris* EO, rich in linalool and oxygenated monoterpenes, has a strong bio-fungicidal activity against the same fungus, completely inhibiting mycelial growth [15, 16]. The EOs extracted from different types of eucalyptus have a unique combination of natural ingredients and medicinal properties. They have a wide range of health benefits, such as decongestant, antispasmodic, anti-inflammatory, antiseptic, antimicrobial, antifungal, antibacterial and antiviral properties [17]. According to the European Pharmacopoeia, *Eucalyptus globulus* EO must contain at least 70% 1,8 cineole for it to be sold in pharmacies because it is this compound which gives it its main expectorant and mucolytic property [18]. Although eucalyptol (1,8 cineole) is responsible for the lingering scent of eucalyptus oils and its many benefits, eucalyptus oils contain several natural chemical compounds that work synergistically to create a range of health benefits. The percentage of most of the components identified in eucalyptus EOs changes significantly with the literature even if generally they are of the 1.8 cineole chemotype [19, 20, 21, 22]**.** They can be of the Globulol [23] or Aromandrene [24] chemotype or other. These compositional differences are likely related to numerous factors that influence the plant's biosynthetic pathways and, therefore, the relative proportion of the main characteristic compounds [25]. In this context, this study aims to determine the chemical composition of OEE harvested in the commune of Seraïdi (north-eastern Algeria) in order to highlight its chemotype and to evaluate certain biological activities (antifungal, antibacterial and antioxidant). Given that the Seraidi region is very rich in *Eucalyptus globulus*, the interest would be to exploit it by producing EO which could be used as a biopesticide, in other words, an alternative to synthetic pesticides and as a natural antioxidant.

2. Materials and methods

2.1. Plant material

The plant material (Eucalyptus globulus) and its leaves were harvested in 2019 (December-April) from different stations of the commune of Seraïdi (wilaya of Annaba, north-eastern Algeria). Freshly collected, they were dried in the shade in a dry and ventilated place away from moisture for two weeks to be used later for essential oil extraction.

2.2. EEO extraction

The extraction is carried out with a Clevenger-type apparatus. The hydro-distillation consists in immersing 100 g of dry leaves in 100 ml of distilled water for 2 h. After obtaining the EO, the latter is decanted from the aqueous layer, dried with anhydrous sodium sulfate ($Na_2 SO_4$) and stored in hermetically sealed bottles at 4°C according to ISO 9235 standards. In the dry state, the yield is achieved by recovering this oil over a 10 min interval ranging from 0 to 90 min. The yield is expressed as a percentage and is given by the following formula [26]:

HRE (%) = M'/M x 100

RHE: yield of essential oil from dry leaves,
M': the mass of essential oil (g),
M: the mass of dry plant material (100 g)

2.3. EEO chemical characterization by GC-MS

The principle of separation is based on a difference in the distribution of the compounds of a mixture between two phases, the mobile phase and the stationary phase (impregnated in the column). In our case, the chemical analysis of EEO was performed by a gas chromatograph coupled to mass spectrometry (GC/MS) equipped with an HP-5MS capillary column (30 m x 0.25 mm) with a film thickness of 0.25 µm, a detector set at 200°C and fed with an H2/Air-gas mixture and an injector set at 275°C with an injection mode in split (leakage ratio: 1/50). Pure helium is used as a carrier with a 0.5 ml/min flow rate. The column temperature is programmed from 50 to 250°C at 4°C/min. The injection volume was 2 ml, and MS was performed at 1 scan s-1 with an ionizing voltage of 70 eV and an ion source temp of 2508. The components were identified by comparing their retention indices with a homologous series of C9-C24 n-alkanes and those of authentic standards [27].

2.4. Antifungal activity evaluation

The antifungal activity was evaluated by the dilution method in a solid medium to determine the inhibition rates and in a liquid medium to determine the MIC (minimum inhibitory concentration for which no mold growth is noted) and MFC [28]. The fungus studied in this work is *Fusarium roseum*, a pathogen responsible for the Fusarium head blight of wheat. It was isolated from lesions developed on the ears of grain taken from a field of cereals and provided by the National Institute of Plant Protection of El Tarf, Algeria (INPV).

