

Original Research Paper

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Comparative phytochemical profile and biological activities of Eucalyptus globulus from two regions of Algeria (Blida and Tizi-Ouzou).

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Article history: Received: March 30th 2025; Revised: September 22nd 2025; Accepted: October 22nd 2025

Abstract

Eucalyptus globulus, a species of the Myrtaceae family and a common medicinal plant in traditional Algerian medicine, is already known for its biological properties and especially its antibacterial and antioxidant properties. Ecological conditions influence pharmacological activity; *Eucalyptus* is therefore an interesting species to study. This work consists of evaluating the biological activity of methanolic extracts and essential oils of *E. coli* leaves. *Globulus* was collected from two regions in northern Algeria: Blida and Tizi-Ouzou. A qualitative phytochemical screening was carried out on the methanolic extracts followed by a quantitative analysis of the polyphenol and flavonoid contents. Antioxidant activity was assessed using the DPPH free radical scavenging assay, and antibacterial activity by the agar diffusion method. Phytochemical analysis revealed a qualitative diversity in the composition of extracts between the two regions. Indeed, extracts from Blida showed higher levels of phenols and flavonoids than those from Tizi-Ouzou. The antibacterial activity of the extracts was found to be high, while the antioxidant activity varied according to geographical origin. This reveals an influence of geoclimatic conditions on the antioxidant power. The results obtained reveal the effect of geographical origin on the chemical composition and biological activities of *Eucalyptus globulus*. These variations must be taken into account in the phytotherapeutic development of the species.

Keywords: *Eucalyptus globulus*, Blida, Tizi-Ouzou, essential oil, antioxidant activity, antibacterial activity.

الخلاصة

يُعد اكالبتوس جلوبيولوس، وهو نوع من فصيلة الآسية نباتاً طبيّاً شائعاً في الطب التقليدي الجزائري، معروفاً بخصائصه البيولوجية، وخصوصاً بخصائصه المضادة للبكتيريا والمضادة للأكسدة. ونظراً لتأثير الظروف البيئية على النشاط الدوائي، فإن شجرة الأوكالبتوس تُعتبر نوعاً جديراً بالدراسة. يهدف هذا العمل إلى تقييم النشاط البيولوجي للمستخلصات الميثانولية والزيت الأساسية لأوراق اكالبتوس جلوبيولوس التي جُمعت من منطقتين في شمال الجزائر: البلدة وتيزي وزو. تم إجراء اختبار كيميائي نباتي نوعي على المستخلصات الميثانولية، تلاه تحليل كمي لمحتوى البوليفينولات والفلافونويدات. كما تم تقييم النشاط المضاد للأكسدة من خلال اختبار النقاط الجذور الحرة، والنشاط المضاد للبكتيريا بواسطة طريقة الانتشار على الوسط المغذي. كشفت التحاليل الكيميائية النباتية عن تنوع نوعي في تركيب المستخلصات بين المنطقتين. إذ أظهرت مستخلصات منطقة البلدة مستويات أعلى من الفينولات والفلافونويدات مقارنة بتلك القادمة من تيزي وزو. كما تبين أن النشاط المضاد للبكتيريا للمستخلصات كان مرتفعاً، بينما اختلف النشاط المضاد للأكسدة حسب الأصل الجغرافي؛ مما يدل على تأثير الظروف الجغرافية والمناخية على القدرة المضادة للأكسدة. وتُظهر النتائج التي تم الحصول عليها تأثير المنشأ الجغرافي على التركيب الكيميائي والأنشطة البيولوجية لنبات اكالبتوس جلوبيولوس، وهي اختلافات يجب أخذها بعين الاعتبار في تطوير الاستخدامات العلاجية للنبات

الكلمات الرئيسية: البلدة، تيزي وزو، زيت عطري، نشاط مضاد للأكسدة، نشاط مضاد للبكتيريا، اكالبتوس جلوبيولوس

Introduction

Interactions between humans and environmental agents, in particular microorganisms, both in symbiosis and pathogenicity, are manifold and sometimes the cause of serious diseases occurring in various contexts. Medicinal plants, due to their long history of use, are a useful resource for disease prevention and treatment worldwide (Cardoso et al., 2019), mainly due to their richness in secondary metabolites

(polyphenols, flavonoids, volatile compounds, etc.) with well-described antioxidant and antimicrobial activities (Hazzit, 2015; Dastmalchi et al., 2008).

In recent years, there has been growing interest in these bioactive natural compounds as substitutes for sometimes harmful synthetic additives. Studies have pointed to plant extracts as sources of antioxidants and antibacterial agents (Gutiérrez et al., 2003; Daker et al., 2008; Karou et al., 2005). Among the most promising species, we will be looking at *Eucalyptus globulus* Labill. *Eucalyptus*, a medicinal plant with antiseptic, has expectorant and antimicrobial properties. *Eucalyptus* essential oil (EO) is rich in 1,8 cineole (eucalyptol), it has strong antioxidant properties and a broad antibacterial spectrum, both of which are related to the active volatile compounds found in its composition (Boukhatem et al., 2017; Damiani et al., 2013 and Luis et al., 2016).

In this context, the aim of the present study was to explore the impact of geographical origin on the phytochemical profile and the antioxidant and antibacterial activity of the essential oil and methanolic extracts of *E. globulus* harvested in the two northern Algerian regions of Blida and Tizi-Ouzou. The study also attempted to specify the biological activities as a function of regional climatic conditions with a view to their therapeutic use.

