https://doi.org/10.31925/farmacia.2026.1.22 **ORIGINAL ARTICLE**

ESSENTIAL OIL PROFILING BY GC-MS AND *IN VITRO* ANTIOXIDANT POTENTIALS EFFECTS OF AN ALGERIAN ENDEMIC PLANT *THYMUS PALLESCENS DE NOÉ*.

HOUSSAM EDDINE MUSTAPHA SADLI [1], ZOUBIR BELMOKHTAR [1,2], MAHFUZ ELMASTAŞ [3], DUYGU MISIRLI [3], YASSINE MERAD [4], OZLEM TAVUKCUOGLU [3],YAKUP CANBAY [3,5], ABDERREZAK DJABEUR [1]

[1]*Laboratory of production and valorization of plants and microorganisms (LP2VM), Department of Biotechnology, Faculty of Natural and Life Sciences, University of Science and Technology of Oran - Mohamed Boudiaf USTO-MB, B.P. 1505, El-Mn'aour, Oran 31000, Algeria*
[2]*Department of Environmental Sciences, Faculty of Natural Sciences, Djilali Liabes University of Sidi-Bel-Abbes, Sidi Bel Abbes 22000, Algeria*
[3] *Hamidiye Faculty of Pharmacy, University of Health Sciences Türkiye, Istanbul, Turkey.*
[4]*Central Laboratory, Hassani Abdelkader Hospital, Université de Sidi-Bel-Abbès (Djillali Liabès), Algeria*
[5]*Department of Pharmacognosy, Faculty of Pharmacy, İstanbul Yeni Yüzyıl University, İstanbul, Türkiye*

**corresponding author: houssam.sadli@univ-usto.dz*

Manuscript received: October 2025

Abstract

This study aimed to analyse the chemical composition of the essential oil of *Thymus pallescens* (TPEO) collected from two distinct geographical areas in Algeria (Aïn Témouchent and Sidi Bel Abbès). Antioxidant activity was also evaluated. Using gas chromatography–mass spectrometry (GC-MS), analysis revealed the carvacrol chemotype (74.927 - 65.629%) as the major component in both TPEO samples, followed by γ-terpinene (5.210 - 7.493%), *p*-cymene (5.152 - 7.795%), and linalool (2.985 - 2.902%) as the main compounds. The chemical composition was identified by comparing the obtained mass spectra with those in the NIST and Wiley libraries, along with retention indices. The evaluation of antioxidant capacity employed four assays (DPPH$^{\bullet}$, ABTS$^{\bullet+}$, FRAP, and CUPRAC). TPEO in both assays (DPPH$^{\bullet}$ and ABTS$^{\bullet+}$) demonstrated the highest activity, with IC_{50} values of 5.42 ± 0.44 - 9.28 ± 0.78 µg/mL for DPPH$^{\bullet}$ and 60 ± 1.70 - 54 ± 3.30 µg/mL for ABTS$^{\bullet+}$. The present data substantiate the chemical consistency of TPEO composition previously documented in the literature. Furthermore, the observed free radical scavenging capacity underscores the prospective utility of *Thymus pallescens* as a viable natural antioxidant in food preservation and pharmaceutical formulations.

Rezumat

Scopul acestui studiu a fost analiza compoziției chimice a uleiului esențial de *Thymus pallescens* (TPEO), recoltat din două zone geografice distincte din Algeria (Aïn Témouchent și Sidi Bel Abbès), precum și evaluarea activității antioxidante *in vitro*. Compoziția chimică a fost determinată prin cromatografie gazoasă cuplată cu spectrometrie de masă (GC-MS), fiind identificat carvacrolul (74,927 - 65,629%) ca principal component în ambele probe, urmat de γ-terpinen (5,210 - 7,493%), p-cimen (5,152 - 7,795%) și linalool (2,985 - 2,902%). Identificarea compușilor s-a realizat prin compararea spectrelor de masă cu bazele de date NIST și Wiley, precum și pe baza indicilor de retenție. Activitatea antioxidantă a fost evaluată utilizând testele DPPH$^{\bullet}$, ABTS$^{\bullet+}$, FRAP și CUPRAC. Uleiul esențial a prezentat o capacitate antioxidantă ridicată în testele DPPH$^{\bullet}$ și ABTS$^{\bullet+}$, cu valori IC_{50} cuprinse între 5,42 ± 0,44 și 9,28 ± 0,78 µg/mL, respectiv între 54 ± 3,30 și 60 ± 1,70 µg/mL. Rezultatele obținute confirmă consistența compoziției chimice a uleiului esențial de *Thymus pallescens* raportată anterior în literatură și evidențiază potențialul acestuia ca sursă naturală de antioxidanți, cu posibile aplicații în industria farmaceutică și alimentară.