2.4.1. Dilution technique in solid medium

The different concentrations of EO chosen (250, 500, 1000, 1500, 2000, 2500, 3000, 3500 and 4000 µg/ml) were mixed with 1 ml of methanol (50%). Each of the methanolic solutions obtained (0.5ml) was added to 20 ml of warm PDA medium. After homogenization, the mixture was poured into Petri dishes. The inoculation was done using Pasteur pipette by central puncture, and all the dishes were incubated for 7 days at 27° C. The mycelial growth was recorded daily. A measurement of the diameters of different colonies was made at the end to calculate the inhibition rate (I'%) [29] as follows:

I'(%) =100 x (dC-dE) / dC

I' (%): inhibition rate expressed as a percentage,
dC: diameter of colonies in the "positive control" plates,
dE: diameter of colonies in the plates containing the essential oil.

2.4.2. Dilution technique in liquid medium

The various solutions having had percentages of inhibition higher than 50% are maintained. A volume of 100 µl of these solutions was added to 900 µl of liquid Sabouraud medium containing the strain to be tested and incubated at 27°C for 7 days. After incubation, the tubes in which no mold growth at the lowest concentrations are determined as the MIC value. Concerning the MFC, we continued the experimentation by taking 50 µl from the tests, presenting a total inhibition to which we added 950µl of liquid and sterile Sabouraud medium. After 7 days of incubation, the subcultures with no apparent growth are determined as the MFC value.

2.5. Antibacterial activity evaluation

The agar diffusion was used to determine the EEO antibacterial activity [7]. The bacterial strain used for this

test is *Pseudomonas savastanoi,* known for its frequent involvement in the contamination of olive tree in the world, particularly in Algeria. Using a Pasteur pipette, we scrape a few colonies from a pure culture of bacteria having a maximum of 24 h and discharge them into 10 ml of sterile physiological water. The opacity of the suspension should be equivalent to 0.5 Mc Farland, which corresponds to 108 CFU/ml, then diluted to obtain an inoculum at 106 CFU/ml [30]. Petri dish poured media (Mueller Hinton medium) is swabbed from the bacterial suspension. Sterile Wattman paper discs of 0.6 mm diameter are soaked with different EEO concentrations (2500, 3000, 3500, 4000 µg /ml) and deposited on the bacterial strain. The plates are maintained for 30 min at a temperature of 25-30°C and are then incubated at 37°C/24 h. Antibacterial activity was assessed by measuring the diameters of bacteria-free zones (expressed in millimeters) formed around the discs using a caliper or ruler.

2.6. Antioxidant activity evaluation

The EO antioxidant capacity was evaluated *in vitro* using the DPPH° radical scavenging assay according to the protocol described by [31]. To 1 ml of the different sample concentrations prepared in ethanol, 250 µl of an ethanolic solution of DPPH (0.2 mM) was added. After incubation for 30 min in the dark at room temperature, the absorbance of the reaction medium is measured at 517 nm. The antioxidant activity of this EO *towards* the DPPH radical was evaluated according to its reduction, resulting in its change from purple to yellow color.

Anti-free radical activity (%) = (A0 -A / A0) x100

A0: absorbance of the DPPH · solution without the sample (negative control),

A: absorbance of the DPPH · solution in the presence of the sample

3. Results

3.1. Extraction kinetics and yield

The extraction kinetics consists of determining the yield as a function of extraction time and aims to set the time needed to extract the maximum amount of oil and avoid losses of time and energy. Figure 1 shows the results obtained from the HRE formula (%). The kinetics of EO extraction from *Eucalyptus globulus* leaves indicates that the latter's yield increases with time and reaches a maximum of 1.58% after 90 min.

3.2. Main compounds detected by GC-MS

The EEO chemical analysis by GC-MS detected 20 compounds (Table 1, Figure 2). This oil is composed mainly of linalool which represents 30.09% of the total oil, followed by b-Linalyl oxide (13.93%), Camphor (12.09%), 1,8-Cineole=eucalyptol (10.95%) and Bergamol (10.03%). Other constituents were identified at

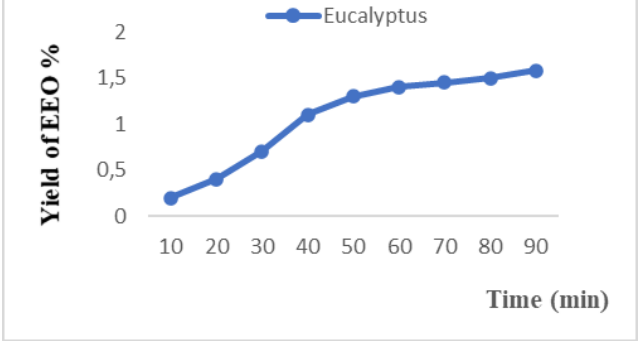

Fig. 1. Extraction kinetics and yield of *Eucalyptus globulus* EO obtained by hydroditillation

Table 1. *Eucalyptus globulus* EO chemical compounds obtained by GC-MS.

