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Original Research Article

The Effect of Altitude on the Chemical Composition, Antioxidant and Antimicrobial Activities of *Eucalyptus globulus* Labill. Essential Oils

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ABSTRACT

Eucalyptus globulus Labill., a medicinal plant native to Eastern Morocco, is renowned for its therapeutic qualities and pleasant aroma. This study aimed to investigate the effect of altitude on the chemical composition, and biological activities of *E. globulus* leaves essential oil. *E. globulus* leaves were collected from three different locations; Nador, Morocco, Taourirt, Morocco, and Ain Benimathar, Morocco with altitudes 7 m, 380 m, and 950 m, respectively. The essential oils were extracted by hydro-distillation. The chemical composition of the oils was determined by GC-MS analysis. The antioxidant potential was assessed by total antioxidant capacity (TAC) and the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. The antimicrobial activity was tested against various bacterial and fungal strains using standard procedures. GC-MS analysis revealed significant variations in the chemical composition of the essential oils. For the sample collected at 7 m altitude, 17 constituents were identified in the essential oil, with the major constituents being *β*-Cymene (17.21%), Eucalyptol (10.88%), *α*-pinene (10.54%), Phellandrene (9.70%), and p-Menth-1-en-4-ol (8.92%). The essential oil of the sample collected at 380 m altitude, contained mainly Eucalyptol (45.53%), 4,6-di-t-Butylpyrogallol (18.27%), *α*-pinene (7.25%), *β*-Cymene (6.24%), and Camphor (5.26%). While the essential oil of the sample collected at 950 m altitude were composed mainly of Eucalyptol (24.92%), Pinocarveol (16.94%), 4,6-Di-t-Butylpyrogallol (14.40%), *β*-Eudesmol (12.50%), and *α*-pinene (11.30%). The oils exhibited significant antioxidant and antimicrobial activities with greater activity against the fungi. This study has highlighted the impact of cultivation altitude on the chemical composition and the biological effect of *E. globulus* essential oil.

Keywords: *Eucalyptus globulus*, Altitude, Essential oil, Phytochemical composition, Antioxidant activity, Antimicrobial activity..

Introduction

Morocco boasts of a rich and diverse botanical landscape, with a large variety of plant species that serve medicinal, endemic, and culinary purposes. The nation's floral diversity is crucial to its overall biodiversity, which thrive across different topographical landscape comprising mountains, plains, valleys, deserts, and coastal areas. This topography provides microclimates that fosters the adaptation of plant species to specific ecological niches.[1,2] Particularly noteworthy is the oriental region of Morocco, characterized by its complex blend of geographical features that endow it with a rich biodiversity.[3] These geographic features support a lot of habitats within the region, thereby fostering the survival of a plethora of flora and fauna. *Eucalyptus globulus* Labill., commonly called blue gum, is a member of the Myrtaceae family and originated from Australia. Due to its numerous health benefits, its cultivation has assumed a global dimension. The plant is a well-known medicinal plant in the oriental region of Morocco, and it is a rich source of essential oil. The essential oil derived from *E. globulus* has found extensive use in aromatherapy and pharmaceuticals due to its well-established therapeutic applications such as in the treatment of cough, influenza, and inflammation. Evidence from empirical research, has shown this essential oil to possess a wide array of biological activities, including but not limited to antioxidant, antimicrobial, anti-inflammatory, antispasmodic, and insecticidal activities.[4–15] The composition of this essential oil and its biological activities have been reported to be greatly influenced by numerous environmental factors.[16,17] Altitude play a major role in determining the impact of these environmental factors on plants. For example, climatic variations, solar radiation, temperature gradients, nutrient availability, and atmospheric pressure are all directly influenced by altitude.[16, 18-20]

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The present study seeks to assess the influence of altitudinal variations on the essential oil extracted from *E. globulus* in the eastern region of Morocco. Three provinces with distinct altitudes; Nador situated at sea level (7 m), Taourirt occupying a moderate elevation (380 m), and Ain Benimathar, situated at an elevated plateau (950 m) were selected for the study. The primary objective was to determine the relationship between altitude and the chemical constituents of the essential oil, as well as its biological activities *vis-à-vis* its antioxidant, antibacterial, and antifungal properties.
The findings from the study could provide useful information that could impact the practical applications of *E. globulus* essential oil as natural medicines, cosmetic formulations, and for industrial use.

