Teucrium polium subsp. *Helichrysoides* (Diels) Maire, native to Central Sahara: phytochimical profile and antioxidant activity of essential oil

Teucrium polium subsp. *Helichrysoides* (Diels) Maire, nativa da Sahara Central: perfil fitoquímico e actividade antioxidante do óleo essencial

Teucrium polium subsp. *Helichrysoides* (Diels) Maire, nativo de Sahara Central: perfil fitocímico y actividad antioxidante del aceite esencial

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ABSTRACT

Tem sido manifestada uma preocupação considerável relativamente à segurança dos produtos químicos utilizados nas indústrias agroalimentar, cosmética e farmacêutica. Esta preocupação está particularmente relacionada com os efeitos secundários potencialmente deletérios dos antioxidantes sintéticos, que têm sido associados a danos no fígado e à carcinogénese. Consequentemente, é imperativo explorar soluções alternativas, com as plantas medicinais a fornecerem um reservatório substancial de moléculas bioactivas que possuem propriedades biológicas. Este estudo investiga a atividade antioxidante do óleo essencial (OE) de Teucrium polium subsp. helichrysoides (Diels), originário do Sara Central e conhecido pelas suas propriedades medicinais. O óleo essencial foi

extraído por hidrodestilação e o seu perfil fitoquímico foi posteriormente determinado por cromatografia gasosa e espetrometria de massa. Para verificar a atividade antioxidante do óleo essencial, foram utilizados três ensaios in vitro: eliminação do radical livre difenil-1-picrilhidrazil, poder antioxidante redutor férrico e branqueamento do β-caroteno. Os resultados demonstraram que o OE apresentava um elevado teor de sesquiterpenos, sendo o β-eudesmol (66,67%) identificado como o principal composto. Este quimiotipo β-eudesmol não foi previamente identificado na bibliografia. Apesar de exibir uma capacidade significativa para eliminar radicais livres e um poder redutor, a atividade antioxidante do OE foi comparativamente mais fraca na inibição da oxidação lipídica. A atividade antioxidante do OE foi mais fraca em comparação com a dos antioxidantes padrão. O OE de Teucrium polium L. subespécie de Hoggar contém um elevado teor de sesquiterpenos oxigenados, particularmente β-eudesmol, álcool sesquiterpenóide, que pode contribuir para as suas propriedades antioxidantes. Este facto sublinha a importância desta molécula nas indústrias agroalimentar, cosmética e farmacêutica.

Keywords: essential oil, β-eudesmol, *Teucrium polium* subsp. *helichrysoides* (Diels) Maire, antioxidant activity.

RESUMO
Tem sido manifestada uma preocupação considerável relativamente à segurança dos produtos químicos utilizados nas indústrias agroalimentar, cosmética e farmacêutica. Esta preocupação está particularmente relacionada com os efeitos secundários potencialmente deletérios dos antioxidantes sintéticos, que têm sido associados a danos no fígado e à carcinogénese. Consequentemente, é imperativo explorar soluções alternativas, com as plantas medicinais a fornecerem um reservatório substancial de moléculas bioactivas que possuem propriedades biológicas. Este estudo investiga a atividade antioxidante do óleo essencial (OE) de Teucrium polium subsp. helichrysoides (Diels), originário do Sara Central e conhecido pelas suas propriedades medicinais. O óleo essencial foi extraído por hidrodestilação e o seu perfil fitoquímico foi posteriormente determinado por cromatografia gasosa e espetrometria de massa. Para verificar a atividade antioxidante do óleo essencial, foram utilizados três ensaios in vitro: eliminação do radical livre difenil-1-picrilhidrazil, poder antioxidante redutor férrico e branqueamento do β-caroteno. Os resultados demonstraram que o OE apresentava um elevado teor de sesquiterpenos, sendo o β-eudesmol (66,67%) identificado como o principal composto. Este quimiotipo β-eudesmol não foi previamente identificado na bibliografia. Apesar de exibir uma capacidade significativa para eliminar radicais livres e um poder redutor, a atividade antioxidante do OE foi comparativamente mais fraca na inibição da oxidação lipídica. A atividade antioxidante do OE foi mais fraca em comparação com a dos antioxidantes padrão. O OE de Teucrium polium L. subespécie de Hoggar contém um elevado teor de sesquiterpenos oxigenados, particularmente β-eudesmol, álcool sesquiterpenóide, que pode contribuir para as suas propriedades antioxidantes. Este facto sublinha a importância desta molécula nas indústrias agroalimentar, cosmética e farmacêutica.

Palavras-chave: óleo essencial, β-eudesmol, *Teucrium polium* subsp. *helichrysoides* (Diels) Maire, atividade antioxidante.

