Research Article

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Wild *Thymus zygis* L. ssp. *gracilis* and *Eucalyptus camaldulensis* Dehnh.: Chemical composition, antioxidant and antibacterial activities of essential oils

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Abstract: Natural substances extracted from plants have been increasingly studied and recognized, recently. Essential oils (EOs), for example, possess antioxidant and antibacterial properties, enabling their application across different sectors like agro-food, pharmaceuticals, and cosmetics. In Morocco, exceptional plant diversity, mirroring the diversity of ecosystems, has not yet revealed all its secrets. Therefore, the aim of this study is to determine the chemical composition and evaluate the antibacterial and antioxidant activities of EOs from *Thymus zygis* L. ssp. *gracilis* and *Eucalyptus camaldulensis* Dehnh. collected in the El Hoceima and Mamora regions, respectively. The EOs were extracted by hydrodistillation employing a Clevenger-type apparatus. Gas chromatography/mass spectrometry (GC/MS) analyses identified 54 constituents representing 92.65% of the total for *T. zygis* and 55 components representing 99.60% for *E. camaldulensis*. The primary components found in the EO of *T. zygis* are δ-terpineol (27.64%), followed by δ-3-carene (15.7%), thymol (14.17%), and dehydrolinalool (4.99%). The main compounds in *E. camaldulensis* EO are 1,8-cineole (43.61%), γ-terpinene (11.71%), α-terpineol (10.58%), and *p*-cymene (4.93%). The antioxidant properties of these oils were investigated by utilization of the 2,2-diphenyl-1-picrylhydrazyl method and the ferric reducing antioxidant power (FRAP) test. The antibacterial activity was assessed against two Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram-negative bacteria (*Micrococcus luteus* and *Escherichia coli*). Both EOs showed significant antioxidant activity but were less effective than reference antioxidants quercetin and catechin. Antibacterial studies demonstrated strong activity of *T. zygis* and *E. camaldulensis* EOs against the

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studied bacteria, as well as good inhibitory properties (minimum inhibitory concentration).

Keywords: chemical composition, antioxidant activity, DPPH, FRAP, antibacterial activity, *Eucalyptus camaldulensis* Dehnh., *Thymus zygis* L. ssp. *gracilis*, essential oil

1 Introduction

Today, concerns regarding public health issues related to foodborne diseases are significant on a global scale. The primary cause for the development of these diseases is the deterioration of food caused by microbial contamination and oxidation of foodstuffs [1]. Presently, the food industries use synthetic preservatives to hinder the oxidation and microbial contamination of packaged food products. Nevertheless, there is a current trend in these industries toward embracing natural antioxidant and antimicrobial compounds, plant extracts, as substitutes for synthetic preservatives. This shift is driven by the adverse impacts of synthetic preservatives on both the human health and the environment [2].

Essential oils (EOs) contain secondary plant metabolites. Distinguished by their strong odor and complex volatile composition, they act as chemical signals controlling a plant's environment [3]. These EOs, comprising a blend of volatile compounds, exhibit a wide range of properties, including antifungal, antibacterial, antimicrobial, and antioxidant activities [4–6]. Due to these biological characteristics, proposals to use for food preservation have been suggested, whether by incorporating them directly into foods or in packaging materials [7]. In Morocco, various plant species, including *Thymus zygis* L. ssp. *gracilis* and *Eucalyptus camaldulensis* Dehnh., have been the subject of extensive chemical and pharmacological studies.

T. zygis is a perennial plant of the Lamiaceae family, known in Morocco as "Zaâitra, whose natural range is restricted to Morocco and the Iberian Peninsula," primarily found in the Mediterranean region, North Africa, Asia, and southern Europe. In Morocco, it is mainly localized in the regions of High Atlas, Anti-Atlas, Middle Atlas, and Middle Atlantic. It is commonly used to treat respiratory infections, colds, acute bronchial conditions, as a food preservative, and in traditional medicine [8,9]. Studies have shown that it improves the sensory properties of milk, reducing lipid oxidation, and increases its nutritional value, including proteins, lipids, dry matter, polyunsaturated fatty acids, as well as cheese [10]. The EOs of this plant are widely used as antiseptic agents in various pharmaceutical fields due to their antioxidant, antimicrobial, anti-inflammatory, antiseptic, anticoagulant, and antispasmodic properties [11]. These activities are attributed to phenolic compounds, particularly thymol, and alcoholic compounds such as δ-terpineol [12].

E. camaldulensis Dehnh. originates from continental Australia and is a member of the Myrtaceae family [13]. It is widely cultivated worldwide and is in the process of naturalization in Morocco [8]. The trees can reach impressive heights, with some exceeding 100 m; the typical height for the most prevalent species is between 40 and 50 m, while others of this genus are more modest in size. Eucalyptus leaves are entire, leathery, and possess a distinct cuticle; they are persistent and emit an aromatic fragrance. Essences of the Eucalyptus genus are acknowledged as significant repositories of secondary metabolites, several of which demonstrate diverse biological activities. Eucalyptus leaves are used as antispasmodics and antipyretics, frequently employed to alleviate respiratory ailments [14,15]. EOs obtained from Eucalyptus have long been used in pharmacy for the production of antiseptics [16]. The chemical analysis of its EOs has revealed the presence of various compounds, such as 1,8-cineole, γ-terpinene, α-terpineol, and *p*-cymene. These compounds indicate that the EOs may have antibacterial, antioxidant, anti-inflammatory, and analgesic properties [17–20].