Keywords: *Thymus pallescens de Noé*, gas chromatography-mass spectrometry, free radical, FRAP

Introduction

The genus *Thymus* (*Lamiaceae*) constitutes one of the most extensive families of aromatic plants, comprising nearly 250 genera and approximately 7,000 species (1). Taxonomic investigations indicate that *Thymus* among the most prevalent genera within *Lamiaceae*, encompassing 315 species distributed worldwide (1, 2). In Algeria, the genus is represented by 17 species and subspecies, which exhibit considerable heterogeneity in their geographical distribution across diverse ecological zones (3, 4). *Thymus* species have been extensively employed in traditional medicine throughout the country, for their antimicrobial, antispasmodic, and antioxidant properties (5).

Thymus pallescens de Noé is endemic plant growing naturally in Algeria, widely distributed throughout

northern Algeria and can be found in various geographical areas (6), has predominantly been characterized regarding its essential oil (EO) composition, with recent studies identifying carvacrol (56 - 70%) accompanied by p-cymene (10.3 - 17.3%) and γ-terpinene (10.8 - 14.2%) as principal volatile markers responsible for significant antimicrobial against *C. albicans* and *S. cerevisiae* and insecticidal activities against *Tribolium confusum* (7-9).

Oxidative stress, defined as the imbalance between reactive oxygen species (ROS) generation and endogenous antioxidant defence mechanisms, represents a fundamental factor in the progression of chronic pathologies including cardiovascular diseases, neurodegenerative disorders, diabetes, and cancer (10). The deleterious effects of ROS contribute to various inflammatory diseases, such as arthritis, asthma, and cardiovascular diseases, whilst also playing a significant role in certain neuroinflammatory disorders, including Parkinson's disease and Alzheimer's disease (11). Consequently, scientific research focusing on combating oxidative stress through the identification and characterisation of bioactive compounds with potent antioxidant properties has become crucial for human health, as these natural agents help reduce the risk of chronic diseases whilst minimising the adverse effects associated with synthetic antioxidants.

In this context, the search for effective natural antioxidants capable of mitigating ROS induced cellular damage has emerged as a major focus of contemporary biomedical and pharmaceutical research. Plant essential oils represent significant secondary metabolites, comprising approximately 10% of total bioactive molecules in certain plants (5), offer a safer, more sustainable approach to ROS management and provide additional therapeutic benefits through their anti-inflammatory, antimicrobial, and antioxidant activities. Their ability to neutralise free radicals, chelate metal ions, and modulate cellular antioxidant enzyme systems positions make them as promising candidates for the development of novel therapeutic agents and functional food additives (12-14).

Thyme (*Thymus* genus) has garnered considerable scientific attention due to its exceptional phytochemical richness and biological activities. Carvacrol (5-isopropyl-2-methylphenol) and thymol (2-isopropyl-5-methylphenol) constitute the principal phenolic monoterpenes of thyme essential oil and are primarily responsible for its diverse biological activities, including potent antibacterial, anti-inflammatory, antifungal, immunomodulatory, antioxidant, and antiviral properties (15, 16). These bioactive compounds have found extensive applications across multiple sectors, including medicine, pharmaceutical manufacturing, food preservation, and cosmetic formulations. The remarkable antioxidant capacity of carvacrol and thymol is attributed to their ability to enhance the activity of endogenous detoxification enzymes involved in ROS neutralisation, whilst simultaneously acting as direct scavengers of superoxide anions ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radicals (•OH), and other reactive species (17). This antioxidant mechanism, enhance the therapeutic potential of thyme essential oil components in the prevention and management of oxidative stress related pathologies.

Despite the recognised bioactivity of *Thymus* species, comprehensive studies investigating the antioxidant potential of *Thymus pallescens de Noé* essential oil remain scarce. Therefore, the present study aims to (i) characterise the volatile compound profile of the hydrodistilled essential oil of *Thymus pallescens* de *Noé* using gas chromatography–mass spectrometry (GC-MS) analysis, (ii) evaluate its *in vitro* antioxidant activity via established radical scavenging assays (DPPH$^{\bullet}$ and ABTS$^{\bullet+}$), and (iii) assess its metal ion chelating capacity through ferric reducing antioxidant power (FRAP) and cupric ion reducing antioxidant capacity (CUPRAC) assays. This analytical phytochemical methodology contributes to the valorisation of this Algerian endemic species as a potential source of natural antioxidant agents with promising applications in the pharmaceutical, food, and cosmetic industries.