RESUMEN
La seguridad de las sustancias químicas utilizadas en la industria agroalimentaria, cosmética y farmacéutica ha suscitado una gran preocupación. Esta preocupación está relacionada sobre todo con los efectos secundarios potencialmente nocivos de los antioxidantes sintéticos, que se han asociado con daños hepáticos y carcinogénesis. Por consiguiente, es imperativo explorar soluciones alternativas, y las plantas medicinales constituyen una importante reserva de moléculas bioactivas con propiedades biológicas. Este estudio investiga la actividad antioxidante del aceite esencial (AE) de Teucrium polium subsp. helichrysoides (Diels), originario del Sáhara Central y conocido por sus

propiedades medicinales. El aceite esencial se extrajo por hidrodestilación y posteriormente se determinó su perfil fitoquímico mediante cromatografía de gases-espectrometría de masas. Para determinar la actividad antioxidante del aceite esencial, se emplearon tres ensayos in vitro: eliminación de radicales libres difenil-1-picrilhidrazilo, poder antioxidante férrico reductor y blanqueo de β-caroteno. Los resultados demostraron que el AE presentaba un alto contenido de sesquiterpenos, siendo el β-eudesmol (66,67%) el principal compuesto identificado. Este quimiotipo β-eudesmol no se había identificado previamente en la bibliografía. A pesar de mostrar una capacidad significativa para eliminar radicales libres y un poder reductor, la actividad antioxidante del AE fue comparativamente más débil en la inhibición de la oxidación lipídica. La actividad antioxidante del AE fue más débil en comparación con la de los antioxidantes estándar. El AE de Teucrium polium L. subespecie de Hoggar contiene un alto contenido de sesquiterpenos oxigenados, en particular β-eudesmol, alcohol sesquiterpenoide, que puede contribuir a sus propiedades antioxidantes. Esto subraya la importancia de esta molécula en las industrias agroalimentaria, cosmética y farmacéutica.

Palabras clave: aceite esencial, β-eudesmol, Teucrium polium subsp. helichrysoides (Diels) Maire, actividad antioxidante.

1 INTRODUCTION

The majority of fats present in foodstuffs are triglycerides, which are susceptible to autoxidation. This results in oxidative deterioration and the development of undesirable flavours, particularly in unsaturated fatty acids. Factors such as light, chlorophyll, metals such as iron and copper, and enzymes such as lipoxygenase accelerate this process (Schaich, 2005). Since the 1940s, synthetic antioxidants such as BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), PG (propyl gallate), and TBHQ (tertiary butyl hydroquinone) have been employed in the food industry. Despite their efficacy, synthetic antioxidants have been the subject of concern regarding their potential adverse health effects. Consequently, over the past two decades, research has increasingly focused on identifying antioxidants derived from plant materials as natural and cost-effective alternatives to synthetic antioxidants to enhance consumer health and ensure food safety (Hashemi et *al.*, 2017; Bolouri et *al.*, 2022).

The genus *Teucrium* is a large and polymorphic group within the Lamiaceae family. The Plant List includes 415 accepted scientific names for this genus, encompassing species, subspecies, varieties, forms, and hybrids, which are classified into ten sections, including *Polium* (Mill.) (Candela et *al.*, 2021).

In Algeria, *Teucrium polium* is represented by 12 subspecies, being particularly prevalent in the Saharan Atlas, here, which grow spontaneously in the stony beds of wadis at altitudes between 1200 and 2600 meters. In the central Sahara, two endemic subspecies are described: The first is *geyrii* Maire, which has white corollas and grey tomentum and is found in Tassili N'Ajjer. The second is *helichrysoides* (Diels) Maire, which has yellow corollas and tomentum and is found in the

Hoggar and Tefedest regions. The germander family is one of the most highly valued plants in the Hoggar region. The Tuaregs are well-acquainted with the plant, which they refer to as 'Tekmezzout'. The plant is used in poultices for wounds and in infusions as a stomachic and antidiarrheal remedy. Locally known as "Kheyata" or "Jaada," these aromatic plants are used in Algerian folk medicine for their hypoglycaemic, hypolipidaemic, anti-inflammatory, antibacterial, and antioxidant properties (Sihoglu Tepe et *al.*, 2021; Benchelah et *al.*, 2004).

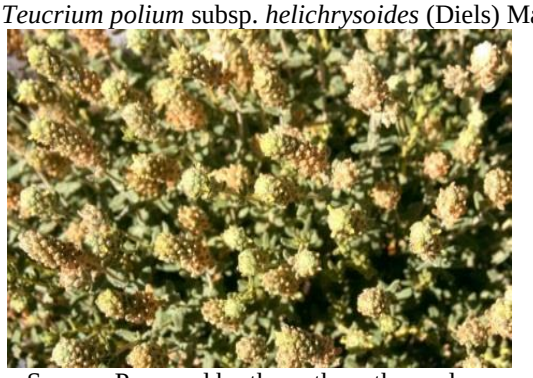
The objective of the present study is to enhance the value of our knowledge of the floral richness of the Hoggar region by investigating the chemical composition and in vitro antioxidant properties of the essential oil from *Teucrium polium* subsp. *helichrysoides* (Diels) Maire subspecies, which has been studied for the first time.