Materials and Methods

Collection of Plant materials and essential oil extraction
During the vernal season of 2023 (March, 2023), foliar samples of *E. globulus* were collected from three discrete geographical coordinates within the Oriental region (Figure 1), specifically Nador, Taourirt, and Ain Benimathar, Morocco. The plant material was identified and authenticated in the Center for Oriental Water Sciences and Technologies (COSTEE), University of Mohammed the First, Oujda, Morocco. Herbarium specimen was deposited, and voucher number HUMPOM667 was assigned. To extract the essential oil, the comminuted plant material was subjected to hydro-distillation at 100°C in a Clevenger apparatus.

Determination of the chemical composition of E. globulus essential oil (EGEO)
The qualitative and semi-quantitative analysis of *E. globulus* essential oils (EGEOs) was performed in a gas chromatograph equipped with a mass spectrometer (MS QP2010, Shimadzu Scientific Instruments, Tokyo) according to procedure previously reported.[21–25]
Briefly, the GC consist of a BPX25 capillary column (30 m x 0.25 mm x 0.25 µm) packed with dimethylpolysiloxane as the solid phase. The injection volume was 1 µL with Helium gas (99.99%) as the mobile phase at a flow rate of 3 mL/min. The oven temperature started at 50°C (held for 1 min) and increased to 250°C at a rate of 10°C/min, and maintained at 250°C for 1 min. The ionization mode was electron impact (EI) at 70 eV, the mass was scanned from 40 to 300 m/z. Compound identification was accomplished by juxtaposing retention times with established standards and comparing the mass spectra data with the fragmentation patterns retrieved from NIST database.[26,27]

Determination of Antioxidant activity
Total antioxidant capacity (TAC)
The total antioxidant capacity of EGEOs was assessed using the phospho-molybdate assay according to the procedure previously reported by Elbouzidi *et al.* (2022).[28] A standard curve was prepared using vitamin C and results expressed in vitamin C equivalents.

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay
A solution of 0.1 mM DPPH in methanol was prepared, and EGEO was prepared at concentrations of 0.2 to 1 mg/mL (0.2, 0.4, 0.5, 0.7, 0.8, and 1 mg/mL). A 2.5 mL of the DPPH solution was mixed with 0.5 mL of each concentration of the essential oils. The mixture was incubated at room temperature in the dark for 30 min. Following the incubation, the optical density of the mixture was measured at 517 nm against a blank. Ascorbic acid was used as the reference standard.[29–31]
The scavenging activity was calculated using the formula:

Percentage free radical scavenging Activity (%) = [(A_blank - A_sample) / A_blank] × 100

Where; A_blank is the absorbance of the DPPH solution without the extract (EGEO), and A_sample is the absorbance of the mixture of DPPH with the extract at different concentrations.

Determination of Antibacterial Activity
Selection of Bacterial Strains
Isolates of two Gram-positive bacteria (*Staphylococcus aureus* and *Micrococcus luteus*) and two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) strains were selected for the study.