Materials and Methods

Plant material

During the flowering phase the aerial parts (flowers and leaves) of *Thymus pallescens de Noé*, which is known as zaatar, were collected in June 2023 from two locations in northwestern Algeria: Tessala Mountain, Sidi Bel Abbès region (N 35°16'38.6", W 0°48′3″, Altitude 1050 meters), and Samour Forest, Tamzoura, Ain Témouchent region (N 35°24'59.99", W 0°38'59.99", Altitude 423 meters), located approximately 400 km southwest of capital (Algiers). Species identification was performed by Dr. Asma El Zerey-Belaskri (University of Oran 1, Ahmed Ben Bella, Ageria), principally based on the taxonomic key of Quézel and Santa 1962, 1963 (18). A voucher specimen has been deposited in the herbarium of the Laboratory of Biotechnology of Rhizobia and Plant Breeding (University of Oran 1, Ahmed Ben Bella, Algeria) under the accession number “LBRAP-Lam126”.

Essential oil extraction procedure

For essential oil extraction, the fresh aerial parts of the plant were air-dried at room temperature in a shaded, well-ventilated area to prevent exposure to direct sunlight and to preserve volatile compounds. The dried flowers and leaves were then subjected to hydrodistillation using a steam distillation apparatus at 90°C for 4 h.

GC-MS for chemical profiling

GC-MS analysis was performed using a Hewlett Packard Agilent 6890 Plus coupled with a quadrupole mass spectrometer (Agilent 5973). The capillary

column type (HP-5MS) has a length of 30 m with an internal diameter of 0.25 mm and a thickness of 0.25 µm, treated with 5% phenyl and 95% dimethyl-polysiloxane.

GC-MS conditions are a temperature of injection at 250°C, 0.2 µL injector volumes in split mode with a split ratio of 1:80, the column temperature started at 45°C for 8 minutes and changed to 250°C at the rate of 2°C/min, and the isotherm temperature held for 10 minutes. The total analysis was 120 minutes.

Compound identification

The identification of different compounds contained in the essential oil is carried out based on the results obtained after the analysis performed. The identification is done by two methods: (i) by comparing the mass spectra obtained with those in GC-MS database (Wiley 7N, NIST02); (ii) by comparing their retention index (RI) relative to the n-alkane series (C8-C30) and the values mentioned in the literature (19, 20).

Antioxidant capacity assays

The antioxidant capacity of the essential oil of *Thymus pallescens de Noé* (TPEO) was tested in the context of its scavenging capacity on DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ assays and its ability to reduce metals on FRAP and CUPRAC assays.

2,2-Diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$) radical scavenging activity

The DPPH$^{\bullet}$ free radical scavenging assay was carried out according to the method of Brand-Williams *et al.* (21). A volume of 0.1 mL of thyme essential oils at various concentrations (5 - 200 µg/mL) was added to 2.9 mL of an ethanolic DPPH$^{\bullet}$ solution (0.06 mM). The mixture was incubated at room temperature for 30 min, and the absorbance was measured at 517 nm. Ascorbic acid was used as a positive control, and ethanol served as the blank.

The DPPH$^{\bullet}$ radical scavenging activity of the essential oils was evaluated at concentrations of 5, 10, 25, 50, 100, and 200 µg/mL. The percentage of radical scavenging activity was calculated using the following equation:

$$\text{Radical Scavenging Activity (\%)} = [(A_{control} - A_{sample})/A_{control}] \times 100,$$

where, A_{contro} is the absorbance of the DPPH$^{\bullet}$ solution without essential oils, and A_{sample} is the absorbance of the sample containing the essential oils.

The percent inhibition values were plotted against the corresponding concentrations of the essential oils. The IC_{50} value, which represents the concentration of the extract required to inhibit 50% of the DPPH$^{\bullet}$ radical was determined by linear regression analysis using the curve.

2, 2'-Azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS$^{\bullet+}$) radical scavenging activity

The ABTS$^{\bullet+}$ cation radical scavenging activity was carried out according to the method of Re *et al.* (22). The ABTS$^{\bullet+}$ radical was prepared in two steps. First, 7 mM ABTS$^{\bullet}$ (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) was dissolved in distilled water. Second, a 2.45 mM potassium persulfate ($K_2S_2O_8$) solution was prepared separately. These two solutions were then mixed to initiate the formation of the ABTS$^{\bullet+}$ radical cation. The resulting mixture was incubated in the dark for 12 - 16 h at room temperature to obtain the ABTS$^{\bullet+}$ solution.

The obtained solution was further diluted with distilled water to achieve an absorbance of 0.65 - 0.75 at 734 nm. Trolox was used as a positive control.

For the assay, 10 µL of each sample and Trolox dilution prepared from a stock concentration of 1000 µg/mL was mixed with 190 µL of ABTS$^{\bullet+}$ solution. The plate was incubated in the dark, and absorbance was measured at 734 nm against the negative control.

The scavenging capacity of ABTS$^{\bullet+}$ was calculated as the following equation:

$$\text{ABTS}^{\bullet+}\text{ Scavenging Activity (\%)} = [(A_{control} - A_{sample})/A_{control}] \times 100,$$

where, A_{contro} is the absorbance of the ABTS$^{\bullet+}$ solution without essential oils, and A_{sample} is the absorbance of the sample containing the essential oils.