2 MATERIAL AND METHODS

2.1 SELECTION AND IDENTIFICATION OF PLANT MATERIAL

In June 2018, during the blossom time, branches of *Teucrium polium* subsp. *helichrysoides* (Diels) Maire (Figure 1) were obtained at the Ilamen station (longitude 5° 29' 30", latitude 23' 14' 26.9") in the Hoggar region (Tamanrasset, Algeria). According to Maire (1952), the plant was identified by scientist (Sahki R.) from the National Forest Research Institute (INRF) at Tamanrasset. Plant material was dried to weight stability in the shade, at ambient temperature and away from light. Dried samples were properly stored in a dry environment.

Figure 1. General appearance of a *Teucrium polium* subsp. *helichrysoides* (Diels) Maire tuft during the blossom time.



Source: Prepared by the authors themselves

2.2 ESSENTIAL OIL EXTRACTION

The air-dried leaf biomass was subjected to hydro-distillation for 4 hours in a Clevenger apparatus in accordance with the European Pharmacopoeia (2008). The yield of essential oil was expressed as w/w with respect to dry matter. The EO was filtered and stored in the dark at 4°C prior to analysis and bioassay testing.

2.3 MASS SPECTROMETRY ANALYSIS

The essential oil of *Teucrium polium* subsp. *helichrysoides* (Diels) Maire was analysed by gas chromatography-mass spectrometry (GC-MS) using a Hewlett Packard 6890 plus gas chromatograph coupled to Hewlett Packard Agilent 5973 mass spectrometry. The capillary column used was HP-5 MS (stationary phase: 5% Phenyl 95% dimethylpolysiloxane) with the following characteristics: length, 30 m; internal diameter, 0.25 mm; film thickness 0.25 µm. The helium was used as the carrier gas at a flow rate through the column 0.5 mL/min. Injection mode was split 1/80, the injector temperature was maintained at 250 °C and the flame-ionisation detector (FID) at 230°C. The oven temperature was programmed as follows: initial temperature 60°C for 8min, programmed rate 2°C/min up to final temperature 250°C with isotherm for 10 min. The injected volume of fixed oils is 0.2 µL. Ionisation energy 70 eV in electronic ionisation mode; ion source temperature 230°C, scan mass range m/z 30-550 and interface line temperature 280°C.

2.4 EVALUATION OF ANTIOXIDANT ACTIVITY

2.4.1 DPPH radical scavenging assay

The antiradical potential of *Teucrium polium* subsp. *helichrysoides* (Diels) Maire essential oil was evaluated using the free DPPH radical assay as described by Kedir et *al*. (2023), with slight modifications. Briefly, 1 mL of DPPH solution (0.1 mM) was combined with 1 mL of essential oil dissolved in ethanol at different concentrations. The mixtures were then incubated at 27°C for 30 minutes. As the reduction occurs, the colour of the solution gradually diminishes, thereby facilitating the efficient tracking of the reaction's progress via the use a UV-visible spectrophotometer at 517 nm. The concentration of DPPH• in the reaction medium was determined using the following equation, obtained through calibration curve:

$$A_{517nm} = 15.033\ [DPPH\bullet]_T - 7.1\times10^{-3}$$

Where $[DPPH\bullet]_T$ is expressed in g/L, with a correlation coefficient of R^2=0.99. The remaining percentage of DPPH (DPPH• rem) for different antioxidants at varying concentrations was calculated using the equation:

$$\%\ DPPH\bullet_{REM} = [DPPH\bullet]_T / [DPPH\bullet]_{T=0}$$

The amount of antioxidant required to reduce the initial DPPH• concentration by 50% was determined and reported as EC50. The positive controls employed were the synthetic antioxidants butylhydroxytoluene (BHT), butylated hydroxyanisole (BHA), and ascorbic acid (vitamin C).

The equilibrium time was determined by examining the kinetics of reactions between the tested essential oils and DPPH• at EC_{50}. The parameter TEC_{50} is defined as the time required to reach equilibrium with an antioxidant concentration equal to EC_{50} (Popovici et *al.*, 2009).

The antiradical efficiency is a parameter that relates the concentration of DPPH• and the time required for the TEC_{50} assay to occur, with the effective concentration EC_{50} of the sample. This results in a constant parameter for each solution (Popovici et *al.*, 2009). The following formula is used to determine this value:

$$ARE = 1/ (EC_{50} \times TEC_{50})$$

where ARE: Antiradical Efficiency, EC_{50}: Median Effective Concentration (mg antioxidant/g DPPH•), ETC_{50} represents the time (min) required for equilibrium to be reached. The antiradical efficiency of the tested solutions was estimated based on the classification proposed by Sanchez- Moreno et *al.* (1998).

2.4.2 Ferric ion reducing antioxidant power (FRAP) assay

The reductive power of *Teucrium polium* subsp. *helichrysoides* (Diels) Maire essential oil was examined adopting the method of Bourkhis et *al.* (2010). The control consists of a solution of a synthetic antioxidant (BHT, BHA, vitamin C). The reductive ability was determined by calculating the EO and standards antioxidant concentration corresponding to an absorbance of 0.5, and reported as an EC_{50} value (µg/mL).