Materials and methods

Study area

Leaf samples from the *Eucalyptus globulus* plant were taken from individuals of the same age and therefore at the same stage of ripening in order to limit the ability of plant senescence to influence phytochemical profiles. The samples were taken from two regions: Tizi-Ouzou and Blida, in northern Algeria, under contrasting climatic conditions.

Station 1: Tizi-Ouzou (district of Azeffoun, Tamgout b'Adrare area).

Located at an altitude of 36.8 m (36°49'01"N, 4°32'01"E), this station is part of an industrial reforestation zone. It has a humid Mediterranean climate with warm winters and an average annual rainfall of 881 mm. The dry period extends from June to mid-September. Climatic data was obtained from the Tizi-Ouzou weather station. The Tizi-Ouzou station, located in the district of Azeffoun (Tamgout b'Adrare area), is part of an industrial reforestation sector covering an area of 7,718 hectares, divided between the localities of Azeffoun and Aghrib (Azeffoun daïra). The area is bounded to the north by the Mediterranean Sea (from Tigzirt to the border with the wilaya of Béjaïa), to the east by the oued Ighzer Mahcène, which marks the border with Béjaïa, and to the south by the villages of Ibhrizène, Igoudjedal, Tifezouin, Ihamziouène, Tiza, Boumassaoud, Cheurfa Bouzeik, Ighil Mahni and the ridge path, and to the west by the ridge line bordering the Azazga district (Figure 1).

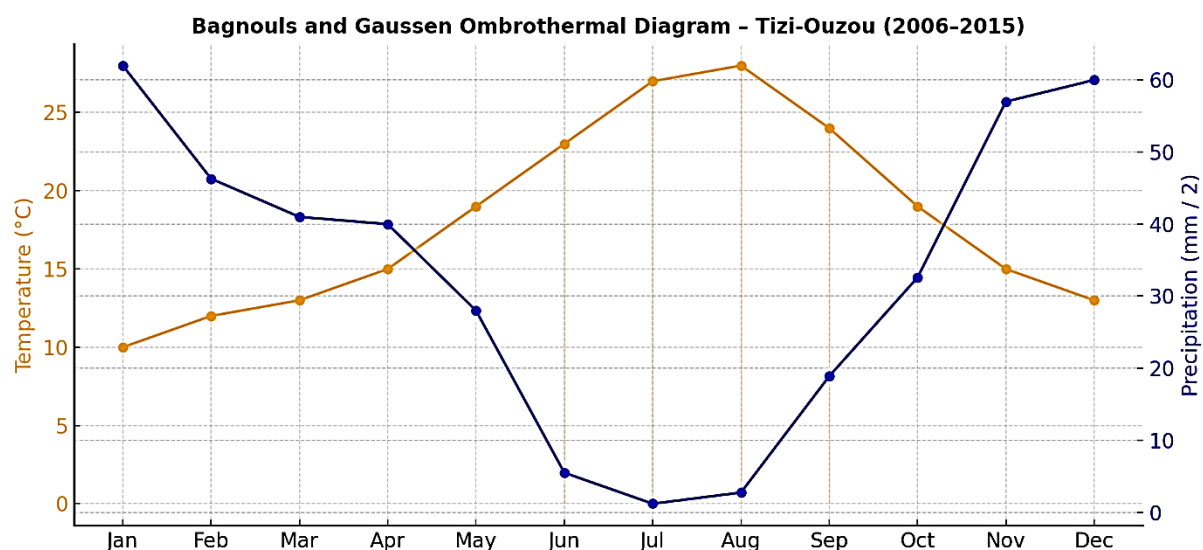


Figure 1: Bagnouls and Gaussen umbrothermal diagram for the station of Tizi-Ouzou 1 (2006-2015).

Station 2: Blida (Chr a National Park, Beni-Ali station).

Located at an altitude of 1040 m (32 26'47"N, 2 52'20"E), this station is set in a mountain forest environment. As there is no weather station nearby, the data used comes from the the station of Medea, which offers similar climatic conditions. The climate is sub-humid Mediterranean, with cool winters and an annual rainfall of around 803 mm. The data are shown in Figure 2.

A comparative bioclimatic analysis based on Emberger's rainfall-temperature quotient (Q2) revealed a difference between the two sites: Tizi-Ouzou is located in the humid zone (Q2 = 126.43), while Blida is classified as subhumid (Q2 = 101.33).

Despite all these disparate climatic characteristics, *Eucalyptus globulus* adapts well to these environments thanks to its rapid growth capacity and low water requirements. These differences in ecological conditions justify comparisons of phytophysical profiles and biological activities according to geographical origin.