In this context, the aim of this study is to analyze the chemical composition of the EOs from *T. zygis* and *E. camaldulensis*, as well as to assess their antioxidant activity by scavenging the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical and the ferric reducing antioxidant power (FRAP). Additionally, the study aims to assess their antibacterial potency against four pathogenic strains, namely *Escherichia coli, Micrococcus luteus*, *Bacillus subtilis,* and *Staphylococcus aureus*. This is part of a broader effort to explore active natural substances for food preservation, targeting oxidative processes, microbial contamination, and prolonging the freshness of food products.

2 Materials and methods

2.1 Plant material

Samples of aerial parts of *T. zygis* and *E. camaldulensis* were collected in June 2021 from the El Hoceima region (Northern Morocco: latitude 35°08′09.8"N; longitude 4°05′ 10.7"W) and Mamora (Northwest Morocco: latitude 34°16′ 16.0"N; longitude 6°25′28.1"W), respectively. Species identification was done at the Scientific Institute of Rabat (Morocco) by Dr. Mohammed Sghir Taleb (a Research

Professor at the Scientific Institute. His research interests are botany, plant ecology, aromatic, and socioeconomy).

2.2 Extraction of EOs

The aerial parts of *T. zygis* and *E. camaldulensis* were subjected to hydrodistillation using a Clevenger-type apparatus [21]. Three distillations were carried by boiling 200 g of plant material with 2 L of distilled water for 3 h each. The resulting EOs were transferred into securely sealed glass bottles and stored at a temperature of 4°C until they were needed for subsequent use. The yields of EO from the samples were determined using the following formula proposed by Marion et al. [22].

$$\text{Yield\%} = (\text{weight of EO obtained by distillation(g)}) / (\text{weight of dry biomass(g)}) \times 100.$$

All experiments were conducted in triplicate.

2.3 Gas chromatography/mass spectrometry (GC/MS) analysis

The EOs were subject to gas chromatographic analysis using a Hewlett-Packard gas chromatograph (HP 6890) equipped with an HP-5 capillary column (30 m × 0.25 mm, film thickness: 0.25 µm), an FID detector, and a fixed injector at 275°C. The oven temperature was set to 50°C for 5 min and then ramped up to 250°C at a rate of 4°C/min. Nitrogen gas was used as the carrier gas at a flow rate of 1.8 mL/min, with a split ratio of 1/50 and a flow rate of 72.1 mL/min. Samples were diluted 1/50 in methanol, and manual injection was performed with a volume of 1 µL.

The chemical composition was determined through GC/MS, conducted on a Hewlett-Packard gas chromatograph (HP 6890) linked to a mass spectrometer (HP 5973). The column employed was a capillary column filled with HP-5MS (5% phenyl methyl siloxane) (30 m × 0.25 mm, film thickness: 0.25 µm). The column temperature was held at 50°C, and the water temperature was set to 0.5°C. The temperature was then increased to 250°C at a rate of 2°C/min. Helium was used as the carrier gas at a flow rate of 1.5 mL/min, and a fraction of the sample was introduced into the system. The split ratio was 1/74.7, with a flow rate of 112 mL/min. Identification of components through MS was confirmed using the NIST 98 spectral library. MS parameters included an ionization voltage of 70 eV, an ion source temperature of 230°C, and a mass scan range of 35–450 m/z. Component identification was further validated by comparing their elution order with their reported retention indices in the literature.

3 Antioxidant activity

3.1 DPPH radical scavenging activity

The trapping capacity of the DPPH radical was assessed using the standard method as reported by Lopes-Lutz et al. [23]. In this method, 1 mL of each methanolic solution of the tested EOs at various concentrations (62.5–1,000 µg/mL) was mixed with 1 mL of a methanolic solution of DPPH (0.3 mM). The mixture was vortexed and then incubated in the dark for 30 min. Absorbance was measured using a spectrophotometer at 517 nm. A positive control was represented by a solution of a standard antioxidant, quercetin, whose absorbance was measured under the same conditions as the samples. The obtained data were used to determine the sample concentration required to trap 50% of DPPH-free radicals (IC50), by plotting the percentage of inhibition against the sample concentrations.

The antioxidant activity was evaluated using the following equation:

$$\text{AA\%} = ([\text{Abs control} - \text{Abs test}]/\text{Abs control}) \times 100,$$

where AA is the antioxidant activity; Abs is the absorbance at 517 nm.

3.2 FRAP assay

This assay is based on the reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}). The reducing capacity of various EOs was determined following the method established by Oyaizu [24]. Various concentrations of samples and the positive control (catechin) were prepared. In each test tube, 1 mL of each sample (1.5–20 µg/mL), 2.5 mL of phosphate buffer (0.2 M, pH = 6.6), and 2.5 mL of potassium ferricyanide complex (1% w/v) ($K_3Fe(CN)_6$) were added. The mixture was incubated in a water bath at 50°C for 20 min. After incubation, 2.5 mL of trichloroacetic acid (10% w/v) was added to stop the reaction. Then, 2.5 mL of the supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of ferric chloride (0.1% w/v) ($FeCl_3$). Absorbance was measured at a wavelength of 700 nm using a spectrophotometer. EC50 represents the concentration value of the oil giving an absorbance of 0.5. All experiments were repeated three times.

3.3 Organisms tested

Bacterial strains *E. coli* (ATCC 8739), *M. luteus* (ATCC 9341), *S. aureus* (ATCC 6538), and *B. subtilis* (ATCC 6633) were

obtained from the Microbiology Laboratory Collection at the Center for Innovation, Research, and Training in Rabat, Morocco. The strains were inoculated from a mother culture maintained on agar at 4°C, onto nutrient agar plates and incubated at 37°C for 24 h.

3.4 Antibacterial activity of EOs

The antibacterial activity of EOs was evaluated by the disc diffusion method, recognized for its reliability and reproducibility. This method involves placing a sterile disc, previously impregnated with EO, on a growing bacterial lawn and measuring the area where bacteria cannot grow; this is indicated by the diameter value of the inhibition zone, signifying the antibacterial activity of the EOs.