The percent inhibition values were plotted against the corresponding concentrations of the essential oils. The IC_{50} value, which represents the concentration of the extract required to inhibit 50% of the ABTS$^{\bullet+}$ cation radical was determined by linear regression analysis using the curve.

Ferric ion reducing antioxidant power assay (FRAP)

The ferric reducing ability was evaluated using the assay described by Benzie and Strain (23). Three separate solutions were prepared for the FRAP reagent: 20 mM $FeCl_3$ in distilled water, 10 mM TPTZ in 40 mM HCl, and 22.8 mM acetate buffer (pH 3.6) prepared using sodium acetate trihydrate in a volumetric flask. The FRAP reagent was prepared by mixing 300 mL of acetate buffer with 3 mL of $FeCl_3$ solution and 3 mL of TPTZ solution.

For the assay, 10 µL of samples prepared at a concentration of 0.2 mg/mL was added to each well, followed by 300 µL of FRAP reagent. The mixture was incubated at 37°C for 4 min, and the absorbance was measured at 593 nm.

The standard curve was obtained by serial dilution of ferrous sulphate solutions (5 mM $FeSO_4$). The reducing power of the plant extracts was expressed as mmol of Fe^{2+} equivalents *per* gram of thyme essential oil (mmol Fe^{2+}/mL essential oil).

CUPRAC (cupric ion reducing antioxidant capacity) assay

The CUPRAC protocol described by Apak *et al.* (24) for the evaluation of antioxidant capacity uses three main solutions. The first solution consisted of

copper (II) chloride dihydrate ($CuCl_2 \cdot 2H_2O$) prepared at a concentration of 7.9 mM in distilled water. The second solution was ammonium acetate (NH_4Ac) buffer prepared at a concentration of 1 M, with the pH adjusted to 7. The third solution contained neocuproine dissolved in ethanol to obtain a concentration of 7.5 mM.

Trolox was used as a standard and prepared by serial dilution of a 2 mM stock solution to obtain a concentration range of 0.05 - 0.35 mM.

Thyme essential oils were prepared at a concentration of 0.156 mg/mL. For microplate analysis, the blank consisted of 50 µL of each of the three prepared solutions and 100 µL of distilled water, whereas for the samples, 100 µL of thyme essential oil replaced the water. After incubation at 37°C for 15 min, followed by an additional 5 min incubation, the absorbance was measured at 450 nm.

The reducing capacity of the extracts was calculated using the standard curve and expressed as mmol Trolox equivalents *per* gram of essential oil (mmol TE/mL essential oil).

Statistical analysis

All assays were performed in triplicate. Results are expressed as mean ± standard deviation (S.D.). Statistical analyses were conducted using one-way ANOVA followed by Tukey's post-hoc test for multiple comparisons. Means denoted by different lowercase letters are significantly different at $p < 0.05$. Analyses were performed using IBM SPSS Statistics 26.

Results and Discussion

Chemical profiling

The extraction yield obtained by hydrodistillation of *Thymus pallescens de Noé* flowers and leaves from Tessala Sidi Bel Abbès (TPTS) and Tamzoura, Aïn Témouchent (TPTM) was 2.99 ± 0.26% (v/w) and 2.42 ± 0.25% (v/w), respectively. Variations in essential oil content have been reported for *Thymus pallescens* collected from different regions of Algeria, with yields ranging from 0.4% to 6.2% (8, 9, 25).

The obtained essential oils were yellow to yellow-brown in colour and exhibited a strong spicy aroma. Variations in essential oil yield and other characteristics of medicinal plants, such as chemical composition and pharmacological activity, may be influenced by several ecological factors, including soil composition, temperature, harvesting period, and altitude (26).

A total of 26 components were identified in the essential oils from both Tessala Sidi Bel Abbès and Tamzoura, Aïn Témouchent, accounting for 98.647% and 99.138% of the total composition, respectively (Table I). The major compounds (> 2%) were carvacrol (74.927 - 65.629%), γ-terpinene (5.210 - 7.493%), *p*-cymene (5.152 - 7.795%), linalool (2.985 - 2.902%), and thymol (2.141 - 6.681%).