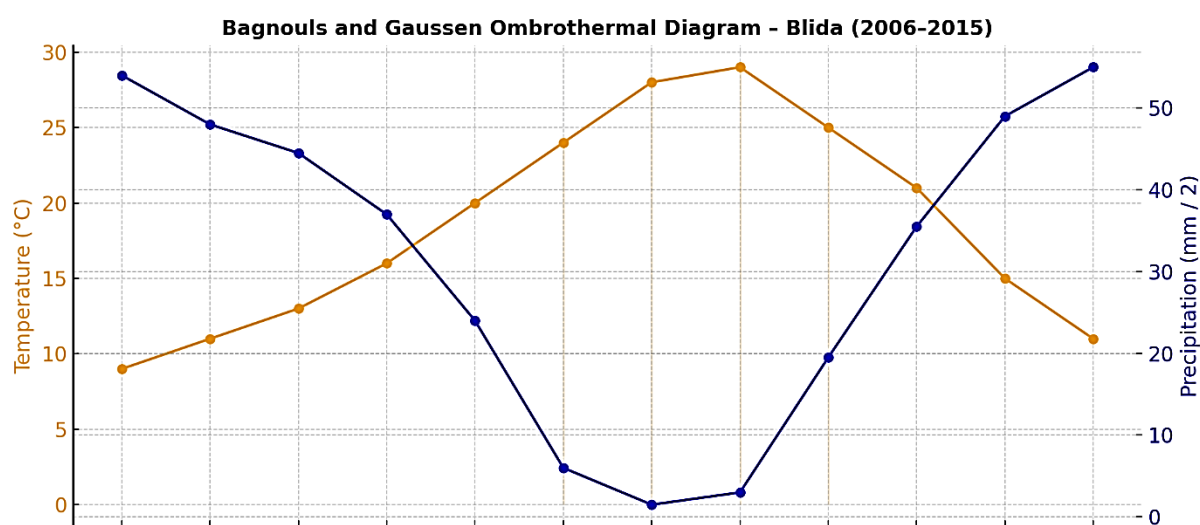


Figure 2: Bagnouls and Gaussen umbrothermal diagram for the station of Blida 2 (2006-2015).

Method

Preparation of leaves (raw material)

Fresh *Eucalyptus globulus* leaves were carefully cleaned by removing impurities, stems and dust. After rinsing with clean water, part of the plant material was dried in the open air and in the shade in order to preserve the heat-sensitive compounds.

Extraction of the essential oil

At the Z ralda educational farm (Algiers), the essential oil was extracted using hydrodistillation in partnership with Biosource DZ. This method involves boiling the plant material in water to release the volatile compounds. The vapours contain the essential oil, which is then condensed in a cooler to be separated by decantation and isolated in the oily phase.

Characterisation of the essential oil

Organoleptic (appearance, colour, odour, flavour) and physico-chemical (yield, refractive index, acidity index, pH analyses) were carried out in accordance with AFNOR standards (1986), providing an initial assessment of the quality and purity of the essential oil obtained.

Preparation of Methanolic Extract

A sample of dry leaves weighing 10 g was first coarsely ground and then macerated in 300 mL of 80% methanol, aided by magnetic stirring at room temperature and protected from light for 24 h. The resulting solution was centrifuged at 6,000 rpm for 10 to 15 min. The supernatant was collected and evaporated to dryness under reduced pressure to obtain the crude methanolic extract (CME). The extraction yield of the crude methanolic extract was determined using the following formula:

Extraction Yield (%) = (Mass of dry extract (g) / Mass of initial dry plant material (g)) × 100

This extraction procedure was performed according to the method described by Sasidharan et al. (2011) with slight modifications.

Qualitative study (phytochemical screening)

A phytochemical screening test was carried out to identify the main families of secondary metabolites. Although this is not an identification of the molecules per se, it indicates which chemical classes dominate in the plant extracts (Seladji, 2015).

Quantitative study

-Determination of total flavonoids

Flavonoids were assayed using the method of Lamaison and Carnat (1990) reported by Djeridane et al. (2006). To 1 mL of methanolic extract was added 1 mL of a 2% aluminium chloride solution (AlCl₃). After homogenisation, the mixture was incubated at room temperature for 15 minutes in the dark. Absorbance was measured at 430 nm using a UV-Vis spectrophotometer.

*Calibration curve

Quantification was carried out by extrapolation on a calibration curve established with quercetin as the standard. Solutions of quercetin were prepared at different concentrations (e.g. 0; 25; 50; 75; 100 µg/mL). The absorbance of each standard solution was measured at 430 nm. The concentration of total flavonoids was expressed as mg quercetin equivalent per gram of dry extract (mg [compound] EQ/g DE).

-Determination of total polyphenols

Total polyphenols were determined using the colorimetric method of Singleton et al. (1999). A quantity of 200 µL of methanolic extract was mixed with 1.5 mL of Folin-Ciocalteu reagent. After 5 min of rest, 1.5 mL of sodium carbonate (Na₂CO₃, 60 g/L) was added. The mixture was vortexed and then incubated at room temperature for 30 minutes. The absorbance was read at 750 nm.

*Calibration curve

Quantification was performed using a calibration curve prepared with gallic acid. Standard solutions of gallic acid were prepared at different concentrations (e.g. 0; 50; 100; 150; 200 µg/mL), and processed using the same protocol as the samples. Results are expressed as mg gallic acid equivalent per gram of dry extract (mg GAE/g DE).

Biological study

Antibacterial activity: For antibacterial evaluation, four standardised reference bacterial strains were used (Table 1).

Table 1. List and characteristics of the microbial strains tested.