2.4.3 β-carotene bleaching (Inhibition of lipid peroxidation) assay

The β-carotene bleaching assay was employed to assess the inhibitory capacity of *Teucrium polium* subsp. *helichrysoides* (Diels) Maire essential oil against lipid peroxidation, as previously documented by Tepe et *al*. (2004). The decolourisation kinetics of the emulsion were monitored at regular time intervals over 48 hours at a wavelength of 470 nm. The percentage of antioxidant activity (AA) of the tested samples (essential oil, BHT, BHA, vitamin C) was calculated using the following equation:

$$AA\ \% = (Abs_{\text{sample 2H}} - Abs_{\text{control 2H}}) / (Abs_{\text{sample 0H}} - Abs_{\text{control 2H}})$$

Where $Abs_{\text{sample 0H}}$ and $Abs_{\text{sample 2H}}$ represent the absorbances in the presence of the tested samples at 0 and 2 hours, respectively, and $Abs_{\text{control 2H}}$ denotes the absorbance without samples at 2 hours.

2.5 DATA ANALYSIS

Each experiment was replicated at least three times and data were expressed as mean ± standard deviation. The data were analysed using the SPSS 18 statistical software package (IBM Corp., 2010). One-way analysis of variance (ANOVA) was performed. A series of post-hoc comparisons were made using the Tukey test to compare means. Different letters (a to d) indicate that the means are significantly different (*** Significant at Tukey test $p \leq 0.05$)

3 RESULTS

3.1 PHYTOCHEMICAL SCREENING OF THE ESSENTIAL OIL

The essential oil obtained from the aerial parts of *Teucrium polium* subsp. *helichrysoides* (Diels) Maire, harvested at its flowering period, was pale yellow in colour, with a yield of 0.8 ± 0.02% (w/w). Through GC/MS analysis, we identified 47 compounds (as presented in Table 1), accounting for 96.64% of the sample. The compounds were listed in ascending order based on their (RI). The essential oil content was primarily composed of oxygenated sesquiterpenes (70.76%), followed by hydrocarbon monoterpenes (14.20%), and a small amount of oxygenated monoterpenes (6.01%) and hydrocarbon sesquiterpenes (5.69%). The essential oil is composed mainly of β-eudesmol, which accounts for 66.67% of the total composition. Smaller amounts of caryophyllene, α- Thujene, and ρ-Cymene are also present, at 3.91%, 3.43%, and 2.53%, respectively. This is the first report of the β -eudesmol *Teucrium polium* L. chemotype, which was not previously identified in the bibliography.

Table 1. Chemical Composition of *Teucrium polium* L. subsp. geyrii Maire essential oil.

Nº	Compounds	(%)	RT	KI_a	KI_b	Nº	Compounds	(%)	RT	KI_a	KI_b
01	α- Thujene	**3.43**	9.26	936	930	25	Cis Linalool oxide	0.22	25.89	1174	1174
02	α-Pinene	1.55	9.59	941	939	26	terpinen 4-ol	2.01	26.18	1178	1177
03	2,4-Thujadiene	0.20	10.20	950	960	27	α-Terpineol	0.16	26.84	1188	1188
04	Sabinene	1.09	11.93	975	975	28	myrtenal	0.07	27.39	1196	1195
05	β-pinène	0.79	12.08	977	979	29	verbenone	0.10	28.41	1210	1205
06	β -myrcène	1.26	13.11	992	990	30	Cis carveol	0.09	29.22	1222	1229
07	α-Terpinene	0.36	14.69	1014	1017	31	Cuminaldehyde	0.14	30.46	1240	1241
08	ρ-Cymene	**2.53**	15.31	1023	1024	32	Carvone	0.11	30.79	1244	1243
09	Limonene	2.11	15.57	1027	1029	33	Carvenone	0.18	31.41	1253	1258
10	Trans-β-Ocimene	0.25	16.99	1047	1050	34	Carvacrol	0.32	34.61	1299	1299
11	γ-Terpinene	0.63	17.63	1056	1059	35	β-caryophellene	**3.91**	42.70	1419	1419
12	Trans-sabinène hydrate	0.08	18.60	1070	1070	36	α-caryophellene	0.78	44.25	1446	1454
13	Trans Linalool oxide	0.13	19.88	1088	1088	37	α-selinene	0.78	46.96	1493	1498
14	Linalool	0.12	20.40	1096	1096	38	Y- cadinene	0.05	47.98	1510	1513
15	α-thujone	0.41	20.76	1101	1102	39	δ-Cadinene	0.16	48.57	1520	1523
16	β-thujone	0.46	21.75	1115	1114	40	Caryophyllenyl alcohol	0.53	50.22	1549	1572