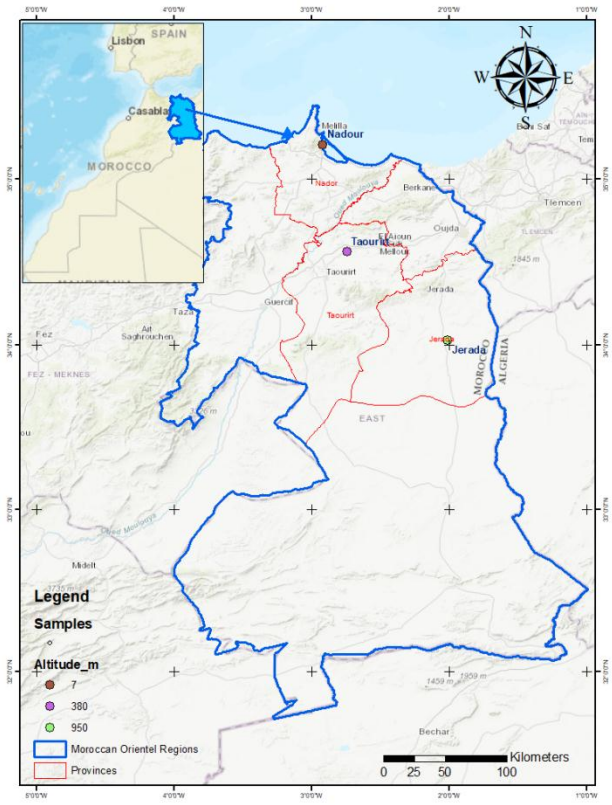

Figure 1: Sampling locations (map prepared with ArcMap 10.5 software)

Disc diffusion assay
The antibacterial activity of the EGEOs was assessed using the disc diffusion technique. The bacterial inoculum was prepared following the procedure prescribed by the National Clinical Laboratory Standards Committee. The inoculum was aseptically placed in Petri dishes containing Sauton agar. Subsequently, circular filter paper discs with a 6 mm diameter were placed on the surface of the Petri dishes. The discs were loaded with 5 mL of the essential oil at different concentration. The Petri dishes were incubated at 37°C for bacterial strains and 25°C for fungal strains for 48 to 72 h. After the incubation period, the Petri dishes were cooled to 4°C and maintaining at this temperature for 2 h. To assess the antibacterial activity, the diameter of the inhibition zones around each filter paper disc was measured. Each of the experiments was done in triplicates.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)
The MIC of the essential oils was determined in Mueller-Hinton culture medium supplemented with 0.15% agar. The essential oils were prepared in concentrations ranging from 4% to 0.0078% in the culture medium. The medium containing the essential oils was placed in 96-well plates. Each well was inoculated with a bacterial culture containing a cell density of 1 x 10^6 cells/mL. Gentamicin (1 mg/mL) was used as the positive control. The plates were incubated at 37°C for 24 h. After the incubation, 15 µL aliquot of a 0.015% resazurin solution was added to the medium, and further incubated for another 2 h at 37°C. The gradual change in colour of resazurin from its initial blue hue to a pink colour due to production of resorufin is an indication of bacterial growth in the medium. The experiment was performed in triplicates.

For the determination of MBC, a 3 µL aliquot each from the wells, including the negative control well was transferred into a nutrient-enriched Muller-Hinton agar growth medium. This medium was then incubated at 37°C for 24 h. Following the incubation period, the MBC was determined as the lowest concentration of the essential oil at which discernible bacterial growth was absent.

Antifungal Activity

Selection and origin of fungal strains

Pure fungal strains consisting of two mold (*Aspergillus niger* and *Penicillium digitatum*), and two yeast (*Candida glabrata* and *Rhodotorula glutinis*) were obtained from the Laboratory of Microbial Biotechnology, Faculty of Sciences, University of Mohammed the First, Oujda, Morocco.

Preparation of inoculum and disc diffusion assay

The molds were maintained in Potato Dextrose Agar (PDA) medium (BIOKAR, France), while the yeasts were maintained in Yeast Extract Peptone Dextrose (YPD) medium. The culture media were incubated at 25°C for seven days for the molds, and at 25°C for 48 h for the yeasts. At the end of the incubation period, the fungal spores were counted using the Thoma hemacytometer, and the cell density was standardized to 2×10^6 spores/mL for the molds and 1×10^6 cells/mL for the yeasts. The assay was performed according to the method prescribed by the National Committee for Clinical Laboratory Standards.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC)

For the determination of MIC for yeast species, the cells were treated with concentrations of the essential oils ranging from 8% to 0.0015% in a 96-well microplate. The plates were Incubated at 25°C for 48 h, after which 15 µL resazurin solution was added to each well, and then further incubated at 25°C for 2 hours. The MIC was determined as the lowest concentration of essential oil at which there was a discernible change in the colour of the resazurin solution from blue to pink, indicative of the onset of growth.

In the case of the mold species, 8% to 0.0015% concentrations of the essential oils were prepared in 0.15% YEG agar. The determination of the MIC followed the same procedure as described above for the yeasts, but the initial incubation period was 72 h. Cycloheximide (1 mg/mL) was used as the positive control.