Table I

Chemical composition and Area percentage of both *Thymus pallescens de Noé* samples

No	Components	TPTS Area%	TPTM Area%	RT/min	RI
1	α-Thujene	-	0.415	5.282	917.87
2	Tricyclene	1.264	-	5.398	920
3	α-Pinene	0.068	1.910	5.467	921.531
4	Camphene	-	0.156	6.015	932.37
5	Verbenene	0.104	0.193	7.353	958.9
6	Myrcene	0.610	1.038	8.728	986
7	α-Phellandrene	0.179	0.132	9.260	996.59
8	p 2-Carene	0.110	-	9.586	1002.17
9	α-Terpinene	1.157	1.696	10.036	1008.53
10	p Cymene	5.152	7.795	10.683	1017.681
11	γ-Terpinene	5.210	7.493	13.126	1052.21
12	Terpinolene	-	0.089	15.247	1082.19
13	m-Cymenene	0.067	-	15.299	1082.925
14	Linalool	2.985	2.902	16.503	1099.94
15	Isoborneol	0.085	0.186	20.365	1154.26
16	Terpinen-4-ol	-	0.124	21.518	1170.478
17	Linalool formate	-	0.061	24.596	1214.49
18	Thymol, methylether	0.297	-	24.720	1236.326
19	Carvacrol, methylether	0.137	0.124	26.482	1242.4
20	Thymol	2.140	6.681	30.339	1299.49
21	Carvacrol	74.927	65.629	32.028	1325.993
22	Bourbonene	0.491	0.177	36.070	1389.47
23	Caryophyllene	1.764	1.093	36.614	1398
24	E-Caryophyllene	0.321	0.124	37.763	1417.05

No	Components	TPTS Area%	TPTM Area%	RT/min	RI
25	Gurjunene	0.105	-	38.654	1431.9
26	Aromadendrene	0.132	-	39.057	1438.616
27	α-Guaiene	-	0.059	39.096	1439.292
28	Aromadendrane (dehydro)	0.133	0.135	40.394	1460.94
29	Muurola-4(14),5-diene (cis)	0.190	-	40.754	1466.9
30	γ-Gurjunene	0.421	0.238	41.264	1475.4
31	D-Germacrene	0.151	0.112	41.835	1484.9
32	γ-Amorphene	-	0.161	42.473	1495.613
33	γ-Cadinene	0.274	-	43.138	1507.018
34	Cadineneether <trans->	-	0.331	46.043	1458.273
	Total EO yields (%)	98.647	99.138		

The distribution of the identified compounds according to terpene classes (Figure 1) shows that both TPTS EO and TPTM EO were dominated by oxygenated monoterpenes (80.571% and 75.792%, respectively), followed by monoterpene hydrocarbons (13.921% and 20.917%), sesquiterpene hydrocarbons (4.155% and 2.100%), and finally oxygenated sesquiterpenes, detected only in TPTM EO at a low concentration (0.33%).
The data revealed that TPTS EO contains higher concentrations of carvacrol compared to TPTM EO. Conversely, TPTM EO exhibits the highest concentrations of hydrocarbon monoterpenes in its composition relative to TPTS. This chemical differentiation is particularly evident in thymol content, with TPTS EO showing a concentration of 2.141%, while TPTM EO demonstrates a higher concentration of 6.681%. Monoterpenes are the most common compounds in thyme essential oils (27). In a recent investigation, *Neli E et al,* 2025 (28) identified thymol as the principal constituent (41,84%) of *Thymus vulgaris* essential oil sourced from Bulgaria. Concurrently, *Bochra M et al*. 2025 (29) demonstrated the co-dominance of thymol (46.03%) and carvacrol (29.24%) in *T. vulgaris* specimens collected from Algeria. Furthermore, numerous studies have demonstrated that carvacrol chemotype and thymol chemotype are only two prevalent monoterpene chemotypes present in *Thymus pallescens* commonly found throughout Algeria, in which the most frequent is carvacrol chemotype (44.4 - 92.63%) with nine studies in nine different regions (8, 21, 25, 30, 31) and one for thymol chemotype (49.3%) (30). Although many thyme species are characterised by the predominance of thymol as their main chemotype, the species were studying, *Thymus pallescens*, is characterised by its high content of carvacrol (74.927 - 65.629%) compared to thymol (2.141 - 6.681%). Even though these two molecules are isomeric phenols, they have the same molecular weight but differ in their physical properties: thymol is crystalline, while carvacrol is liquid. This difference makes carvacrol more volatile than thymol (32, 33). This characteristic explains the particularly intense odour of *Thymus pallescens* (rich in carvacrol) compared with odour of some other thyme species.