17	Dehydro sabina ketone	0.25	22.14	1121	1120	41	Germacrene D-4-ol	0.13	51.66	1574	1575
18	α-Campholenal	0.17	22.52	1126	1126	42	Spathulenol	0.06	51.78	1576	1578
19	trans-Dihydro-β-terpineol	0.13	23.31	1137	1138	43	β - Caryophyllène oxide	1.57	52.04	1580	1583
20	Trans-Pinocarveol	0.21	23.52	1140	1139	44	Humulene epoxide II	0.96	53.44	1604	1608
21	Camphor	0.21	23.88	1146	1146	45	τ-Cadinol	0.33	55.56	1641	1640
22	Sabina ketone	0.27	24.75	1158	1159	46	β-Eudesmol	**66.67**	56.09	1650	1650
23	β-Terpineol	0.07	24.98	1161	1163	47	α-Bisabolol	0.51	58.22	1687	1685
24	Umbellulone	0.09	25.67	1171	1171		Total		96.64%		
Oxygenated Sesquiterpenes							70.76%				
Hydrocarbons Sesquiterpenes							5.69%				
Hydrocarbons Monoterpenes							14.20%				
Oxygenated Monoterpenes							6.01%				

Source: Prepared by the authors themselves

3.2 ANTIOXIDANT ACTIVITY

3.2.1 DPPH radical scavenging assay

The antioxidant activity of *Teucrium polium* subsp. *helichrysoides* (Diels) Maire essential oil and synthetic antioxidants (BHT, BHA, vitamin C) was evaluated using a spectrophotometer. The results demonstrate that the remaining percentage of DPPH• is inversely proportional to the concentration of the essential oil of the plant under investigation. As the concentration of the essential oil increases, the remaining percentage of DPPH• decreases until a plateau is reached, corresponding to the near total exhaustion of the DPPH• present in the reaction medium (Figure 2A). The essential oil of Hoggar *Teucrium polium* L. *helichrysoides* subspecies demonstrated a remaining percentage of DPPH• of 18.49 ± 0.26% at a concentration of 4500µg/ml.

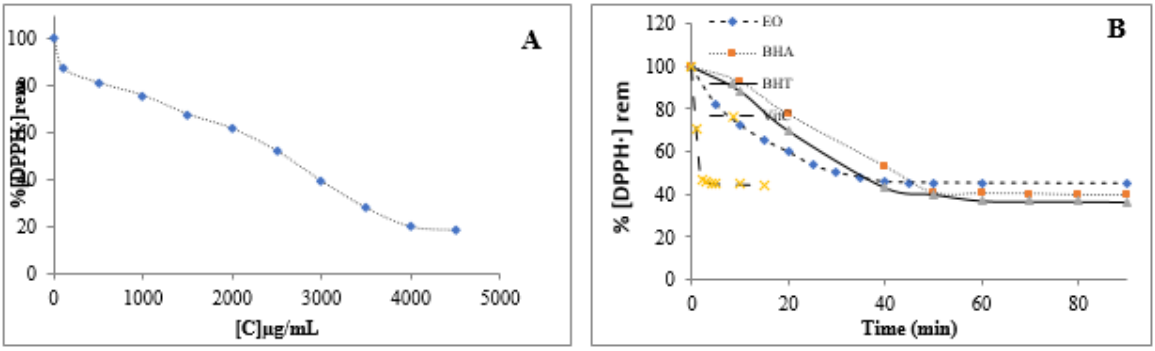
In order to overcome the influence of concentration, the reactivity is estimated by the effective concentration EC_{50} of the antioxidant. The EC_{50} index is defined as a 50% reduction in the activity of DPPH• in the reaction medium. This is inversely proportional to the antioxidant capacity of the compound in question. Therefore, the higher the antioxidant capacity, the smaller the value of EC_{50}. The essential oil of *Teucrium polium* subsp. *helichrysoides* (Diels) Maire has been demonstrated to convert the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH•) to a stable diphenylpicrylhydrazine (DPPH-H) with an EC_{50} of 2609.26±33.35 µg/mL. This process has been shown to result in significantly ($p \leq 0.05$) lower antioxidant activity and capacity to trap the DPPH free radical compared to synthetic antioxidants (Table 2). It appears that the most effective antioxidant is vitamin C (ascorbic acid), with an EC_{50} of 1.91 ± 0.015µg/mL.

The reduction kinetics of $[DPPH•]_{Rem}$ obtained for the EC_{50} concentration of the essential oil and synthetic antioxidants are shown in Figure 2B. The results obtained and the estimation of the TEC_{50} provided by Brand-Williams et *al.* (1995) indicate that the synthetic antioxidant (vitamin

C) reacts more rapidly with DPPH•. It requires 1.85 minutes to decrease the concentration of the free radical by 50%. In contrast, the reaction rates of BHT and BHA are significantly slower, with reaction times of 35.29 minutes and 43.12 minutes, respectively. The essential oil under examination displays an intermediate reaction profile, with a TEC_{50} of 25.20 minutes.

The essential oil was found to exhibit biphasic reaction kinetics, with two distinct zones. The first zone, which was observed within the first 25 minutes, displayed a high kinetic trapping zone of the radical. The second zone, which displayed a low kinetic trapping zone of the DPPH radical or a tendency towards equilibrium, was also observed.

Figure 2. A. The remaining [DPPH•] reduction kinetics as a function of essential oil concentration. B. The [DPPH•] remaining kinetics reduction at the IC_{50} concentration over time.