To accomplish this, 15 mL of TSB (tryptic soy broth) agar medium was poured into each Petri dish, and 100 µL of bacterial suspension with a density equivalent to 0.5 McFarland standard (108 CFU/mL) was deposited. Sterile filter paper discs of 6 mm diameter were impregnated with 5 µL of EO and placed on the inoculated Petri dishes. Additionally, ampicillin (100 µg/mL), penicillin (100 µg/mL), and tetracycline (300 µg/mL) were employed as positive reference standards to ascertain the susceptibility of the tested strains. The Petri dishes were then incubated at 37°C for 24 h. Following incubation, the diameter of the inhibition zone was measured in millimeters. All experiments were conducted in triplicate [25].

3.5 Determining the minimum inhibitory and bactericidal concentrations

The minimum inhibitory concentration (MIC) of EOs was assessed through the microdilution method using 96-well microplates [26]. It corresponds to the lowest concentration of the EO that entirely inhibits the visible growth of the tested microorganism following incubation. Accordingly, from stock solutions of EOs, various dilutions of each EOs were prepared in TSB medium to achieve a final volume of 50 µL for each well. Then, 50 µL of microbial inoculum with a concentration of 10^8 CFU/mL (equivalent to 0.5 McFarland) was added to a series of dilution concentrations. After 24 h of incubation at 37°C, 10 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was added to each well as an indicator of bacterial growth. After another incubation at 37°C for 30 min, microbial growth was revealed by a change in the color from yellow to violet. Minimum bactericidal concentrations (MBCs) were determined by streaking negative wells on TSB agar. The MBC value represents the concentration of the EO at which no growth is observed upon subculture after incubation at 37°C for 24 h [27].

3.6 Statistical analysis

Statistical analysis was carried out using GraphPad Prism version 8.0 for Windows (Graphpad Software Inc., San Diego, California, USA). The data presented are expressed as mean ± standard deviation derived from three independent extractions. A one-way analysis of variance (ANOVA) was performed to evaluate the data, followed by Tukey's test for determining differences in mean values, with the significance level set as $p \leq 0.05$.

4 Results and discussion

4.1 EO yields

The yield of EOs extracted from *T. zygis* and *E. camaldulensis* through hydrodistillation is 0.57 and 3.18% EOs, respectively. Different yields have been obtained for *T. zygis* EOs in previous studies: in the Meknes region in Northeast Morocco, the yield is 1.3% [28]; in Spain, the yield ranges from 0.4 to 0.8% [29]; while it is higher in Khénifra in the Middle Atlas (Morocco) at approximately 3.87% [30]. Similarly, for *E. camaldulensis*, the EO yield varies from one region to another: in Iran, it is 2.10% [24]; in Egypt, it is only 0.9% [31]; and it is even lower in Italy, at 0.31% [32].

4.2 Chemical composition of EOs

Chromatographic analyses using GC and GC/MS identified 54 compounds in the EO of *T. zygis* (Table 1), accounting for 99.65% of the total composition. The monoterpene fraction was predominant (94.18%) compared to the sesquiterpene one (5.47%). It exhibited a high content of δ-terpineol (27.64%), followed by δ-3-carene (15.7%), thymol (14.17%), dehydrolinalool (4.99%), *trans*-carvone oxide (4.13%), and α-pinene (3.98%) as major constituents. The EOs of *T. zygis* from the Rif region of Morocco (El Hoceima) are notably distinct from other previously studied sources. For instance, the EOs of *T. zygis* from Ifrane to Tigrigra (Middle Atlas) are characterized by predominantly thymol (1.8–47.10%), *p*-cymene (14.8–19%), carvacrol (12–57.5%), and γ-terpinene (3.1–11.90%) [33]. In Serbia, the EO of this species is marked by high concentrations of thymol (35%) and *p*-cymene (24.1%) [34]. Conversely, the EOs of *T. zygis* from Spain are