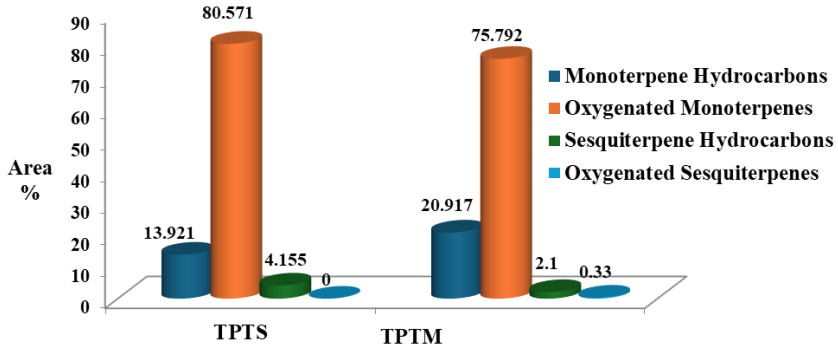

Figure 1.
Terpenes group distribution in both *Thymus pallescensde Noé* plants

The results also reported the appearance of hydrocarbon monoterpenes in TPTS EO and TPTM EO (13.921 - 20.917%) in different proportions, especially *p*-cymene (05.152 - 7.795%) and *γ*-terpinene (05.210 - 7.493%) in high concentrations. These two molecules are precursors of carvacrol, contributing to its biosynthetic pathway. *γ*-terpinene, which represents a cyclic form of geranyl diphosphate (GDP), is converted to carvacrol by the action of cytochrome P450 monooxygenases (34). Dehydrogenated or enzymatically oxidized *γ*-Terpinene to form the monocyclic monoterpenes *p*-

cymene (Conversion of *γ*-Terpinene to *p*-Cymene); after that, carvacrol is produced by the hydroxylation of *p*-cymene (35).

Upon comparative analysis of our findings with studies conducted on the same species across diverse geographical regions, we observed an inverse correlation between *p*-cymene and carvacrol content. This inverse relationship explains the notably low *p*-cymene concentration observed in our investigation, characterised by a high carvacrol content, compared with results reported in previous research (8, 9). Our observations correspond to those reported by Hadef *et al.,* (36) in their study about *Thymus numidicus*.

This distinctive variation in chemical composition characterises TPTS EO and TPTM EO and may be influenced by various ecological factors, including altitude, soil composition, temperature, humidity, light intensity, and other climatic conditions (37). These environmental parameters directly affect the activity of a family of terpene synthase (TPSs) enzymes, which are responsible for the synthesis of carvacrol, thymol, *p*-cymene, *γ*-terpinene, from the independent pathway, MAV, the mevalonic acid pathway (38).

Antioxidant capacity

Four *in vitro* antioxidant activity assays were used to evaluate the total antioxidant capacity (TAC) of TPTS EO and TPTM EO, namely the DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ radical scavenging assays, as well as the ferric reducing antioxidant power (FRAP) and cupric reducing antioxidant capacity (CUPRAC) assays. These complementary methods provide insight into the mechanisms by which the constituent compounds of the essential oils interact with each assay system.

DPPH$^{\bullet}$ radical scavenging activity. The DPPH$^{\bullet}$ radical scavenging assay showed that the essential oils of both plants (TPTS and TPTM) exhibited low IC_{50} values, ranging from 5.42 ± 0.44 to 9.28 ± 0.78 µg/mL. For comparison, the IC_{50} value of ascorbic acid was 66.93 ± 16.24 µg/mL. The strong antioxidant activity observed for both TPTS and TPTM essential oils, with significantly higher activity for TPTS EO, can be attributed to their richness in bioactive compounds and their ability to neutralise free radicals, thereby limiting oxidative processes.

ABTS$^{\bullet+}$ radical scavenging activity. In the ABTS$^{\bullet+}$ assay, the blue radical cation was reduced to a colourless form through interaction with the bioactive compounds present in the essential oils of TPTS and TPTM. The analysis revealed IC_{50} values of 54.0 ± 3.30 µg/mL for TPTM EO and 60.0 ± 1.70 µg/mL for TPTS EO. For reference, the IC_{50} value of the positive control, Trolox, was 100.0 ± 8.62 µg/mL. These results indicate a high antioxidant potential of the *Thymus pallescens* essential oils.

Ferric Ion Reducing Antioxidant Power assay. The antioxidant capacity of TPTS EO and TPTM EO was further evaluated using the FRAP assay, which determines the ability of antioxidants to reduce metal ions. This assay is based on the reduction of ferric ions (Fe^{3+}) to ferrous ions (Fe^{2+}), accompanied by a colour change from yellow (Fe^{3+}-TPTZ complex) to blue.

The analysis revealed values of 3.35 ± 0.40 and 2.80 ± 0.21 mmol Fe^{2+} equivalents/mL for TPTS EO and TPTM EO, respectively, at a concentration of 0.156 mg/mL. These results clearly demonstrate the superior reducing capacity of TPTS EO compared with TPTM EO.

Cupric Ion Reducing Antioxidant Capacity assay. The bioactive compounds present in both TPTS EO and TPTM EO demonstrated the ability to reduce Cu(II) ions to Cu(I) ions, reflecting the oxidative conversion efficiency of non-enzymatic antioxidants. This reduction was evidenced by a visible colour change from blue (CUPRAC reagent) to yellow–orange in the presence of neocuproine (2,9-dimethyl-1,10-phenanthroline).