Number	Retention time	Content (%)	Compound
1	5.479	0.19	Alpha-Pinene
2	6.697	0.95	3-Octanone
3	6.806	0.20	Beta-Myrcene
4	6.881	0.24	Anhydrolinalool oxide
5	7.378	0.88	Hexyl Acetate
6	7.973	10.95	**1,8-Cineol (Eucalyptol)**
7	8.105	0.39	Cis-Ocimene
8	8.420	0.27	Beta-Ocimene
9	9.221	13.93	**b-Linalyl oxide**
10	9.696	8.82	**Epoxylinalol**
11	10.108	**30.09**	**Linalool**
12	10.331	1.30	1-Octen-3-yl acetate
13	11.510	12.09	**Camphor**
14	11.779	0.44	Cis-Dihydrocarvone
15	12.288	5.71	Borneol
16	12.940	1.41	Hexyl butyrate
17	13.106	1.11	(-)-alpha-Terpineol
18	15.052	10.03	**Bergamol**
19	16.184	0.52	Lavandulyl acetate
20	19.194	0.48	Geranyl acetate

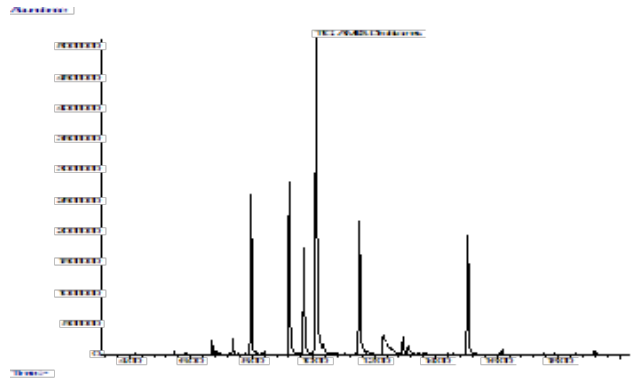

Fig. 2. GC-MS profile of *Eucalyptus globulus* EO.

relatively medium levels, such as epoxylinalol (8.82%), Borneol (5.71%), Hexyl Butyrate (1.41%), 1-Octen-3-Yl Acetate (1.30%) and alpha-Terpineol (1.1%). On the other hand, the chemical identification revealed quite low contents of other constituents, which do not exceed 1% of the total. It therefore turns out that the EO extracted from eucalyptus leaves from the Seraidi region (Eastern Algeria) is of linalool chemotype and not eucalyptol like the majority of eucalyptus oils.

3.3. Antifungal activity determination

Figure 3 shows the effect of the EEO on the mycelial growth of *Fusarium roseum*. It can be seen that after 7 days of incubation, an inhibitory effect of the applied doses is recorded, which reaches its maximum (100%) at the two highest concentrations, i.e., 3500 and 4000 µg/ml. Also, we notice that up to the concentration of 2000 µg/ml, the inhibition percentages do not exceed 50%, which is confirmed by the density of the Fusarium mycelium, which is reduced by half compared to the control (Figure 4). Finally, the concentrations 2500 and 3000 µg/ml have an average inhibition percentage that oscillates between 60 and 70%, with an apparent reduction of the mycelium compared to the control.

3.4. Antibacterial activity determination

From the values listed in Table 2, it can be seen that the inhibition diameter increases with increasing EEO concentrations. It reaches 11 mm at the lowest concentration (2500 µg/ml) and its maximum at the highest concentration (4000 µg/ml), where it is double or 22 mm.

Evaluation of MIC and FMC on liquid media using doses from 2000µg/ml to 4000µg/ml showed that the 2500µg/ml dose is the MIC and the 4000µg/ml dose represents the FMC where no growth was detected. These results are consistent with those obtained on incorporated media, where the 4000 µg/ml dose was found to be the most toxic to the plant pathogenic fungus.