For the determination of the MFC, an aliquot (3 µL) of culture from wells where growth was notably absent was transferred into the YEG medium and incubated at 25°C for 48 h for the yeast strains, and 72 h for the mold strains.

Data Analysis

Data were expressed as means ± standard deviations of three independent measurements. Statistical analysis was carried out utilizing the IBM Statistical Package for Social Sciences (IBM SPSS version 23). Data were subjected to one-way analysis of variance (ANOVA). Differences between means were determined by post hoc tests. P-value ≤ 0.05 was regarded as significant.

Results and Discussion

Yield of essential oils

Table 1 shows the yields of the essential oils extracted from *E. globulus* leaves. The yields ranged from 0.25% to 2.80%. from the results, significant variation in the essential oil yield according to the cultivation altitude of *E. globulus* was observed. Plants cultivated at the highest altitude of 950 m gave the highest essential oil yield. This was closely followed by the plants cultivated at an intermediate altitude of 380 m, while those cultivated at the lowest altitude of 7 m gave the least essential oil yield. This observation suggests the potential impact of the cultivation altitude on the essential oil yield of *E. globulus* leaves.

Chemical composition of E. globulus Essential Oil

The essential oil derived from the leaves of *E. globulus* cultivated at 7 m contained a total of 17 compounds as detected by the GC-MS analysis. The key constituents were *β*-Cymene (17.21%), Eucalyptol (10.88%), *α*-pinene (10.54%), Phellandrene (9.70%), and p-Menth-1-en-4-ol (8.92%). On the other hand, the essential oil obtained from *E. globulus* leaves at an altitude of 380 m contained 13 compounds identified by GC-MS, with the major constituents being Eucalyptol (45.53%), 4,6-di-t-Butylpyrogallol (18.27%), *α*-Pinene (7.25%), *β*-Cymene (6.24%), and Camphor (5.26%). The essential oil obtained from *E. globulus* leaves at the highest altitude of 950 m had only 9 components, with the principal constituents being Eucalyptol (24.92%), Pinocarveol (16.94%), 4,6-di-t-Butylpyrogallol (14.40%), beta-Eudesmol (12.50%), and *α*-Pinene (11.30%).

These observations deviate significantly from the study of Kalemba *et al* ,[32] who conducted an inquiry into the essential oil extracted from *E. globulus* native to Algeria. In their investigation, they reported 1,8-cineole (48.6%), globulol (10.9%), trans-pinocarveol (10.7%), and *α*-terpineol (6.6%) as the major constituents of *E. globulus* essential oil. Based on these observations, it is clear that the chemical composition of the essential oil from *E. globulus* is highly influenced by the location and altitude of cultivation.

Researches have shown the inherent ability of *E. globulus*, and other plant species to undergo adaptive changes in their chemical make up in response to prevailing environmental conditions. This adaptive change became particularly evident in instances where heightened Eucalyptol production occurred at elevated cultivation altitude. This adaptation is speculated to be a defensive mechanism by which plants protect their leaves as well as other plant tissues from environmental stresses such as increased exposure to ultraviolet radiation.[33] The correlation between elevated Eucalyptol content and high altitude offers a plausible rationale for the observed variations in essential oil composition based on the altitude of the location where the plant grows or it's cultivated.

Following the interplay between environmental factors and essential oil composition, the findings from the present study has contributed to the body of knowledge pertaining to the defensive adaptation mechanisms exhibited by *Eucalyptus globulus*. It has also provided insights into the ability of *E. globulus* to navigate and negotiate through alterations in its external milieu.

The chromatograms of the gas chromatography - mass spectrometry (GC-MS) analysis are shown in Figure 2 (A, B, C), while a comprehensive list of these compounds is presented in Table 2.

Antioxidant activity

Evaluation of the antioxidant activity of *E. globulus* essential oils using the DPPH and TAC assays showed significant variation in the antioxidant activity of the essential oils (EO1, EO2, and EO3) based on the cultivation altitude of the plant. The IC_{50} values from the DPPH assay were 4.13 ± 0.005 µg/mL, 2.84 ± 0.04 µg/mL, and 1.98 ± 0.05 µg/mL, for EO1, EO2, and EO3, respectively. The IC_{50} values from the TAC assay for EO1, EO2, and EO3 were 23.42 ± 0.60 µg AA/mL, 53.42 ± 0.40 µg AA/mL, and 64.47 ± 0.70 µg AA/mL, respectively. These IC_{50} values suggest a high and altitude-dependent antioxidant potential of the essential oils of *E. globulus* leaves (Table 3).