Strains	Forms	Gram	Family	Main infection caused
<i>Escherichia coli</i> ATCC 25922	Bacilli	Negative	Enterobacteriaceae	-Dysenteriform diarrhoea -Gastroenteritis -Urinary tract infections
<i>Staphylococcus aureus</i> ATCC 25923	Cocci	Positive	Micrococcaceae	-Gastroenteritis -Urinary tract infections. -Osteomyelitis and arthritis
<i>Pseudomonas aeruginosa</i> ATCC 27853	Bacillus	Negative	Pseudomonadaceae	-Nosocomial infections -Surgical wound infections -Pulmonary infections
<i>Bacillus cereus</i> ATCC 6633	Bacillus	Positive	Bacillaceae	-Food poisoning

The strains were stored at $\pm 5^{\circ}\text{C}$ in the refrigerator in tubes containing slanted storage agar. We used the following culture media for these antimicrobial tests: Nutrient agar (NG): a non-selective medium for isolating and maintaining bacterial strains, for subculturing strains; and Mueller Hinton agar (MH): a microbiological growth medium that is commonly used for antibiotic and essential oil susceptibility testing of microorganisms, the aromatogram test.

Assessment of the antimicrobial activity of essential oil and methanoic extract: The antimicrobial activity of the essential oil and methanolic extract of *Eucalyptus globulus* was assessed using the disc diffusion method (or aromatogram), which is similar to the protocols for the classic antibiogram. Mueller-Hinton agar plates were prepared, poured and dried (16 Petri dishes).

The tests were performed on 4 bacterial strains: *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginos* and *Bacillus cereus*.

Plating: Mueller-Hinton agar plates (16 Petri dishes) were prepared, poured and dried. Each plate was inoculated by flooding with 2 mL of bacterial suspension standardised to 0.5 McFarland, then homogenised by circular movements. Excess suspension was removed using a sterile Pasteur pipette.

The plates were left to dry at room temperature for 10-15 minutes.

Disk deposition: Sterile Whatman filter paper disks were soaked with 5 μL of extracts (essential oil or methanolic extract, undiluted):

EO (Blida)

EO (Tizi-Ouzou)

Methanolic extract (Blida)

Methanolic extract (Tizi-Ouzou)

Three discs per plate were placed on the agar surface using sterile forceps. The discs were lightly pressed to ensure good contact. The plates were then incubated at 37°C for 24 hours, with the lid facing downwards.

Reading and interpretation of results

After incubation, antibacterial activity was determined by measuring the diameter of the zones of inhibition (in mm) around each disc. The results were interpreted according to the criteria defined by De Billerbeck (2007):

$D < 6 \text{ mm}$: Resistant

$6 \text{ mm} < D < 13 \text{ mm}$: Intermediate activity;

$D > 13 \text{ mm}$: Sensitive activity

The results were also completed by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

Assessment of antioxidant activity using the DPPH method: The antioxidant activity of essential oils and methanolic extracts was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging method, following the procedure described by Brand-Williams et al. (1995), with a few modifications.

-Preparation of the DPPH solution

A solution of DPPH was prepared at a concentration of 0.004% (40 $\mu\text{g/mL}$) in methanol. This fresh solution was stored in the dark until use.

-Sample preparation

The essential oils were tested undiluted (pure), at a rate of 5 μL per test, while the methanolic extracts were prepared at concentrations of 0.5 mg/mL to 2 mg/mL in methanol. A volume of 200 μL of sample was mixed with 1 mL of DPPH solution.

-Incubation and reading

After shaking, the tubes were incubated for 30 minutes at room temperature in the dark. Absorbance was measured at 517 nm using a UV-Visible spectrophotometer against a methanol blank. Methanol alone with DPPH was used as the control.

-Calculation of antioxidant activity

The absorbance inhibition of the DPPH radical was calculated using the following formula:

$$\text{Inhibition (\%)} = \left(\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \right) \times 100$$

A_{control}: Absorbance of the DPPH solution without sample.

A_{sample} : Absorbance of the DPPH solution with plant extract.

Vitamin C (ascorbic acid) was used as a positive reference antioxidant, tested under the same conditions at the same concentrations.

Statistical analysis

The data obtained from the different tests (extraction yield, total phenolic and flavonoid contents, antibacterial and antioxidant activities) were expressed as the mean $\pm$ standard deviation (n = 3). A comparative statistical analysis was performed to evaluate significant differences between the two study regions (Tizi-Ouzou and Blida), regardless of the type of biological activity analyzed. The student's t-test for independent samples was used to compare the means of the two groups. The significance threshold was set at $p < 0.05$, indicating a statistically significant difference.

Results and discussion

Results of analyses carried out on the two essential oils

Evaluation of organoleptic properties

The organoleptic parameters are summarised in Table 2.

Table 2. Organoleptic characteristics of *Eucalyptus globulus* essential oil.

	Appearance	Colour	Odour
AFNOR, 2000 (EO <i>E. globulus</i>)	Clear liquid, Fluid and mobile	Very pale yellow to Transparent	Fresh and spicy
Azeffoun essential oil (Tizi-Ouzou)	Clear fluid	Very pale yellow to Transparent	Fresh and pleasant
Beni-Ali essential oil (Blida)	Clear liquid	Pale yellow to Transparent	Spicy

The organoleptic characteristics (colour, odour, appearance, etc.) of the essential oils of *Eucalyptus globulus* collected in the regions of Blida and Tizi-Ouzou which were subjected to statistical analysis using Student's t-test. The result obtained was $p = 0.03$, which is below the significance level of 0.05. This difference is statistically significant, suggesting that the organoleptic characteristics of the oils differ significantly between the two regions. These differences can be attributed to exogenous factors such as altitude, climatic conditions, soil composition and the time of harvest, which influence the sensory quality and chemical composition of the essential oil. In this way, the organoleptic profile of the oils could reflect the specific environmental characteristics of each harvesting area, which could also be relevant to potential industrial or therapeutic uses in the region.