3.5. Antioxidant activity determination

Figure 5 shows the inhibition rate of DPPH measured as a function of EEO. It can be seen that the inhibition percentages increase with increasing EEO concentrations applied to reach a maximum of 82% at the highest concentration of 100mg/ml. These values nevertheless remain lower than those recorded for ascorbic acid (84.45%). These results also allowed us to determine the IC50 for *Eucalyptus globulus* EO, equivalent to 17 mg/ml.

4. Discussion

The enthusiasm for essential oils (EOs) has persisted for several years, extending beyond mere olfactory pleasure. Their use now encompasses a range of beneficial and even therapeutic effects for both humans and nature. It is in this perspective that the present study was carried out and whose aim was to characterize the EO of the leaves of *E. globulus* harvested in the region of Seraidi in eastern Algeria in order to bring out the chemotype of the oil studied and also to highlight some biological properties.

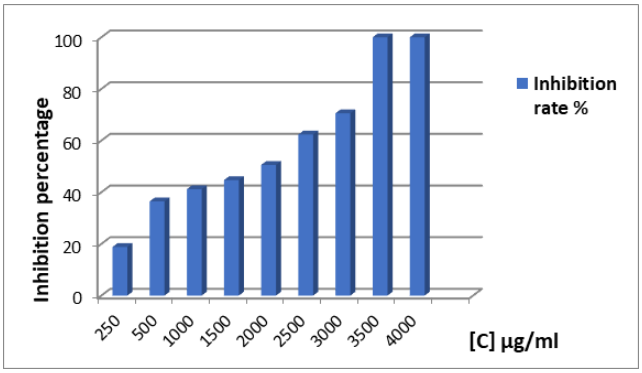

Fig. 3. EEO effect on *Fusarium roseum* mycelial growth (antifungal activity).

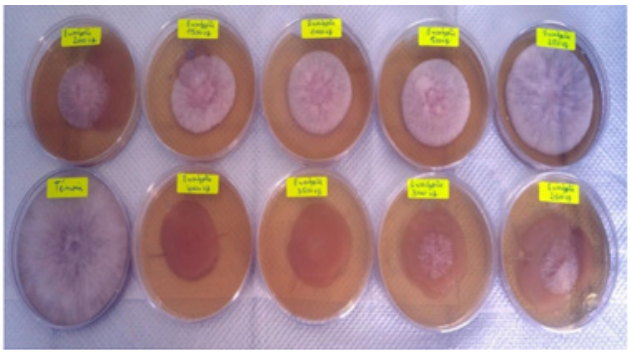

Fig. 4. Effect of *Eucalytus globulus* EO on the radial growth of *Fusarium roseum* in solid medium.

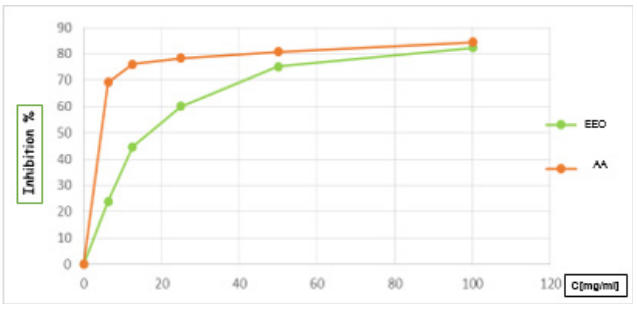

Fig. 5. Determination of the *Eucalyptus globulus* EO antioxidant activity compared to ascorbic acid (AA).

Table 2. Effect of *Eucalyptus globulus* EO on the growth of the bacterial strain *Pseudomonas savastanoi.*

Concentration of EO (µg/ml)	2500	3000	3500	4000
Diameter of inhibition (Pseudomonas savastanoi)	11mm	14mm	18mm	22mm