Table 1: Yields of Eucalyptus globulus Leaves Essential Oils

Essential oil	EO1	EO2	EO3
Yield (% w/w)	0.25 ± 0.06 [a]	1.20 ± 0.05 [b]	2.8 ± 0.02 [c]

All values represent mean ± SD (n = 3). Significant differences are indicated by different letters (a, b and c) in the same row ($p < 0.05$). EO1: Essential oil extracted from plants located at an altitude of 7 m. EO2: Essential oil extracted from plants located at 380 m altitude. EO3: Essential oil extracted from plants located at an altitude of 950 m

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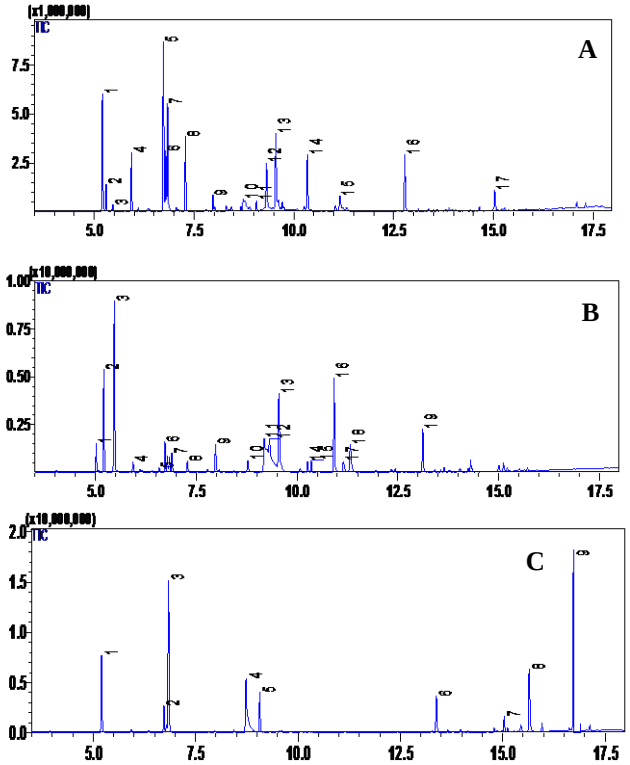

Figure 2: GC-MS Chromatogram of Essential Oil Extracted from the leaves of *E. globulus*. (A): Plants at 7 m altitude, (B): Plants at 380 m altitude, (C): Plants at 950 m altitude

These findings underscore the pivotal role played by altitude in the antioxidant activity of the investigated essential oils. Heightened levels of stress encountered by plants at certain altitudes, particularly at elevated altitudes, stimulate the increased biosynthesis of bioactive secondary metabolites responsible for endowing their essential oils with high antioxidant properties.[7,8] These bioactive secondary metabolites has the capacity to neutralize free radicals, thus effectively protecting the cells and tissues from the damaging effect of oxidative stress due to excessive free radicals.[34–36]

The observed elevation-related increase in antioxidant activity of the essential oils of *E. globulus* leaves can be attributed to the adaptive response of *E. globulus* to stressful environmental conditions, including increased ultraviolet radiation and other altitude-associated stressors. The results from the present study corroborate the findings from previous research which showed the role of altitude in chemical compositions of plants, and consequently their biological activities.[37]

Antibacterial activity

The results from the antibacterial activity screening showed that *E. globulus* leaves essential oils possess antibacterial activity against both Gram-positive and Gram-negative bacteria. The inhibition zone diameter ranged from 8.75 to 30.00 mm. The essential oil designated as EO1, which originated from plants cultivated at 7 m altitude, exhibited inhibition zone diameter of 14.5 mm, 13.5 mm, 11 mm, and 13.5 mm against *Staphylococcus aureus, Micrococcus luteus, Escherichia coli,* and *Pseudomonas aeruginosa*, respectively. On the other hand, EO2, sourced from plants thriving at 380 m altitude, showed the most potent antibacterial activity against *Staphylococcus aureus*, with inhibition zone diameter of 30 mm. It showed significantly lower activity against the other organisms as demonstrated by the inhibition zone diameters of 11.5 mm, 11 mm, and 11 mm against *Micrococcus luteus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, respectively.