Assessment of physico-chemical properties

•Refractive Index

$t' = 22.1^{\circ}\text{C}$ for the essential oil from Azeffoun (Tizi-Ouzou), with $I = 1.461$

$t' = 24.1^{\circ}\text{C}$ for the essential oil from Beni-Ali (Blida), with $I = 1.475$

•Acid Value

0.05 mL = volume of K_{OH} used for Azeffoun (Tizi-Ouzou)

0.1 mL = volume of K_{OH} used for Beni-Ali (Blida)

56.1 = molar mass of K_{OH}

0.27 g = mass of oil from Azeffoun (Tizi-Ouzou)

0.2 g = mass of oil from Beni-Ali (Blida)

0.1 = molarity of K_{OH}

The acid value is calculated using the formula:

$$AV = \frac{56.1 \times 0.1 \times V_{K_{OH}}}{m(EO)} \quad AV = \frac{m(EO)}{56.1 \times 0.1 \times V_{K_{OH}}}$$

It was found that the extraction yield of *Eucalyptus globulus* essential oil from the Tizi-Ouzou region is 0.23%, while the yield from the Blida region is 0.18% (Table 3).

Table 3. Summary table of the physicochemical characteristics of the essential oil of *Eucalyptus globulus*.

Property	Azeffoun essential oil	Beni-Ali essential oil	Reference (AFNOR 2000)
Yield	0.23%	0.18%	/
Ph	4	4	4-6
Refractive index	1.462	1.476	1.460- 1.476
Rotatory power	0	0	(-5 ; +2)
Acidity value	1.03	2.80	0,84-3,74

The organoleptic characteristics of the essential oils extracted from the leaves of *Eucalyptus globulus* in the Blida and Tizi-Ouzou regions are markedly different, particularly from the chromatic and olfactory points of view. This disparity is the result of numerous exogenous and endogenous factors that have an impact on their composition and yield (Garnéro, 1991; Bruneton, 1999).

Endogenous factors include the vegetative stage, the plant organ distilled, hybridisation, genetic mutations, polyploidy and chemical polymorphism (Belyagoubi, 2006; Aprotosoaie et al., 2010). Exogenous factors include environmental conditions such as temperature, humidity, light, rainfall, soil type (edaphic conditions), geographical origin, harvest date, cultivation techniques, extraction method and plant material storage conditions (Mohammedi, 2006; Pinto et al., 2006; Olle and Bender, 2010).

The physico-chemical data recorded for the two essential oils (Table 3), on the whole, correspond well to the reference values set by AFNOR standards.

The acidity index, which is an indicator of the degradation of the oils during storage, was evaluated at less than 2 for both samples, reflecting good conservation and therefore a low level of free acids (Boukhatem, 2014). The refractive index (purity indicator) was within the norms; low values of this indicator are favourable for use in the cosmetic sector, as this indicates low light scattering (Boukhatem, 2014).

Therefore, because the organoleptic properties combined with the results of the physico-chemical analyses indicate that the essential oils produced in the two areas are of good quality, and that they have been well preserved, they can be used for pharmaceutical or cosmetic purposes.

Assessment of biological properties

Antibacterial activity

The diameters of the zones of inhibition of our two essential oils are summarised in Table 4.

Table 4. Diameters of the zones of inhibition of *Eucalyptus globulus* essential oils.

Bacterial strain	Diameters of inhibition zones in mm		Result	
	EO from Azeffoun (EO _A)	EO from Beni-Ali (EO _B)	EO _A	EO _B
<i>Escherichia coli</i>	26 mm	13 mm	Sensitive	Sensitive
<i>Staphylococcus aureus</i>	25 mm	15 mm	Sensitive	Sensitive
<i>Pseudomonas aeruginosa</i>	7 mm	8 mm	Intermediate	Intermediate
<i>Bacillus cereus</i>	17 mm	21 mm	Sensitive	Sensitive

The results of the inhibition zone measurements are shown in Table 8. These results were used to assess the antibacterial activity of essential oils extracted from *Eucalyptus globulus* in the Tizi-Ouzou and Blida regions. Each test used a single concentration (5 µL) of pure (undiluted) essential oil on a Wattman paper disc.

Statistical analysis of antibacterial activity measured by inhibition diameters showed a highly significant difference between extracts from the two regions ($p = 0.002 < 0.01$). These results show that antibacterial efficacy might highly depend on the geographical origin of the plant material and the different qualitative and quantitative variations in the bioactive compounds.

However, the essential oil from the region of Tizi-Ouzou showed strongly marked inhibitory activity against *Escherichia coli* (26 mm) and *Staphylococcus aureus* (25 mm), moderate activity against *Bacillus cereus* (17 mm) and low activity against *Pseudomonas aeruginosa* (7 mm), which is a good result. With the exception of *Pseudomonas aeruginosa*, Gram-positive strains showed good activity of this essential oil, which is in line with data in the literature (Traoré et al., 2013; Elaissi et al., 2011).

The essential oil extracted from Blida clearly showed good inhibitory power on *Bacillus cereus* (21 mm), moderate power on *Staphylococcus aureus* (15 mm) and *Escherichia coli* (13 mm), and was weak on *Pseudomonas aeruginosa* (8 mm).