Indeed, eucalyptus essence is extracted by steam stripping from the leaves, and the yield of the EO extraction operation generally ranges from 1 to 3% [32], which is in perfect agreement with the values found in this study. Similarly, [24, 33, 22, 19] reported similar yield values, which are 1.6%, 1.89%, 1.31/1.49% and 1.21% respectively. [34] recorded an EEO extraction yield from plants harvested in the Bejaia (central Algeria) region of about 2.53%, which is significantly higher than the value noted in the extraction of the studied oil. Several studies conducted in Algeria revealed different EEO extraction yields from one region to another, such as [35] in the region of Constantine with a rate of 0.93%, [36] in El-Tarf with 1.65% and [37] in Constantine with 2.5%. This difference in extraction yields depends on several factors that can affect the EO contents, such as environmental, agronomic, age, genotype and geoclimatic factors [38]. In addition, the extraction method and conditions can influence the recovery percentages [39]. At the same time, the characterization of EEO revealed the presence of about twenty different compounds. In reality, not all varieties of Eucalyptus have the same constituents, but some are present in the majority of cases, and these are very often the ones that are responsible for the therapeutic benefits attributed to Eucalyptus [40]. Indeed, 1,8-cineole or eucalyptol generally represents 70-80% of the EO, and the other constituents are diverse, numerous and minority [32]. This active ingredient (1,8- cineole) is the genetic marker of the *E. globulus* species. Eucalyptus's medicinal properties include pinene, limonene, and at least 250 other compounds, including citronellal, cryptone, and piperitone [41]. However, the studied EO turns out to be different with respect to the contents of chemical compounds where the majority compound is Linalol (monoterpene) and not 1.8- Cineole. Several authors have reported this difference. For example, [23] found that Globulol was the dominant compound with 23.6%, followed by monoterpene 1.8 cineole with 19.8%. Similarly, [26] reported the predominance of aromadendrene (31.2%) and global (10.7%), followed by 1.8-cineole (14.5%). It is also noted that the composition of the EO extracted from the leaves of *E. globulus* harvested in the region of Seraïdi (East Algerian) is different from that extracted from plants harvested in other regions of Algeria, where studies have shown that the majority compound is still 1,8 cineole as in Constantine (East Algerian) [35], Blida [42] and Tizi-Ouzou [43]. Similarly, the chemical composition of *E. globulus* EOs collected in geographically distant areas (Morocco, Brazil, Argentina) is rich in 1.8 cineole. The latter's rate varies from country to country, where the values are respectively 22.4, 83.9 and 62.1% [19, 20, 22]. This variability depends on pedoclimatic factors that thus influence the chromatographic profile of plant extracts but also on harvest periods, temperature, sunshine duration, rainfall, altitude and soil nature [32]. On the other hand, variation between compound groups may also result from the co-regulation of several biosynthetic genes, controlling complex terpene profiles in different parts of the plant [44].

Concerning the biological properties of EEO, several authors have studied their antifungal activity. [45] evaluated this oil against various fungal species and found antifungal activity against *Aspergillus flavus*, *Aspergillus niger, Aspergillus parasiticus, Aspergillus spp*, *Candida albicans, Fusarium oxysporum*, Mucor spp, *Penicillium digitatum, Rhizopus nigricans, Rhizopus solani, Saccharomyces cerevisiae and Trichophyton spp.* Similarly, [30] reported this oil's MIC values ranging from 2.25 to 9 mg/ml. These observations are in good agreement with our results, where we noted a MIC of 2500µg/ml. [46] reported that *E. globulus* EOs were twice as effective (inhibition diameter: 14-46 mm) as nystatin, a drug used to treat fungal infections on the skin, mouth, vagina, and intestinal tract. [47] also demonstrated the antifungal activity of E. *globulus* EO on two species of Aspergillus: *Aspergillus flavus* and *Aspergillus parasiticus*. The authors added mycelia of both species to a solution A containing *E. globulus* essential oil and a solution B containing only 1,8-cineole. The experiment showed that the EEO had more than a significant effect on inhibiting the growth of mycelia of both species of Aspergillus. Moreover, 1,8-cineole alone has no effect on the mycelia proving that 1,8-cineole is not the only one responsible for the antifungal effect of the EEO. A synergy of molecules gives this action [18], which supports our results. The present study detected antifungal activity for virtually all applied doses against Fusarium mycelial proliferation despite the reported low levels of 1,8-cineole compared to the other components. This antifungal activity is, therefore, probably linked to its chemical profile, particularly the presence of linalool as a major component. The latter is a monoterpene alcohol widely present as a major constituent of essential plant oils, particularly lavender and coriander and it is known for its antifungal and antibacterial properties [48]. Several authors [49,50] have suggested that linalool acts on membrane integrity which disrupts ergosterol biosynthesis as it could also block the cell cycle of the target strain. Also, linalool has the potential to significantly improve the antimicrobial efficacy of other essential oils allowing for a reduction in their concentration in final products [51]. Some authors have attributed the antimicrobial power of EOs to the presence of oxygenated monoterpenes at high levels [52], which can cause alterations on the walls of target strains by their high solubilization [53]. At the same time, the presence of other substances, such as b-Linalyl oxide, bergamot and Camphor, should not be neglected, and their synergy can have an antifungal effect on these fungi. Camphor, for example, is used as an antitussive, antimicrobial, antiviral and analgesic agent, insecticide, and skin penetration enhancer [54].