Table 2: Chemical Composition of the Essential Oils of Eucalyptus globulus collected from three different altitudes

S/N	Compound	% Area		
		EO1	EO2	EO3
1	*α*-Pinene	10.54	7.25	11.30
2	*β*-cymene	17.21	6.24	4.18
3	Eucalyptol	10.88	45.53	24.92
4	Pinocarveol	-	-	16.94
5	Isovaleric acid,isopentyl Ester	-	0.63	-
6	Globulol	-	2.31	2.71
7	Beta-eudesmol	-	-	12.50
8	Camphol	0.96	1.09	-
9	D-Limonene	5.51	4.56	-
10	Camphor	3.33	5.26	-
11	Benzene.1.2-dimethoxy- 4-(2-propenyl)-	4.88	-	-
12	Crypton	-	4.18	-
13	Phellandral	9.70	1.33	-
14	(-)-alloaromadendrene	5.58	2.54	13.37
15	Thiopivalic acid	2.59	-	-
16	Sabinen	0.49	-	-
17	*γ*-terpinen	6.95	-	-
18	Linalool	1.61	-	-
19	Linalyl anthranilat	6.28	-	-
20	*α*-thujenal	2.19	-	-
21	*p*-menth-1-en-4-ol	8.92	0.81	-

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Table 3: Antioxidant potential of the Essential Oils of *Eucalyptus globulus* leaves

Test	IC_{50}			
	EO1	**EO2**	**EO3**	**Ascorbic Acid**
TAC (µg AA/mL)	64.47 ± 0.60	53.42 ± 0.4	23.53 ± 0.70	-
DPPH Assay (µg/mL)	4.13 ± 0.05	2.48 ± 0.04	1.98 ± 0.05	21.06 ± 0.001

Table 4: *Antibacterial activity of E. globulus Essential Oils*

Bacterial strains		***S. aureus***	***M. luteus***	***E. coli***	***P. aeruginosa***
Essential oils	EO1	14.5 ± 0.7	13.5 ± 0,7	11.0 ± 0.9	13.5 ± 0.7
	EO2	30.0 ± 0.7	11.5 ± 0.7	11.0 ± 0.9	11.0 ± 0.7
	EO3	14.5 ± 0.9	11.0 ± 0.9	8.75 ± 0.7	9.5 ± 0.4
Gentamicin, IZ (1 mg/mL)		19.5	21.5	22.5	20.5
MIC (% v/v)	EO1	0.5	4	8	2
	EO2	8	8	8	16
	EO3	8	4	2	16
MBC (% v/v)	EO1	16	16	16	16
	EO2	16	16	16	16
	EO3	16	16	16	16

All values represent the mean ± standard deviation (n = 3). EO1: Essential oil extracted from plants located at an altitude of 7 m. EO2: Essential oil extracted from plants located at 380 m altitude. EO3: Essential oil extracted from plants located at an altitude of 950 m.

Table 5: Antifungal Activity of Essential Oils from the Three *E. globulus* Ecotypes

Fungal strains		***P. Digitatum***	***A. Niger***	***C. glabrata***	***R. glutinis***
Essential oils	EO1	24.0 ± 0.7	22.0 ± 0.4	18.0 ± 0.4	15.0 ± 0.1
	EO2	28.0 ± 0.9	24.0 ± 0.7	30.0 ± 0.9	16.0 ± 0.4
	EO3	31.0 ± 0.9	27.0 ± 0.7	30.0 ± 0.4	16.0 ± 0.4
Cycloheximide, IZ (1 mg/mL)		22.90	22.30	21.50	21.0
MIC (% *v/v*)	EO1	1	1	4	4
	EO2	0.5	0.5	2	2
	EO3	0.25	0.25	1	1
MFC (% *v/v*)	EO1	8	8	8	8
	EO2	4	4	4	4
	EO3	4	4	4	4

All values represent the mean ± standard deviation (n = 3). EO1: Essential oil extracted from plants located at an altitude of 7 m. EO2: Essential oil extracted from plants located at 380 m altitude. EO3: Essential oil extracted from plants located at an altitude of 950 m.