The antimicrobial activity observed could be attributed to the chemistry of the oils, in particular to the presence of 1,8-cineole, aromadendrene, globulol, and limonene. Aromadendrene, which is lipophilic and interacts with membrane proteins (Mulyaningsih et al., 2010), is thought to be involved in the bactericidal action observed in synergistic interaction with 1,8-cineole. Differences in activity on a regional scale could be explained by variations in composition due to ecological factors (climate, altitude, soil), genetic factors (chemical polymorphism) and technical factors (extraction method, condition of plant material, etc.), as reported by Bruneton (1999) and Akin et al. (2010).

Antioxidant activity results

The methods used to measure the antioxidant activity of essential oils are generally based on the ability of an antioxidant to inhibit the discoloration of a specific reagent, such as DPPH (2,2-diphenyl-1-picrylhydrazyl), and the decrease in absorbance of which is measured spectrophotometrically. The essential oil of *Eucalyptus globulus* exhibited moderate antioxidant activity, clearly lower than that of vitamin C, which is known for its strong free radical scavenging capacity. The essential oil from Tizi-Ouzou appeared slightly more active than that of Blida. This relatively low activity may be attributed to the chemical nature of the dominant compounds, particularly hydrocarbon monoterpenes. According to Martins et al. (2014), monoterpenes such as α -pinene, β -pinene, limonene, β -myrcene, sabinene, and terpinolene possess antioxidant properties, although their action depends on the DPPH free radical neutralization mechanism. Other studies (Ruberto & Baratta, 2000) have shown that some of these monoterpenes are only weakly active, depending on the reagent and method used. However, as essential oils are complex mixtures, their biological activity generally results from the synergistic action of several compounds. Olayinka et al. (2012) reported that terpinene derivatives show moderate activity, while p-cymene and pinenes are not very active. On the other hand, certain phenolic compounds such as thymol, carvacrol, and to a certain extent 1,8-cineole, are known for their high antioxidant potential.

The antioxidant properties observed in the oils may therefore be linked to the presence of hydroxyl functions in polyphenols and flavonoids, which are strong electron donors. These substances are recognized for their ability to neutralize free radicals, supporting the idea that essential oils can serve as natural antioxidants, particularly in food preservation (Bruneton, 1999; Richard and Peyron, 1992).

Evaluation of methanoic extracts

Qualitative study (phytochemical screening)

Preliminary assessment of the phytochemical composition of *Eucalyptus globulus* leaf extracts revealed the presence of a number of chemical families represented in Table 5.

Table 5. Results of phytochemical screening carried out on the methanoic extract of *Eucalyptus globulus* leaves.

	Metabolism	Tizi-Ouzou aqueous extract	Blida aqueous extract
Secondary metabolism	Alkaloids	+++	+++
	Anthraquinones	+++	-
	Tannins	+++ (gallic)	+++ (gallic)
	Polyphenols	+++	+++
	Flavonoids	+++	+++
	Saponosides	+++	+++
Additional test	Reducing compounds	+++	+++
	Anthocyanins	++	--
	Cardiotonic glycosides	+++	--
	Organic acids	-	+++

(+++) Strongly positive. (++) Moderately positive. (-) Absent.

Phytochemical screening of *Eucalyptus globulus* reveals:

-A remarkable presence of secondary metabolites for the Tizi-Ouzou methanoic extract, namely alkaloids, anthraquinones, tannins, polyphenols, flavonoids, saponosides, a presence of reducing compounds, cardiotonic glycosides, and a moderate presence of Anthocyanins and a total absence of organic acids.

-A very remarkable presence of secondary metabolites for methanoic extracted from the region of Blida, namely alkaloids, tannins, polyphenols, flavonoids, saponosides, a presence of reducing compounds, organic acids, and a total absence of cardiotonic glycosides, anthocyanins and anthraquinones.

Quantitative study

The total polyphenol and flavonoid content are shown in Table 6. The choice of assaying this family of bioactive chemical substances is justified by their antioxidant and antimicrobial effects (Li et al., 2003).

Table 6. Total polyphenol and flavonoid content of *Eucalyptus globulus* extract.

Methanolic extract	Total polyphenols in mg/g Mv	Total flavonoids in mg/g Mv
Blida sample	11.07	8.72
Tizi-Ouzou sample	10.45	6.68

Flavonoids: The flavonoid assay results shown in Table 7 for our two plant extracts from Tizi-Ouzou (Azeffoun (A)) and Blida (Beni-Ali (B)) show that the highest value is for the methanolic extract from Blida, which is 8.72 mg/g Mv and 6.68 mg/g Mv for the Tizi-Ouzou sample.

Table 7. Summary of flavonoid levels in the two aqueous extracts from the two regions.

Sample	Abs	[c] in mg/g Mv
A ₁	1.994	6.75
A ₂	1.992	6.74
A ₃	2.07	6.55
A = 6.68 ± 0.11 ; B = 8.72 ± 0.15		
B ₁	2.570	8.83
B ₂	2.490	8.54
B ₃	2.560	8.80