Also, we wanted to highlight the antibacterial potential of this oil against a bacterial strain (*Pseudomonas savastanoi*) which is known to be devastating for the olive tree. [55] classified antibiotics by their inhibition diameter values (resistant: D<6 mm; intermediate: 13 mm>D >6 mm; sensitive: D >13 mm) and used this range to determine the antibacterial activity of EOs by comparing their diameters with those of the antibiotics. By comparing with this range, it appears that the strain used in our experiment is sensitive, and this is from the EEO concentration of 3000 µg/ml, where the diameter of inhibition is 14mm, while the concentration of 2500 µg/ml proves to be moderately active with a reported diameter of inhibition of 11 mm. These results are in agreement with several studies. [56] reported the antibacterial activity of *E. globulus* and *E. radiata* EOs against gram-negative strains *(Acinetobacter baumannii).* Similarly, [57] reported the antibacterial potential of *E. globulus* EO against the *Staphylococcus aureus* strain. Indeed, the antibacterial properties of EOs and their major components are effective in controlling the

spread of some bacterial agents [58] where several authors have confirmed their bacteriostatic and bactericidal effects even sometimes at very low concentrations [59]. EOs can act on the cell's outer envelope and the cytoplasm, which will disrupt bacterial structures and lead to an increase in permeability due to an inability to separate EOs from the bacterial membrane [60]. Therefore, these oils' mechanism of action can be attributed to the interaction between their components and the constituents of the cell membrane [61]. Their effectiveness is related to their hydrophobicity, which allows them to integrate into the membrane and mitochondrial lipids, making them permeable, and leading to cellular contents leakage [62]. Several molecules present in EOs have antibacterial properties, such as phenols (carvacrol, thymol and eugenol), alcohols (linalool) and aldehydes (cinnamaldehyde). The *E. globulus* EO used in this work is not very rich in 1,8-cineole. However, it is effective against the *Pseudomonas savastanoi* strain, even though many studies have revealed the ineffectiveness of this oil against *Pseudomonas aeruginosa,* which proves the antibacterial activity depends on the sensitivity of the strain used. Moreover, the studied oil is rich in linalool, which is known to have excellent antibacterial activity. This linalool activity disrupts bacterial cell walls by inhibiting enzyme activity and suppressing the translation of certain regulatory gene products [63]. It has been reported that several factors can influence the antibacterial effects, such as the chemical composition of the EO tested, the experimental method used and the bacterial strain tested. Their antibacterial action depends on the major compounds, synergistic and/or additive effects and minor compounds present [64].

For antioxidant activity, several EOs are known to possess good antioxidant properties, which can be exploited to protect other materials, such as foods and their rancidity [65]. In addition, linalool has been shown to have an anti-free radical and lipid peroxidation inhibiting effect, whose properties could make it a potential alternative to synthetic antioxidants. For example, using coriander essential oil or linalool can improve the shelf life of foods, which is extremely beneficial for the food industry, given the natural origin of these compounds. These attributes are due to the inherent ability of some of their components, especially phenols that prevent oxidative modification through free radical neutralization, oxygen scavenging or peroxide decomposition through their antioxidant activities [48]. Our results differ from those found using commercialized EOs of Tunisian *E. globulus* leaves I(C50 : 0.057- 0.048 and 0.048 mg/ml) [31] and also EOs hydrodistilled from Indian *E. citriodora* leaves (IC50 values were 0.425 ± 0.006; 0.087 ± 0.009; 0.01 ± 0.008 g/ml) for the three antioxidant tests performed [66] and this compared to the IC50 recorded during our study which is largely higher than these values (17 mg/ml). The IC50 is inversely proportional to the anti-radical action (anti-DPPH). A low IC50 corresponds to high antioxidant activity and vice versa, suggesting that the *E. globulus* EO of Seraïdi is endowed with rather moderate anti-radical activity. The antioxidant activity is attributed to the chemical composition of essential oils. However, it can be due to one of the majority constituents, other minority constituents, or synergy between them. The studied EO contains monoterpenes at various levels. In previous studies, oils with a monoterpene predominance have shown a rather modest activity. For example, [67] attributed the modest antioxidant potential of *Laurus nobilis* EO to the presence of oxygenated monoterpenes, including 1,8-cineole, which was considered a weak antioxidant with a fairly high IC50 of 9.360 mg/ml. Again, this difference in activity can be attributed to the difference in chemical composition, environmental, agronomic, age and geoclimatic factors, extraction techniques, storage conditions and experimental extraction conditions [42].