Source: Prepared by the authors themselves

3.2.2 Antiradical efficiency

The antiradical efficiency has been defined, which combines the two parameters (EC_{50} and ETC_{50}) to facilitate the characterisation of a substance's antioxidant behaviour. The parameters for calculating antioxidant activity are summarised in the table 2.

Table 2. The parameters required for the calculation of ARE by the DPPH method.

Substances	EC_{50} (µg/mL)	EC_{50} (g/kg DPPH)	ETC_{50} (min)	ARE (10^{-3})	Classification
Essential oil	2609.26±33.35 [a]	65231.58±613.39	25.20 ±0.29 [a]	0.0005[a]	Low
BHT	5.04±0.044 [b]	126.14±0.11	35.29±0.34 [b]	0.224[b]	Low
BHA	5.30±0.049 [b]	132.59±0.86	43.12±0.19 [b]	0.175[b]	Low
Ascorbic acid	1.91±0.015 [c]	47.90±0.27	1.85 ±0.03 [c]	11.28[c]	Very high
Significance	***	***	***	***	

Different letters (a to c) indicate that means are significantly differences (one-way ANOVA analysis of variance, Tukey test *** Significant at $p \leq 0.05$ level).

Source: Prepared by the authors themselves

The antiradical efficiency, as classified by Sanchez- Moreno et *al.* (1998), is notably low for the essential oil and high to very high for the tested synthetic antioxidants. However, the antiradical efficiency of the essential oil is significantly ($p \leq 0.05$) lower than that of the synthetic antioxidants.

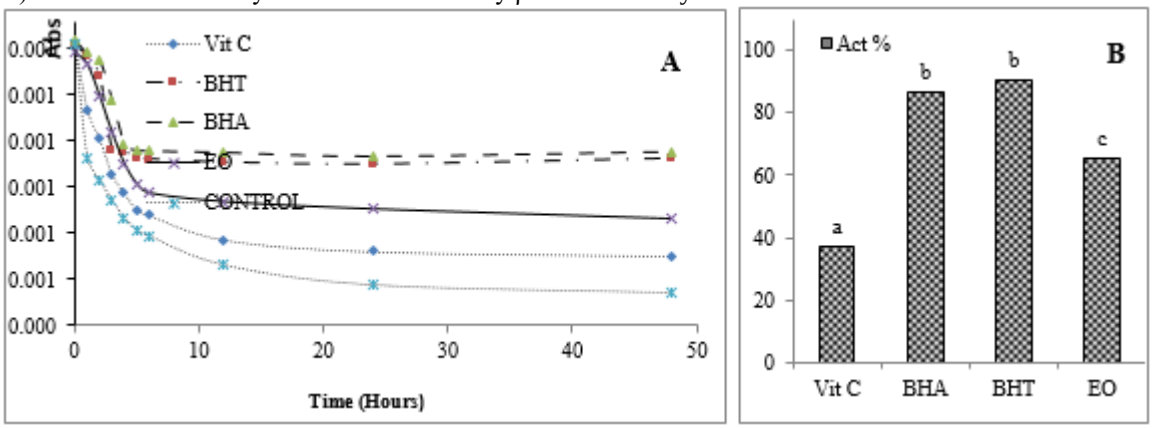
Consequently, when the results are compared, the antioxidant activity of the substances tested by DPPH essay is ranked in the following order: Ascorbic Acid >BHT>BHA > Essential oil.

3.2.3 β-carotene bleaching (Inhibition of lipid peroxidation) assay

The study on the kinetics of β-carotene bleaching demonstrated that the essential oil, synthetic antioxidants (BHA, BHT, ascorbic acid), and the negative control exhibited analogous absorbance patterns, indicating comparable interpretations (Figure 3A). At the initial time point (t_0), the absorbance is approximately 0.75, which reflects the absence of free radicals. Subsequently, there is a gradual decline in absorbance over the subsequent two hours, which can be attributed to the emergence of free radicals. Following a period of 12 to 48 hours, the bleaching process reaches a stationary state, with β-carotene fully degraded and the yellow colour irreversibly lost. This is referred to as the 'exhaustion time'. The negative control demonstrated a rapid degradation of β-carotene, which can be attributed to the absence of antioxidant protection against oxidation.

The essential oil failed to achieve total inhibition of the coupled oxidation of linoleic acid and β-carotene. At a concentration of 2 mg/ml, the inhibition rate demonstrated was 67.62 ± 1.64%, which was significantly lower ($p \leq 0.05$) than the inhibition rates of BHT (90.65 ± 1.46%) and BHA (86.83 ± 1.88%). However, it was significantly higher ($p \leq 0.05$) in comparison to Vitamin C, which demonstrated an inhibition rate of 37.49 ± 1.56% (Figure 3B).

Figure 3. A. The kinetics of β-carotene bleaching by essential oil and synthetic antioxidants. **B.** Antioxidant activity (Act %) of essential oil and synthetic antioxidants by β-carotene assay.