This assay allows us to evaluate the total antioxidant capacity of TPTS EO and TPTM EO, with results expressed in mM Trolox Equivalents *per* mL (mmol TE/mL). The analysis revealed inhibitory concentration values of 1.22 ± 0.08 - 4.06 ± 0.23 mmol TE/mL for 0.156 mg/mL concentration of TPTS EO and TPTM EO. These data showed clearly that TPTM EO established superior antioxidant efficacy.

The ABTS$^{\bullet+}$ and CUPRAC data listed in Table II demonstrated that antioxidant capacities of the EO (TPTM) rich in carvacrol (65.629%) and thymol (6.681%) were significantly a little better than that (TPTS) rich in carvacrol (74.927%) with a lower percentage of thymol (2.141%) compared to TPTM.

Table I

Antioxidant capacity results of both *Thymus pallescens de Noé* essential oils

Simple	DPPH$^{\bullet}$ (µg/mL)	ABTS$^{\bullet+}$ (µg/mL)	FRAP (mmol Fe^{2+}Eq/mL)	CUPRAC (mmol TE/mL)
TPTS	5.42 ± 00.44^{a}	60 ± 01.70^{a}	3.35 ± 00.40^{b}	1.22 ± 00.08^{a}
TPTM	9.28 ± 00.78^{b}	54 ± 03.30^{a}	2.80 ± 00.21^{a}	4.06 ± 00.23^{b}
AA	66.93 ± 16.24^{c}	-	-	-
TOX	-	100 ± 08.62^{c}	-	-

Data expressed as the mean ± SD. The experiment was performed in a minimum of three replicates, lowercase letters ^{a,b,c}, representing significant differences at $p < 0.05$

These results may prove the previous literature that when carvacrol and thymol work synergistically, their similar chemical structures can neutralise free radicals and reduce metal with greater efficacy (39). This was reported in Taibi *et al*. (40) study and confirmed by two assays, DPPH$^{\bullet}$ and ABTS$^{\bullet+}$, through results obtained after investigating the antioxidant capacity of carvacrol and thymol individually, followed by examining their equimolar mixture effect. The study revealed synergistic effects that enhanced antioxidant capacity.This effect is not only due to the synergistic interaction between carvacrol and thymol. γ-Terpinene (5.210 - 7.493%), *p*-cymene (5.153 - 7.795%), and linalool (2.902 - 2.985%) are also present in TPEO at significant concentrations and may act synergistically with carvacrol (41).

Under some experimental conditions, the interaction between carvacrol and thymol can result in a decrease in overall antioxidant capacity; this is scientifically termed an antagonistic effect (41). Many studies have touched upon this, notably in the research conducted by Rúa J *et al*.; Miloš and Makota (42, 43). Considering that the results obtained proved the antagonistic effect, where more mixtures showed a decrease in antioxidant capacity. Taibi *et al*. work (40) provides further confirmation of that effect; their results suggest an antagonistic effect between thymol and carvacrol in oxygen radical absorbance capacity (ORAC) assays. This may provide initial hypotheses to explain the observed decrease in antioxidant capacity (Table II) in our research in both DPPH$^{\bullet}$ and FRAP assays compared to ABTS$^{\bullet+}$ and CUPRAC assays for TPTM EO (DPPH$^{\bullet}$ = 9.28 ± 0.78 µg/mL, FRAP = 2.80 ± 0.21 mmol Fe^{2+}Eq/mL) when compared to TPTS EO.

Conversely, an enhancement in antioxidant effect was observed for TPTS in both assays DPPH$^{\bullet}$ and FRAP, with an IC_{50} of (5.42 ± 0.44 µg/mL - 3.35 ± 0.40 mmol Fe^{2+}Eq/mL) compared to ABTS$^{\bullet+}$ and CUPRAC. In light of that and based on the results of the chromatographic analysis, which revealed a higher carvacrol content in TPTS (74.927%) compared to TPTM (65.629%), and from literature data examining the existing relationship between strong antioxidant capacity and high carvacrol concentration in *Thymus* species (44).

Sun *et al*. (16) research demonstrated that Tqp01 (*Thymus quinquecostatus* var. *przewalskii*), containing 72% carvacrol, exhibited the strongest antioxidant activity among 10 species and 24 varieties experimented in their study. In the same context, much other research conducted by scientists proved statistically the significant positive correlation between carvacrol, their analogues, and different antioxidant assays: DPPH$^{\bullet}$, ABTS$^{\bullet+}$, FRAP, and TBARS (measurement of lipid peroxidation by thiobarbituric acid reactive substances). In this context, Ting He *et al*. (45) reported a positive correlation with DPPH$^{\bullet}$ (- 0.96372, $p < 0.05$) and with ABTS$^{\bullet+}$ (- 0.96479, $p < 0.05$) for carvacrol acetate; this effectively makes thyme EO one of the most effective antioxidants among plants EO (46-48).