Table 1: Chemical composition of EOs of *T. zygis* and *E. camaldulensis*

No	Compounds	RI	Area %	
			Thymus zygis* L. ssp. *gracilis	***E. camaldulensis* Dehnh.**
1	Heptanal	901	0.14	—
2	Tricyclene	921	1.45	—
3	α-Thujene	924	0.05	**3.49**
4	α-Pinene	932	**3.98**	0.32
5	Norbornen-2-ol	939	0.11	—
6	Verbenene	961	**1.65**	—
7	Sabinene	968	0.13	0.25
8	*trans*-Pinane	969	0.13	—
9	β-Pinene	974	0.24	0.35
10	Myrcene	988	0.07	—
11	2-Octanol	994	—	0.1
12	δ-2-Carene	1,001	0.37	0.1
13	δ-3-Carene	1,008	**15.7**	0.06
14	α-Terpinene	1,014	0.42	—
15	*p*-Cymene	1,020	**2.44**	**4.93**
16	1,8-Cineol	1,026	—	**43.61**
17	*E*-β-Ocymene	1,044	**1.85**	—
18	γ-Terpinene	1,054	0.72	**11.71**
19	*cis*-Sabinene hydrate	1,065	—	0.13
20	*cis*-Linalool oxide	1,067	0.6	—
21	Camphenilone	1,078	**1.38**	0.85
22	Terpinoline	1,086	0.48	**2.3**
23	Linalool	1,095	0.16	—
24	*trans*-Sabinene hydrate	1,098	—	0.27
25	6-Camphenol	1,111	0.23	—
26	Dehydrolinalool	1,131	**4.99**	—
27	*trans*-Dihydro β-terpineol	1,134	—	0.39
28	*cis*-Pinene hydrate	1,139	—	1.42
29	δ-Terpineol	1,162	**27.64**	1.16
30	Thujanol	1,164	**1.12**	—
31	*cis*-Linalool oxide	1,170	0.93	—
32	Terpinene-4-ol	1,174	—	**3.91**
33	*iso*-Verbanol	1,176	0.31	—
34	*neo*-Verbanol	1,182	0.84	—
35	α-Terpineol	1,186	—	**10.58**
36	γ-Terpineol	1,199	—	0.22
37	Verbinone	1,204	0.05	—
38	*trans*-Piperitol	1,207	—	0.11
39	*trans*-Carveol	1,215	**2.14**	—
40	*cis*-Sabinene hydrate acetate	1,219		0.37
41	*cis*-Carveol	1,226	**1.77**	—
42	Pulegone	1,233	—	**1.02**
43	Carvone	1,239	—	0.23
44	*trans*-Sabinene hydrate acetate	1,253	—	0.13
45	*iso*-3-Thujanol acetate	1,267	—	0.33
46	*trans*-Carvone oxide	1,273	**4.13**	0.6
47	*neo-iso*-3-Thujanol acetate	1,281	—	0.2
48	Thymol	1,289	**14.17**	—
49	*p*-Cymen-7-ol	1,290	—	**1.35**
50	*trans*-Verbenyl acetate	1,291	0.06	—
51	*iso*-Verbanol acetate	1,308	0.31	—
52	δ-Terpinyl acetate	1,316	—	0.08
53	δ-Elemene	1,335	—	0.95
54	α-Terpinyl acetate	1,346	—	0.51
55	α-Copaene	1,374	0.08	—
56	β-Longipinene	1,400	0.07	—
57	Longifoline	1,407	—	0.05
58	*E*-Caryophyllene	1,417	**2.75**	0.35
59	Carvone hydrate	1,422	—	0.26
60	γ-Elemene	1,434	0.07	—
61	Aromadendrene	1,439	—	0.24
62	α-Humulene	1,452	0.12	0.23
63	Sesquisabinene	1,457	0.17	—
64	9-*epi*-*E*-Caryophyllene	1,464	—	0.09
65	β-Thujaplicin	1,475	0.16	—
66	γ-Muurolene	1,478	—	0.25
67	β-Selinene	1,489	0.39	—
68	δ-Selinene	1,492	—	0.2
69	α-Muurolene	1,500	—	0.38
70	β-Bisabolene	1,505	0.13	—
71	γ-Cadinene	1,513	0.27	0.07
72	δ-Cadinene	1,522	—	0.09
73	Elemol	1,548	—	**2.11**
74	β-Calacorene	1,564	—	0.22
75	Caryophyllenyl alcohol	1,570	0.21	—
76	Germacrene D-4-ol	1,574	—	0.07
77	*trans*-Sesquisabinene hydrate	1,577	0.22	—
78	Caryophyllene oxide	1,582	**2.24**	0.3
79	Davanone	1,587	—	1.1
80	*cis*-β-Elemenone	1,589	0.12	—
81	Widdrol	1,599	—	0.23
82	*trans*-β-Elemenone	1,601	0.09	—
83	*cis*-Isologifolanone	1,612	—	0.08
84	*trans*-Isologifolanone	1,625	—	0.28
85	α-Acorenol	1,632	—	0.15
86	*cis*-Cadin-4-en-7-ol	1,635	0.69	—
87	*epi*-α-Muurolol	1,644	—	0.18
88	β-Eudesmol	1,649	0.35	—
89	α-Eudesmol	1,652	0.28	—
90	Dihydroeudesmol	1,661	—	0.54

(Continued)

Table 1: *Continued*

(Continued)

Table 1: *Continued*

No	Compounds	RI	Area %	
			Thymus zygis* L. ssp. *gracilis	***E. camaldulensis* Dehnh.**
91	14-Hydroxy-*Z*-caryophyllene	1,666	0.39	—
92	Davanol acetate	1,689	0.09	—
93	2*E*,6*Z*-Farnesol	1,714	—	0.1
Total			99.65%	99.6%
Monoterpenes			94.18%	90.57%
Sesquiterpenes			5.47%	9.03%
Phenols			19.73%	0.11%
Aldehydes			0.14%	—
Ketones			7.42%	4.04%
Alcohols			37.55%	22.22%
Ethers			—	43.61%
Hydrocarbons			34.93%	29.03%
Esters			0.06%	0.59%

No: In order of elution on HP-5ms; Components: Components identified based on retention indices and mass spectra; RI: Retention indices are calculated experimentally using a homologous series of C9–C28 alkanes; –: Not detected.

predominantly linalool (39.37%), terpinen-4-ol (15.92%), β-myrcene (7.95%), γ-terpinene (6.36%), and borneol (5.17%) [35]. Research conducted in Spain similarly revealed that thymol (48.59%), *p*-cymene (18.79%), γ-terpinene (22.85%), and linalool (4.31%) constitute the primary components in the EOs of *T. zygis* [36]. However, other authors have found that *T. zygis* EO from Serbia has predominantly linalool (38.0%), 4-terpineol (10.1%), *p*-cymene (6.9%), and β-myrcene (4.9%) [37].