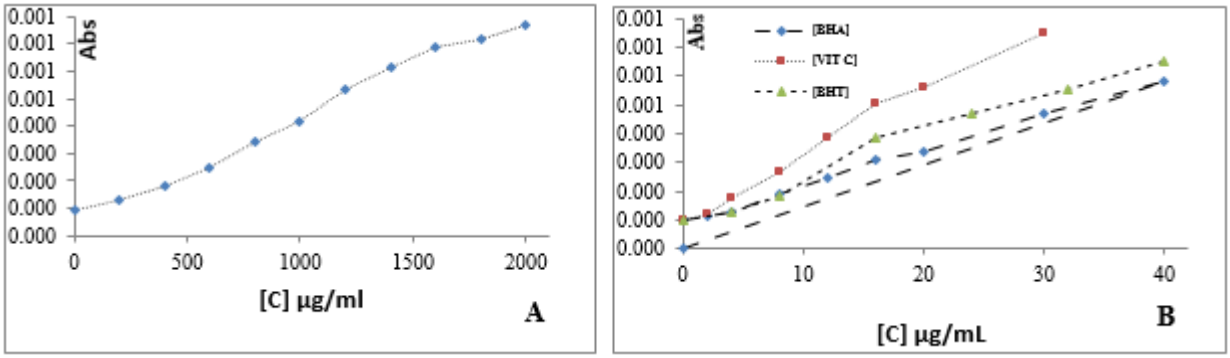
Source: Prepared by the authors themselves

3.2.4 Ferric ion reducing antioxidant power (FRAP) assay

The FRAP method employs an electron transfer mechanism to inactivate free radicals. This process assesses reducing agents that reduce the Fe^{3+} ferricyanide complex to Fe^{2+} in an acidic environment via electron transfer. The generation of Fe^{2+} can be quantified by measuring the formation of Perl's Prussian blue at 700 nm (Jayanthi and Lalitha, 2011). The dose- dependent

curves, which illustrate the reducing power of *Teucrium polium* subsp. *helichrysoides* (Diels) Maire essential oil and synthetic antioxidants as positive controls, are presented in Figures **4A** and **4B**. At a concentration of 2000 µg/mL, the essential oil exhibited the highest reducing power, with an absorbance value of A700=0.768.

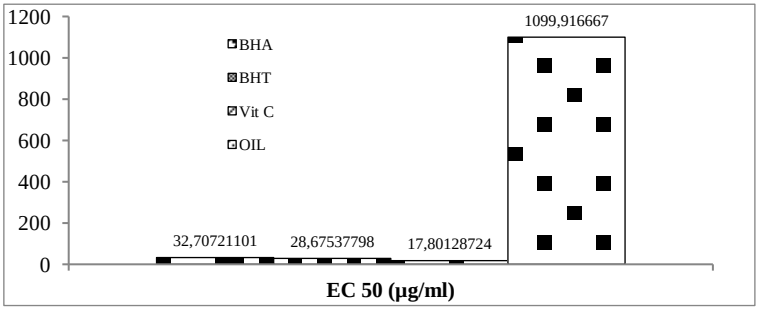
Figure 4. Reducing power of the tested essential oil **(A)** and synthetic antioxidants **(B)**



Source: Prepared by the authors themselves

The figure 5 indicates that the studied essential oil exhibited minimal antioxidant activity, with an EC_{50} value of, 66 µg/mL, in comparison to the antioxidant activity of the standard antioxidants (BHA, BHT, Vitamin C), which had EC_{50} values ranging from 17.80 to 32.71 µg/mL.

Figure 5. Median Effective Concentration (EC_{50})



Source: Prepared by the authors themselves

4 DISCUSSION

This study represents the inaugural documentation of β-eudesmol as a predominant compound in *Teucrium polium* L., as it has not been previously referenced in the scientific literature. These results are consistent with previous studies, which indicate that the essential oils of *Teucrium* species are predominantly composed of sesquiterpenoids (De Martino et *al.*, 2020). According to the Köppen-Geiger classification, the Hoggar region is classified as a hot desert climate (BWh). According to Tsusaka et *al*. (2020), induce the production of sesquiterpenoids, in response to biotic and abiotic stresses, as well as soil acidity. The studies also show that the absolute content of β-eudesmol varies depending on environmental factors such as biological and abiotic stress. *Teucrium*

polium L. essential oil exhibits significant chemical polymorphism even among individuals of the same species with identical genomes and phenotypes. Most of the minor compounds present in the volatile oil of *Teucrium polium* subsp. *helichrysoides* (Diels) Maire have also been identified in *Teucrium polium* L. from various geographical locations in Algeria, albeit at varying concentrations. For instance, the essential oil of *Teucrium polium* L. from Mascara is dominated by limonene, which is only weakly present in our essential oil at a level of 2.11% (Maizi et *al.*, 2019). Hammoudi et *al.* (2013) reported that the the *Teucrium polium* L. subsp. *geyrii* Maire from Hoggar contained mainly sesquiterpene hydrocarbons. They also found that the Hoggar region had a limonene chemotype. The Djelfa region's essential oil contains a significant amount of β-pinene (13.26%) and limonene (12.65%) as reported by Touaibia et *al.* (2021). In the Tlemcen region, the essential oil is dominated by Germacrene D, according to Bendjabeur et *al.* (2018). Meanwhile, Kerbouche et *al.* (2015) identified δ-cadinol chemotype in a species of *Teucrium polium* L. collected from the wilaya of M'sila. The essential oil under study contains 3.91% β-caryophyllene, making it the second most abundant compound. El Atki et *al.* (2020) identified it as the primary constituent of the essential oil extracted from *Teucrium polium* subsp. *Aurum* species in Morocco.