No work has been published on the quantitative aspect of flavonoids in the *Eucalyptus globulus* plant, although several investigations have been based on the characterisation of these compounds from leaf and bark extracts (et al., 2006). The most abundant flavonoids in the leaves are essentially aglycone flavonols (quercetin, myricetin and kaempferol) and glycosides such as quercetin derivatives (quercetin-3-glucoside, quercetin-3-rhamnoside), kaempferol (kaempferol-3-rhamnoside, kaempferol-3-rutinoside) and myricetin (myricetin-3-glucoside, myricetin-3-rutinoside) (Cadahía et al., 1997). However, flavonoids were not detected in the fruit (Yang et al., 2004; Guo and Yang, 2006). This large difference could be explained by the region in which the plant is grown, the assay method, the sensitivity and purity of the reagents used and the harvesting period (Zrira et al., 1994). The t-test also showed a significant difference between the two regions in terms of total flavonoid content ($p = 0.04 < 0.05$). This result suggests that flavonoid accumulation (due to climatic influences and environmental parameters such as humidity, temperature and light) differs from region to region. Several factors can have an impact on the qualitative and quantitative distribution of phenolic compounds in the fractions, in particular climatic and environmental factors, the geographical area (drought, soil), the harvesting period, the development stage, the part of the plant used, the species, as well as the extraction and quantification method (Miliauskas et al., 2004; Ebrahimi et al., 2008). The choice of solvent used can also influence the polyphenol and flavonoid content (Lee et al., 2003).

Total polyphenols: The polyphenol levels for our two plant extracts from Azeffoun (A) and Beni-Ali (B) are shown in table 8.

Table 8: Summary of polyphenol levels in the two aqueous extracts from the two regions.

Sample	Abs	[c] in mg/g Mv
A ₁	2.80	10.25
A ₂	2.89	10.58
A ₃	2.88	10.54
A = 10.45 ± 0.18 ; B = 11.07 ± 0.046		
B ₁	3.02	11.05
B ₂	3.02	11.05
B ₃	3.04	11.13

From the total polyphenol content values summarised in Table 8, it can be seen that the methanolic extract from the Blida sample has a higher content of 11.07 mg/g Mv compared with that of the methanolic extract from Tizi-Ouzou, which is 10.45 mg/g Mv.

The results show that the difference between the two regions is highly significant in terms of total phenolic compound levels in the methanolic extracts ($p = 0.037 < 0.05$). This difference could be due to the impact of climate and soil on the biosynthesis of secondary metabolites. Plants from Blida contained more phenolic compounds.

Zin et al (2006) reported that polyphenols are found in different parts of the plant, such as the bark, leaves, stems, fruit, pollen grains, roots, seeds and flowers. Moreover, the distribution of these compounds within the same plant varies from one organ to another. Depending on the physiological stages of their development (Macheix et al., 2005), the variability in the distribution of phenolic compounds in the three parts of *E. globulus* may also be due to the regulation of their synthesis and accumulation, which are influenced by environmental factors like climate and soil (Close et al., 2004; Pereira et al., 2005), as well as by the effect of the season (Salminen et al., 1999; Salminen et al., 2004). Bututhisakul et al. (2008) reported that extracts with a high yield do not necessarily have the highest polyphenol content.

Biological study

Antioxidant activity

The antioxidant activity of the extract was assessed in vitro using the DPPH free radical reduction method. In the presence of anti-free radical compounds, the DPPH radical is reduced and changes colour to yellow, resulting in a decrease in its absorbance (Table 9).

Table 9: Percentage reduction of the DPPH free radical in the two aqueous extracts.

Compounds: plant solution (hydro-methanolic extract)	Test wavelength 515 nm	Abs of DPPH = 0.911
Abs A ₁	0.120	86.82 %
Abs A ₂	0.116	87.26 %
Abs A ₃	0.114	87.48 %
AA% Azeffoun = 87.18 ± 0.33 ; AA% Blida = 88.98 ± 0.16		
Abs B ₁	0.099	89.13%
Abs B ₂	0.102	88.80 %
Abs B ₃	0.100	

It can be seen that the two methanoic extracts studied have antioxidant activity that varies from one sample to another and are capable of trapping the DPPH radical. Both extracts showed anti-free radical activity towards the DPPH radical. For the same concentration of methanolic extract, the inhibition percentage was 88.98% and 87.18% for the Blida and Tizi-Ouzou samples respectively. It can therefore be seen that the methanolic extract from Blida has the greatest inhibitory power with respect to the DPPH radical compared with that from Tizi-Ouzou. Our samples show less inhibition compared with ascorbic acid, which is the standard antioxidant used to measure antioxidant activity, due to its ability to release hydrogen molecules (Bougandoura and Bendimerad, 2013). These results suggest that *E. globulus* extract contains free radical scavengers acting as primary antioxidants. The action of these antioxidants is thought to be due to their ability to donate hydrogen atoms or electrons derived mainly from the hydroxyl of the A ring of flavonoids (Lee et al., 2007). This difference can be justified by the total polyphenol concentration as outlined in Table 9. The high antioxidant activity of the extracts from the samples analysed is due to their high total polyphenol and flavonoid content. Ghedadba et al (2014) demonstrated that the biological mechanisms of polyphenolic compounds have been attributed to their antioxidant properties through several possible mechanisms, such as their ability to scavenge free radicals, break free radical chain reactions by directly reducing peroxides, and stimulate enzymatic activities of antioxidant defense (Figure. 3).

Antibacterial activity

We studied the in vitro antimicrobial activity of methanoic extracts from the two regions of *Eucalyptus globulus* leaves using the disc diffusion method on a solid agar medium, Mueller-Hinton for bacteria. The antimicrobial activity of the extracts was estimated in terms of the diameter of the zone of inhibition around the discs containing the extracts to be tested against two bacteria (Table 10).

Figure 3: Antioxidant activity of methanoic extracts and *Eucalyptus globulus* EO.