Furthermore, in the EO of *E. camaldulensis*, 55 constituents were identified, comprising 99.60% of the total compounds. The EO of *E. camaldulensis* extracted in this study is rich in 1,8-cineole (43.61%), γ-terpinene (11.71%), α-terpineol (10.58%), *p*-cymene (4.93%), terpinen-4-ol (3.91%), and α-thujene (3.49%), with a high monoterpene content (90.57%) compared to sesquiterpenes (9.03%) (Table 1). It is neither qualitatively nor quantitatively comparable to previous research on the same species. Indeed, the EO extracted from *E. camaldulensis* collected from Algeria is characterized by high contents of eucalyptol (24.260–72.718%), aromadendrene (2.655–8.796%), globulol (1.871–8.247%), and spathulenol (0.940–7.304%) [38]. For instance, the EO of *E. camaldulensis* from Nicosia is predominantly characterized by β-phellandrene (30.6%), α-phellandrene (10.3%), spathulenol (9.3%), *p*-cymene (8.2%), and bicyclogermacrene (6.1%) [39]. Meanwhile, the oil from Taounate in Northern Morocco is co-dominated by 1,8-cineole (34.16%), (−)-spathulenol (21.21%), α-pinene (6.73%), and α-guajene (5.51%) [40]. In Senegal, the EO of this species revealed high contents of 1,8-cineole (52.6%), α-terpineol (6.6%), *cis*-*p*-mentha-1-(7)-8-dien-2ol (5.1%), and *trans*-*p*-mentha-1-(7)-8-dien-2ol (4.8%) [41]. Conversely, a research in Malaysia [42] showed that the EO of this species is rich in γ-terpinene (57.4%), terpinen-4-ol (16.2%), and *o*-cymene (15.7%). The highest levels of 1,8-cineole (76.93%) were reported in Brazil, followed by β-pinene (11.49%) and α-pinene (7.15%) [18].

The variability in the chemical composition of the EOs of *T. zygis* and *E. camaldulensis* can be ascribed to various factors such as climate, soil type, harvest season, humidity levels, temperature, exposure duration, water stress, solar radiation, as well as preservation and extraction methods, which play a significant role. Additionally, the plant's genetic characteristics and growth cycle also contribute to this chemical diversity [18,43,44].

4.3 Antioxidant activity

Antioxidants play a vital role by competing with free radicals and inhibiting the propagation of oxidation reactions [45]. In this study, we examined the EOs of *T. zygis* and *E. camaldulensis* using two antioxidant testing methods: DPPH radical scavenging and FRAP assays. DPPH is commonly utilized to assess the ability of radical scavenging property of various studied substances (Figures 1 and 2). It is a straightforward, rapid, and highly replicable technique [46]. The results presented in Table 2 indicate that the antioxidant efficacy of *T. zygis* EO surpasses that of *E. camaldulensis*, with respective values of 57.292 ± 0.001 µg/mL and 238.851 ± 0.001 µg/mL, while quercetin (reference antioxidant) exhibited higher activity with IC50 = 5.499 ± 0.019 µg/mL. Our results demonstrate higher antioxidant activity than those obtained in previous studies, such as in Spain, where the antioxidant activity of *T. zygis* on DPPH yielded an IC50 value of 0.90 ± 0.03 mg/mL [47]. Conversely, in another study conducted in Spain, different tests were employed to evaluate the antioxidant capacity of *T. zygis* EOs containing a high proportion of thymol, showing strong antioxidant activity with an IC50 value of 27.7 ± 0.3 µmol/g [48]. For *E. camaldulensis*, our findings are consistent with previous studies investigating the antioxidant potential of *E. camaldulensis* growing in Tunisia, where a reported IC50 value was 342 µg/mL [49]. In Pakistan, EOs obtained from the leaves of this tree, extracted by two different methods, showed IC50 values of 16.21 ± 0.97 µg/mL by hydrodistillation and 19.89 ± 0.79 µg/mL by supercritical fluid extraction [50]. Similarly, the EO extracted from *E. camaldulensis* in Turkey revealed stronger activity, exhibiting an IC50 value of 4.096 ± 0.724 µg/mL [51].

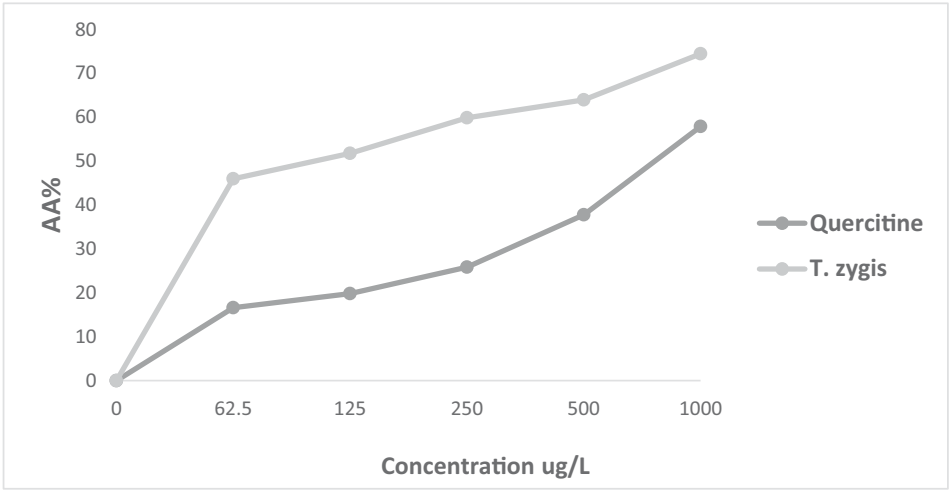

Figure 1: Inhibition percentage of the DPPH radical as a function of different concentrations of *T. zygis* L. EO and the positive control quercetin.