In the study of essential oils, the chemical characterization of Eucalyptus (Eucalyptus globulus) leaf essential oil reveals significant insights into its biological activities, including antifungal, antibacterial, and antioxidant properties. Recent research highlights the importance of understanding the chemical profiles of plant-derived compounds, as variations in composition can greatly influence their efficacy. For instance, Ghanbari et al. (2024) demonstrated that the qualitative evaluation of proteins in garlic clones can impact their biological effects, suggesting that similar approaches could enhance our understanding of eucalyptus oil's active constituents [68]. Furthermore, Nasiri et al. (2023) explored the essential oil profile of Echinophora cinerea, emphasizing how the chemical makeup of different plant parts contributes to their medicinal properties [69]. These studies underscore the necessity of thorough chemical analysis to optimize the therapeutic applications of essential oils like that from Eucalyptus globulus.

Finally, our EO does not contain much eucalyptol (10.95%) and cannot be used in pharmacies as an expectorant or mucolytic because the European Pharmacopoeia prohibits it (requires 70% eucalyptol). However, with its component different from most eucalyptus EOs and with Linalool as the majority compound, we could still be able to integrate it into certain natural products for therapeutic use such as antifungal or antibacterial. Likewise, we could suggest this EO for use as a Bio-pesticide in combination with other fungicides and bactericides in order to reduce the harmful impact of the latter on the environment and human health. On the other hand, for antioxidant power, this activity reaches its maximum at high doses and it would be better to use other EOs with strong antioxidant potential.

5. Conclusion

This paper addressed the chemical composition of the EO extracted from Eucalyptus (*Eucalyptus globulus*) leaves and its antifungal, antibacterial and antioxidant potential. The results indicate that the EO extracted from plants harvested in Seraïdi (north-eastern Algeria) is of chemotype linalool and thus differs from most EO of Eucalyptus leaves, generally of chemotype eucalyptol. Nevertheless, it has a strong antimicrobial activity because it is effective against the pathogen of fusarium head blight (*Fusarium roseum*) as well as against the pathogen of tuberculosis of the olive tree (*Pseudomonas savastanoi).* This activity depends essentially on the mixture of chemical substances it contains rather than on a single component. The results show that the studied EEO is rich in linalool and oxygenated monoterpenes, giving it a strong bio-fungicidal activity. However, as an antioxidant, the results revealed a modest activity of the latter even if the major component of the studied oil is linalool, which is known to have a good antioxidant activity, which allows us to confirm once again the hypothesis that it is rather the antagonistic or synergistic effect of the substances that give the EO its properties and not one of its components.

It would be interesting, for future research, to determine other biological activities (insecticide, anti-parasitic) and highlight the different chemical groups responsible for the different activities in order to use them directly as bio-pesticides instead of EOs. Also, test different combinations such as HE/antibiotic, HE/fungicide and HE/insecticide, in order to identify economically and ecologically reliable solutions while respecting human health and the environment, and therefore practical exploitation of the results for the manufacture of bio-pesticide.

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Conflict of interest
The authors declare no conflict of interest.

Authors' contributions
Haj-Moussa Hakim carried out all the manipulations in the laboratory and also took care of the harvesting of the eucalyptus leaves, he also contributed to the writing of the manuscript. Benamara Sara and Benhalima Hadia contributed to elaborate the work. Benaliouche Fouzia provided the fungal and bacterial strains for the tests. Sbartai Ibtissem made contributions from the interpretation of the results to the drafting and correction of the manuscript for publication. Sbartai Hana conceived the study and guided the work.

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