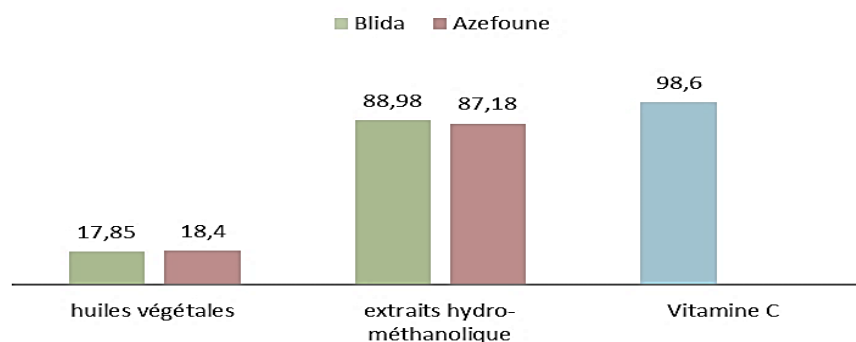


Figure 3: Antioxidant activity of methanoic extracts and *Eucalyptus globulus* EO.

Table 10. Diameters of the inhibition zones of the methanoic extract.

Bacterial strain	Diameters of inhibition zones in mm		Result	
	Azeffoun	Beni-Ali		
<i>Escherichia coli</i>	14 mm	9 mm	Sensitive	Intermediate
<i>Staphylococcus aureus</i>	13 mm	9.5 mm	Intermediate	Intermediate
<i>Pseudomonas aeruginosa</i>	11.4 mm	12.6 mm	Intermediate	Intermediate
<i>Bacillus cereus</i>	11 mm	15.6 mm	Intermediate	Sensitive

There is a significant difference between the two extracts in the antimicrobial power of the methanoic extract from Tizi-Ouzou compared with the extract from Blida for the *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* strains. But for the *Bacillus cereus* strain, there is a larger zone of inhibition in the Blida extract compared with the Tizi-Ouzou extract. The diameter of the zone of inhibition differs from one bacterial strain to another and from one extract to another. The variation in the antibacterial activity of the extracts is explained by variations in their chemical composition. Flavonoids are known for their antibacterial properties. Indeed, they are capable of inhibiting the growth of various types of *Escherichia coli* bacteria (Ulanowska et al., 2007). This is confirmed by the presence of a higher level of flavonoids in the methanoic extract from Blida than in the extract from Tizi-Ouzou. The method used to assess antibacterial activity also influences the results (Natarajan et al., 2005). They are in agreement with several studies on medicinal plant extracts, where Gram-negative bacteria show greater resistance to plant extracts than Gram-positive bacteria (Arias et al., 2004; Khan et al., 2009; Oliviera et al., 2008). This difference is linked to the presence of an envelope comprising a cell membrane rich in lipopolysaccharides and a cell wall, which limits the access of antimicrobial agents to their target in bacterial cells, since the antimicrobial agents are in contact with the cell envelope. Unlike Gram-positive bacteria, which are unprotected against external agents (Oumaskour et al., 2012).

Conclusion

Medicinal plants are a reliable and valuable source of active ingredients, recognised for their therapeutic and biological properties. They are widely used in the agri-food, pharmaceutical, medical and cosmetic sectors. It is in this context that the boom in essential oils and plant extracts over the last decade testifies to the scientific and industrial interest in this natural resource.

In this work, we studied the physicochemical and biological properties of the essential oil and methanolic extracted from the leaves of *Eucalyptus globulus*, and harvested in two regions of northern Algeria, Tizi-Ouzou and Blida. The aim was to study the influence of climatic conditions on the biochemical composition and biological activity of this medicinal species. The physicochemical analyses of the essential oils obtained by industrial hydrodistillation showed that the parameters complied with AFNOR (2000) standards for refractive index, pH and acidity index at the two sampling sites.

Similarly, to assess antibacterial activity in vitro, in four reference strains (*Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Pseudomonas aeruginosa*), an inhibitory effect was demonstrated significantly according to the orientation of the first two strains, reserving for the two regions a different antibacterial effect on the same spectrum of resistance; and even between the two regions, significantly established differences unambiguously suggesting the influence of the environment on the antimicrobial efficacy of essential oil.

In terms of antioxidant activity, the results obtained using the DPPH radical scavenging method also revealed significant variation between the two geographical origins.

The essential oil from Tizi-Ouzou has a better activity than that from Blida. This variability may be due to the different composition of monoterpenes and volatile phenolic compounds. On the other hand, the possible qualitative analysis presented at the phytochemical screening stage of the methanolic extracts was fruitful in confirming the richness of secondary metabolites in both extracts. Quantitative analysis revealed a higher content of total phenolic compounds and flavonoids in the extract taken from Blida, corroborating good antioxidant activity, while the Tizi-Ouzou extract was modestly less active. The antibacterial activity of the methanolic extracts was significantly better against Gram-negative strains, showing a significant difference in inhibition of the exposed extracts between the two sites.

In conclusion, the results suggest that climatic factors have a considerable influence on the chemical composition and biological activities of *Eucalyptus globulus*, and that the essential oil and methanolic extract of this antioxidant- and antimicrobial-rich species are promising alternative sources of natural preservatives for use in the food industry.

Author's Contributions

Author 1: Data analysis, drafting the article.

Author 2: Data analysis, drafting the article.

Author 3: Drafting the article.

Author 4: Data analysis.

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