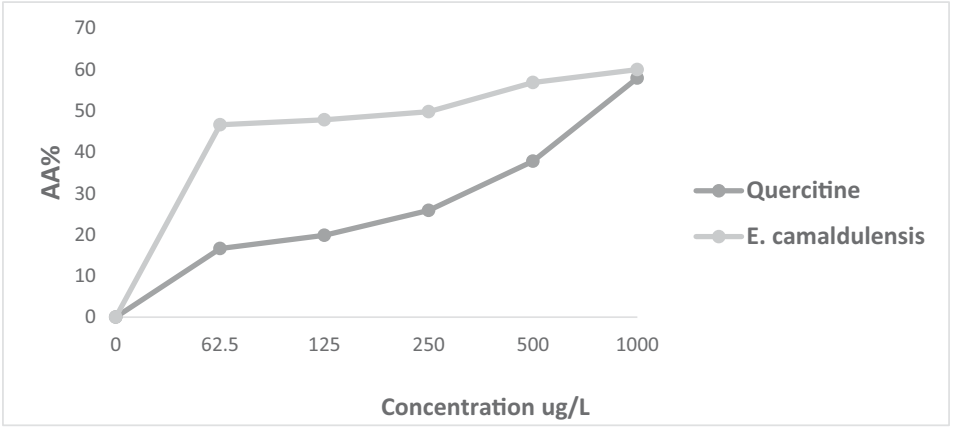

Figure 2: Percentage inhibition of the DPPH radical in relation to different concentrations of *E. camaldulensis* Dehnh. EO and the positive control quercetin.

Table 2: Evaluation of the antioxidant activity of essential oils, as assessed by DPPH radical scavenging and FRAP tests

	DPPH (IC50 = µg/mL)	FRAP (EC50 = µg/mL)
Thymus zygis L. ssp. *Gracilis*	57.292 ± 0.001	7.207 ± 0.001
E. camaldulensis Dehnh.	238.851 ± 0.001	11.834 ± 0.001
Quercitin	5.499 ± 0.019	
Catechin		13.904 ± 0.023

The AA% of *T. zygis* remains significantly higher than the AA% of quercetin even at high concentrations, while that of *E. camaldulensis* at a concentration of 1,000 µg/mL approaches that of quercetin.

The FRAP test was utilized to evaluate the reducing capacity of EOs. This method was developed to quantify the EOs' capability to reduce the ferric iron (F^{3+}) in the $K_3Fe(CN)_6$ complex into ferrous iron (Fe^{2+}) (Figures 3 and 4). The iron reduction capacity is directly correlated with the increase in the sample concentration [52]. The reducing power of *T. zygis* and *E. camaldulensis* through the FRAP test yielded EC50 values of 7.207 ± 0.001 µg/mL and 11.834 ± 0.001 µg/mL, respectively (Table 2). These values differ from those of the natural antioxidant (catechin), which exhibits an EC50 value of 13.904 ± 0.023 µg/mL (Table 2). Some previous studies have also reported a strong reducing power of *T. zygis* EOs from Spain against the $K_3Fe(CN)_6$ complex with an EC50 value of 49.56 ± 0.09 mg/mL [47]. Studies conducted in Burkina Faso on *E. camaldulensis* EO showed significant reducing power with an EC50 value of 6.47 ± 1.34 mg/mL [53].

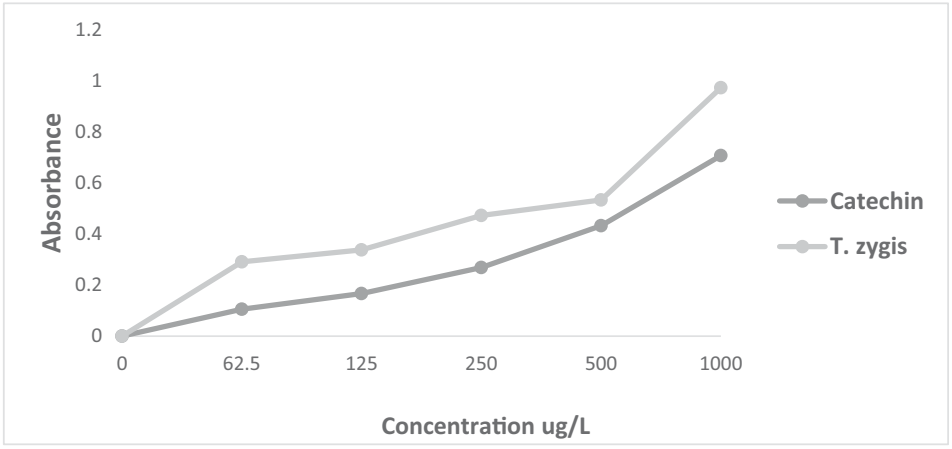

Figure 3: Reducing ability of *T. zygis* L. EO and the positive control catechin.

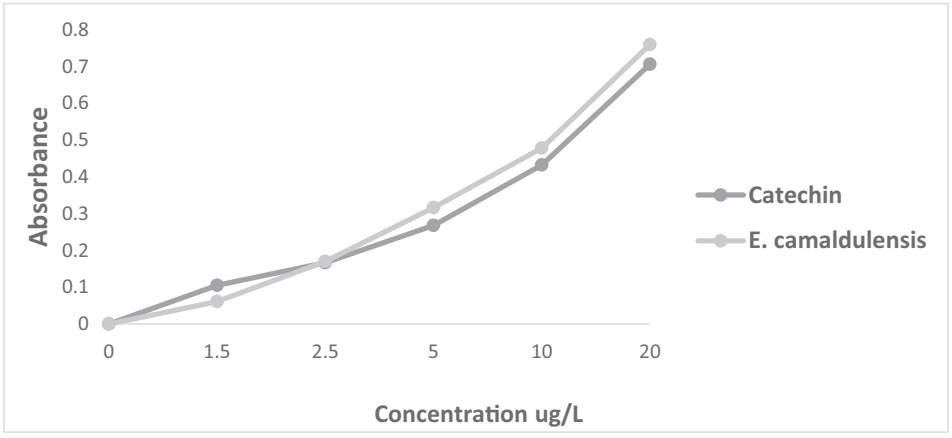

Figure 4: Reducing power of *E. camaldulensis* Dehnh. EO and the positive control catechin.