For the essential oil EO3, extracted from plants grown at an elevated altitude of 950 m, inhibition zone diameters of 14.5 mm, 11 mm, 8.75 mm, and 9.5 mm against *Staphylococcus aureus*, *Micrococcus luteus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, respectively were observed. It is noteworthy that the essential oils showed variable minimum inhibitory concentrations (MICs) reflective of the susceptibility of the bacteria to the essential oils. EO3 exhibited the lowest MIC of 8% against *S. aureus* which indicate the most potent antibacterial activity compared to the other essential oils (Table 4).

However, with respect to the Minimum Bactericidal Concentration (MBC), all the evaluated essential oils exhibited bactericidal activity at concentrations of 16%. This shows that the essential oils exhibited relatively moderate antibacterial activity across the spectrum of the tested bacterial strains. This observation contradicts the findings from a previous study which showed varied MBCs of the essential oil against the different bacteria strains.[38]

The present study suggests a possible role of cultivation altitude in the antibacterial activity of EGEOs.

Essential oils extracted from plants cultivated at locations such as Nador and Ain Benimathar, exhibited less effective antibacterial activity compared to their counterpart sourced from Taourirt. This observed variation might be attributed to the differences in the chemical constitution of the essential oils, which is a direct consequence of the altitudinal differences in the locations where the plant is cultivated.

The findings presented in this study have therefore supported the significant role of altitude in determining the chemical composition and, by extension, the antibacterial activity of *Eucalyptus globulus* essential oil. However, it is important to note that other environmental and geographical factors could exert their influence on the chemical composition of essential oils, and consequently impact their antibacterial properties.

Antifungal activity

The assessment of the antifungal activity of *Eucalyptus globulus* essential oils involved the use of four distinct fungal strains: Rhodotorula glutinis and Candida glabrata, representing yeast strains, Aspergillus niger and Penicillium digitatum, representing mold strains. The results of this assessment are presented in Table 5. The results showed that *E. globulus* essential oils possess remarkable antifungal

potential. At 4% (v/v), EO1 demonstrated a high potential in inhibiting the growth of all tested fungal strains, with the inhibition zone diameter ranging from 15 to 24 mm. In a similar manner, EO2 manifested significant antifungal activity, with inhibition zone diameter ranging from 16 to 30 mm. The MICs for EO2 were lower than the MICs of EO1, indicating that EO2 has higher antifungal activity against all the tested fungal strains compared to EO1. On the other hand, EO3 extracted from plants cultivated at an altitude of 950 m exhibited the highest antifungal activity, with inhibition zone diameter ranging from 16 to 31 mm. Among the three essential oils, EO3 had the lowest MICs, which indicated that it has the most potent activity against the tested fungal strains. This remarkable antifungal activity can be attributed to the bioactive chemical constituents of EGEOs. This assertion has also been corroborated by findings from a previous study.[39] It was also observed that the essential oils extracted from the plants at higher altitudes (EO2 and EO3) had lower MFCs against all test fungal strains.

The findings from the antifungal activity screening have further confirmed our proposition that cultivation altitude have significant influence on the essential oil composition of *E. globulus*, which in turn influence their biological activity. Based on this, it is therefore important to further explore the mechanism that underpins the relationship between altitude, chemical composition and the biological activity of *E. globulus* essential oils.

Conclusion

This study has undertaken a comprehensive exploration of the intricate interplay between altitude and the chemical composition and biological activities of essential oil derived from *Eucalyptus globulus* leaves. The findings from this study did not only reaffirm the antioxidant activity of *Eucalyptus globulus* essential oil but also revealed its significant antibacterial and antifungal potential.

Furthermore, the study has found out that altitude play a significant role in the chemical composition of essential oil of *Eucalyptus globulus* leaves, as well as their antioxidant and antimicrobial properties. This influence is rooted in the adaptive changes of the plant's secondary metabolites in response to various environmental stressors that can result from altitudinal differences in the location of the plant cultivation.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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