In the literature, many studies were reported on the antioxidant activities of essential oil *Thymus* species. *Thymus pulegioides* essential oil evaluated in the finding of Jianu *et al*. (49) demonstrated antioxidant capacity for DPPH$^{\bullet}$ assay comparable to our findings, with an IC_{50} value of 19.13 ± 0.61 µg/mL. Our results are consistent with those reported by Fatima *et al*. (50), whose study demonstrated an IC_{50} value of 6.13 µg/mL for *Thymus zygis*. A comparative analysis of literature data from Djermane N *et al*. (51) shows IC_{50} values for FRAP and CUPRAC assays of > 200 µg/mL and 464.83 ± 3.69 µg/mL, respectively, for *Thymus ciliatus* from Algeria. Laftouhi A *et al*. (52) in their study on *Thymus vulgaris*, reported an IC_{50} value of 244.64 ± 1.34 µg/mL for the FRAP assay. Additionally, the ABTS$^{\bullet+}$ assay exhibited strong antioxidant activity with notably low IC_{50} values of 1.66 ± 0.1 µg/mL and 6.12 ± 0.03 µg/mL for *Thymus sipyleus* Boiss (53), which are superior to our results. In contrast, the same assay in Djermane N *et al*. (51) research exhibited weaker antioxidant activity compared to our results, with an IC_{50} value of 303.71 ± 7.63 µg/mL.

When the results obtained by *Thymus pallescens* essential oil are compared with those of the positive control used in the same assay, a higher antioxidant activity is observed for the tested oil. Nevertheless, this comparison should be interpreted with caution, as it remains subject to several limitations.

In addition to synergistic factors, essential oils exert their antioxidant activity through multiple other mechanisms due to their complex chemical composition, including phenolic radical scavenging and metal chelation. In contrast, commonly used positive controls such as ascorbic acid and trolox are single, well-characterised molecules acting predominantly through specific antioxidant pathways. Furthermore, the strongly hydrophilic nature of ascorbic acid differs substantially from the lipophilic character of essential oils, resulting in distinct distributions within the assay medium, which can directly influence the measured antioxidant response (54-56). These fundamental differences complicate the direct comparison of IC_{50} values between essential oils and the positive control, even when assessed under identical experimental conditions. Also the essential oil clinical application, is limited by poor physicochemical characteristics: low water solubility, high volatility, environmental instability

(light, heat, oxygen sensitivity), and inadequate skin permeation (57). To address these challenges, advanced nano- and lipid-based delivery systems have been developed to enhance aqueous solubility, chemical stability, controlled release, and bioavailability whilst reducing toxicity and environmental degradation (58).

In the same context of *in vitro* antioxidant evaluation, previous studies have shown that reaction kinetics differ considerably among antioxidant compounds. Fast-reacting reference standards may display lower IC_{50} values due to their rapid attainment of equilibrium, whereas the antioxidant activity of natural constituents, which often react more slowly, may be underestimated in fixed end point measurements. Therefore, kinetic behaviour and reaction time should be taken into account when interpreting antioxidant activity and comparing results with the positive control (59).

Hence, the $DPPH^{\bullet}$, $ABTS^{\bullet+}$, FRAP and CUPRAC tests are simple chemical methods that do not reflect the complexity of biological systems. The active concentrations observed *in vitro* may not be achievable *in vivo* due to the low bioavailability of volatile compounds from essential oils. Furthermore, these tests do not account for hepatic metabolism, tissue distribution, or interactions with endogenous antioxidant systems. Studies on cellular and animal models are necessary to confirm the physiological relevance of these results.

Conclusions

That *Thymus pallescens de Noé* from western Algeria, with its white flowers harvested in two steps at altitudes of 1051 and 423 meters, respectively. Its essential oil obtained by hdrodytillation, demonstrates a rich source of bioactive oxygenated monoterpenes, particularly carvacrol and thymol followed by hydrocarbon monoterpenes γ-terpinene, p-cymene, linalool and exhibits significant antioxidant activity *in vitro*. The synergistic effect between carvacrol as the major compound in TPEO and other bioactive compounds allows for a reduction of reactive oxygen species (ROS), which leads to enhanced antioxidant efficacy, protection against oxidative stress and activation of cellular defence pathways. Compared to other *Thymus* species in Algeria, *Thymus pallescens* from Tessala Mountain, Sidi Bel Abbès and Samour Forest, Tamzoura, Ain Témouchent showed superior antioxidant activity, particularly in $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ assays. These findings suggest to this plant essential oil its potential use in pharmaceutical applications or food preservation, pending further *in vivo* evaluation.

Acknowledgement

Not applicable.

Funding

Not applicable.

Availability of data and materials

The data generated in the present study are included in the figures and/or tables of this article.

Ethics approval and consent to participate

Not applicable.

AI use disclosure

Not applicable.

Conflict of interest

The authors declare no conflict of interest.

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