The antioxidant activity of EOs predominantly depends on their chemical composition. The reported antioxidant activity for *T. zygis* and *E. camaldulensis* EOs may be linked to their elevated percentage of monoterpenes. The difference in the antioxidant capacity observed between *T. zygis* and *E. camaldulensis* EOs stems from their different chemical profiles, as they possess complex chemical behavior and different functional groups and polarities [54]. Certainly, the antioxidant activity of *T. zygis* EO is primarily attributed to its elevated levels of phenols and alcohols, such as δ-terpineol and thymol, which are recognized for their robust antioxidant properties [48].

4.4 Antibacterial activity of EOs

The objective of this study is to determine whether the EOs extracted from *T. zygis* and *E. camaldulensis* possess antibacterial activity against four pathogenic bacteria (*E. coli, M. luteus, B. subtilis*, and *S. aureus*) and subsequently compare it to that of antibiotics. The diameters of the inhibition zones against the tested bacteria were measured and are presented in Table 3. The EOs of *T. zygis* and *E. camaldulensis* exhibited potent antibacterial activity, as the inhibition zones exceeded 15 mm for all studied bacteria. Additionally, Gram-positive bacteria (*S. aureus* and *B. subtilis*) showed stronger sensitivity in contrast to Gram-negative bacteria (*E. coli* and *M. luteus*). However, the tested bacteria showed no sensitivity to antibiotics such as ampicillin and penicillin, as no inhibition zones were detected. On the other hand, all tested strains exhibited very strong sensitivity to the antibiotic tetracycline. These results align with those reported by other researchers, notably Smahane et al. [55], who showed that the EO of *T. zygis* effectively inhibits the growth of bacteria like *S. aureus* (37.33–46.33 mm), *E. coli* (28.67–29.33 mm), and *Pseudomonas*

Table 3: Antimicrobial activities of EOs against various bacterial strains assessed using the disk diffusion method

Microorganisms		ID (mm)				
		***T. zygis* L. ssp. *gracilis* (5 µl/disc)**	***E. camaldulensis* Dehnh. (5 µl/disc)**	**Tetracycline**	**Ampicillin**	**Penicillin**
Gram-negative bacteria	*E. coli* (ATCC 8739)	16.11 ± 0.96	19.11 ± 0.30	24 ± 0.33	NZ	NZ
	M. luteus (ATCC 9341)	16,22 ± 0.30	21.78 ± 0.30	20.67 ± 0.44	NZ	NZ
Gram-positive bacteria	*B. subtilis* (ATCC 6633)	17,11 ± 0.30	22.56 ± 0.59	21.67 ± 0.44	NZ	NZ
	S. aureus (ATCC 6538)	18.33 ± 0.89	25.00 ± 0.22	25.5 ± 0.33	NZ	NZ

NZ: no measurable zone of inhibition; ID: inhibition diameter; antibiotics: tetracycline, ampicillin, and penicillin.

aeruginosa (10 mm). Furthermore, Ballester-Costa et al. [47] demonstrated that the EO of *T. zygis* exhibits stronger antibacterial effects against strains of *Listeria innocua* (45.37 mm), *Serratia marcescens* (16.96 mm), *Pseudomonas fragi* (26.61 mm), *Pseudomonas fluorescens* (27.72 mm), *Aeromonas hydrophila* (19.35 mm), *Shewanella putrefaciens* (20.85 mm), *Achromobacter denitrificans* (23.92 mm), *Enterobacter amnigenus* (17.98 mm), *Enterobacter gergoviae* (13.92 mm), and *Alcaligenes faecalis* (33.85 mm).

Moreover, Diriye et al. [56] reported that the EO extracted from *E. camaldulensis* showed notable efficacy against *E. coli* (21 mm), *S. aureus* (24 mm), *Salmonella typhi* (23 mm), and *Klebsiella pneumoniae* (18 mm). Additionally, the EO derived from this tree exhibited antibacterial activity against various pathogenic strains associated with dental caries, including *Streptococcus mutans* (18.8 mm), *Lactobacillus rhamnosus* (19.7 mm), and *Actinomyces viscosus* (21.3 mm) [57].

4.5 MICs and MBCs

The results derived from the disc diffusion method were corroborated by those obtained using the MIC method (Table 4). The EOs of *T. zygis* and *E. camaldulensis* exhibited a potent inhibitory effect against the tested strains, particularly *B. subtilis* and *S. aureus*; these strains showed high sensitivity to *T. zygis* EO with MIC values of 0.062 and 0.125, respectively. On the other hand, strains of *M. luteus* and *E. coli* demonstrated higher resistance, with corresponding MIC values of 0.25 and 0.5. Furthermore, the MIC of *E. camaldulensis* EO against the growth of the tested bacteria (*S. aureus* and *B. subtilis*) falls within the range of 0.25 (Table 4); these bacteria proved to be highly sensitive. In contrast, strains of *M. luteus* and *E. coli* exhibited resistance, with respective MIC values of 0.5 and 1.

Indeed, the inhibitory activity of *T. zygis* EO has been previously studied. Its inhibitory effect has been demonstrated by Afonso et al. [58] against *P. aeruginosa, S. aureus, E. coli, Salmonella typhimurium*, and *Staphylococcus epidermidis*. Later, this activity was confirmed by Radi et al. [59], who tested this EO against *Acinetobacter baumannii*, *S. aureus*, *Enterobacter cloacae, Shigella dysenteriae*, *E. coli*, and *S. typhi*. Previous studies have documented the inhibitory activity of *E. camaldulensis* EO against *S. aureus* and *E. coli* [60,16]. Research on the antimicrobial activity of *E. camaldulensis* EO containing a high proportion of 1,8-cineole has shown strong antibacterial activity. Additional studies have determined the MIC of this compound against the bacteria under investigation, with values of 3.125 µL/mL

Table 4: MIC and MBC of *T. zygis* and *E. camaldulensis* EOs against *S. aureus*, *B. subtilis*, *M. luteus*, and *E. coli*

	CMI/CMB (µL/mL)							
	***M. luteus* (ATCC 9341)**		***B. subtilis* (ATCC 6633)**		***E. coli* (ATCC 8739)**		***S. aureus* (ATCC 6538)**	
	CMI	CMB	CMI	CMB	CMI	CMB	CMI	CMB
T. zygis L. ssp. *gracilis*	0.25	4	0.062	2	0.5	8	0.125	2
E. camaldulensis Dehnh.	0.5	—	0.25	4	1	—	0.25	8

for *E. coli* and 6.25 µL/mL against *S. aureus*, as well as 6.25 µL/mL against *Salmonella enteritidis* [61]. Furthermore, the MIC of the identical compound against the bacterium *S. aureus* was assessed to be 23.43 µg/mL [62].

The EO of *T. zygis* exhibits a significant bactericidal effect against both Gram-positive bacteria *S. aureus* and *B. subtilis*, with an MBC value of 2 µL/mL. Regarding the Gram-negative bacteria, *M. luteus* is sensitive to the bactericidal effect of *T. zygis* EO, with an MBC value of 4 µL/mL, but *E. coli* shows resistance at these concentrations. On the contrary, the EO of *E. camaldulensis* demonstrates moderate bactericidal activity against both Gram-positive bacteria *B. subtilis* and *S. aureus*, with an MBC value of 8 µL/mL. Additionally, it exhibits a bacteriostatic effect on the Gram-negative bacteria *M. luteus* and *E. coli.*

The extensive and noteworthy antimicrobial efficacy observed in the tested *T. zygis* EO could be ascribed to its elevated concentrations of δ-terpineol (27.64%) and thymol (14.17%), which are the principal constituents of *T. zygis* EO. Indeed, thymol functions by disrupting the bacterial cell wall and plasma membrane, as well as by interacting with membrane proteins. Specifically, it disrupts ATP production within the cell by inhibiting the Krebs cycle, thereby impeding its ability to return to normal function following exposure to thymol [10].

On the other hand, the results of the study conducted by Li et al. [61] revealed that 1,8-cineole, the primary component of *E. camaldulensis* EO, induces significant degradation of the outer membrane, decrease in the cytoplasm, and can alter the physical characteristics of both Gram-negative bacteria and Gram-positive. Furthermore, research conducted by Jaradat et al. [63] demonstrated that *p*-cymene causes changes in intracellular pH and ATP levels, suppressing the microbial growth. δ-Terpineol, terpinol-4-ol, and α-terpineol result in the disruption of membrane and cell wall integrity, which changes the permeability and leads to the leakage of intracellular substances [12]. Therefore, the antibacterial effects observed in the EOs of *T. zygis* and *E. camaldulensis* could be elucidated by the molecular interaction between the functional groups of their constituents and bacterial cell walls. Moreover, the considerable antibacterial efficacy could be attributed to the potential synergistic interaction among these constituents.

Indeed, Gram-negative bacteria possess a rigid outer membrane characterized by a high content of lipopolysaccharides and a complex structure, which restricts the diffusion of hydrophobic compounds through this membrane. Conversely, Gram-positive bacteria lack this membrane, which accounts for the resistance of Gram-negative bacteria and the sensitivity of Gram-positive bacteria [40].

Phenols and alcohols are recognized as highly potent chemical compounds with broad-spectrum antibacterial properties. Indeed, phenols and terpene alcohols constitute roughly 50% of the overall composition of *T. zygis* EO. These molecules are widely recognized for their high effectiveness as antimicrobial agents [33,58,59]. Even though the EO of *E. camaldulensis* leaves lacks phenolic compounds and contains a low concentration of alcoholic compounds, it may still exhibit significant antibacterial activity. The active principle, 1,8-cineole, belonging to the ether group, is known for its interesting antibacterial properties [16,50].

5 Conclusions

This study aimed to characterize the chemical composition of EOs from two Moroccan plants, *T. zygis* and *E. camaldulensis*, and assess their antioxidant and antibacterial properties. The results indicate a more significant antioxidant efficacy for *T. zygis* compared to *E. camaldulensis*. Additionally, both EOs exhibited substantial antibacterial properties. Among the four tested strains, *S. aureus* and *B. subtilis* exhibited the highest sensitivity. The antibacterial activity of *T. zygis* and *E. camaldulensis* EOs surpassed that of antibiotics such as ampicillin and penicillin, which showed no inhibition zones. However, all tested strains displayed strong sensitivity to tetracycline antibiotics. The antibacterial activity of *T. zygis* and *E. camaldulensis* EOs is contingent upon their concentration, chemical composition, and specific strains under investigation. The extensive utilization of antibiotics has prompted substantial adaptability among bacterial strains and the emergence of

multi-resistant strains, explaining the resistance of bacteria to ampicillin and penicillin.

Nevertheless, natural antioxidants like *T. zygis* and *E. camaldulensis* EOs could provide a beneficial value to prevent undesirable health issues compared to potentially hazardous synthetic antioxidants. The effectiveness of *T. zygis* and *E. camaldulensis* EOs makes them a potential solution for preserving food products against oxidation and microbial contamination. They might find applications in pharmaceutical, cosmetic, and food industries to extend the shelf life of various products.

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