Gorgini Shabankare et *al.* (2015) demonstrated that different bioclimatic stages significantly influence the phytochemical diversity of Teucrium polium L. in Iran. Sub-humid and semi-arid populations exhibited higher levels of β-caryophyllene, while arid regions showed greater concentrations of farnesene and linalool. Hechachna et *al.* (2023) additionally highlighted the potential for selective harvesting at specific periods to yield essential oils with distinct chemotypes. Asgharipour and Shabankare (2017) also demonstrated that the chemical composition of essential oils varies by phenological stage. During full flowering, the essential oils are dominated by sesquiterpenes, while monoterpenes are prevalent during vegetative growth. Furthermore, Jalali et *al.* (2021) emphasised that the extraction method and plant parts employed can influence the essential oil profile. Boulila et *al.* (2008) also demonstrated that genetic factors contribute to phytochemical variation in Tunisian *T. polium* L. populations. Recent advancements, such as the integration of RAPD markers with statistical tools, have enhanced the ability to differentiate genotypes, suggesting that future studies should integrate morphological, chemical, and molecular markers (Barra, 2009).

4.1 ANTIOXIDANT ACTIVITY

The complexity of phytochemicals precludes the possibility of accurately assessing the total antioxidant activity plant extracts using a single method. It is therefore essential to employ a minimum of two methods to evaluate the overall antioxidative effects of plant extracts (Brand-

Williams et *al.*, 1995). The in vitro antiradical activity of essential oil from *Teucrium polium* subsp. *helichrysoides* (Diels) Maire was evaluated using three spectrophotometric assays: DPPH, β-carotene bleaching with linoleic acid, and FRAP tests. It is noteworthy that this is the first report on the antioxidant activities of the essential oil from this subspecies.

The essential oil of *Teucrium polium* subsp. *helichrysoides* (Diels) Maire exhibited a significant capacity to scavenge DPPH free radicals, with an EC_{50} value of 2609.26±33.35 µg/mL, which was in accordance with the results of the reducing power assay, which yielded an EC_{50} value of 1099.92± 5.21 µg/mL. However, it demonstrated comparatively weaker performance in inhibiting lipid oxidation, achieving only 65.54 ± 1.64% inhibition of linoleic acid oxidation at 2 mg/mL. Overall, Tukey's test clearly indicate that this essential oil is significantly ($p \leq 0.05$) less effective as an antioxidant compared to the standard antioxidant.

In a recent study, Chen et al. (2023) posited that the low antioxidant activity observed in essential oils is likely attributable to the prevalence of non-phenolic compounds, which exhibit markedly weak antioxidant properties. The DPPH• test is qualitative and, while it follows the principle outlined by Brand-Williams et *al*. (1995), discrepancies in analytical protocols may impact the reliability of antioxidant activity comparisons with previous findings in the literature.

The DPPH radical scavenging ability of the essential oil under study appears to be significant in comparison to that documented by Maizi et *al*. (2019), who reported low antioxidant activity for essential oils isolated from *Teucrium polium* L. during flowering, with IC_{50} values of 16.14 and 9.2 mg/mL, Similarly, El Atki et *al*. (2020) reported comparable effects for oils derived from two subspecies of *Teucrium polium* L. (*aurum* and *polium*), with IC_{50} values of 3.7 and 7.2 mg/mL, respectively.

The antioxidant capacity of the studied essential oil can be attributed to the presence of certain components with antioxidant activity. It has been indicated that essential oils which are rich in monoterpenes, oxygenated monoterpenes, and/or sesquiterpenes tend to demonstrate a greater and higher antioxidative potential (Tepe et *al.*, 2004). According to Ruberto and Baratta (2000), these compounds are primarily responsible for the antioxidant potential of essential oils due to their ability to act as hydrogen or electron donors, leading to the reductive transformation of the free radical DPPH• DPPH• into a stable molecule DPPH-H, and consequently, the formation of a yellow colouration. As elucidated by Popovici et *al.* (2009), the process of free radical scavenging by antioxidants occurs through two distinct mechanisms. The first is rapid kinetics, which is characterised by the release of a hydrogen atom from the hydroxyl group. The second is slower kinetics, which involves the release of an electron.

The essential oil under examination is primarily composed of oxygenated sesquiterpenes. It

is widely acknowledged that sesquiterpenes typically exhibit a reduced antioxidant capacity in comparison to monoterpenes. Indeed, essential oils comprising a high proportion of oxygenated compounds have been demonstrated to exhibit superior antioxidant properties (Okoh et *al.*, 2011). However, oxygenated compounds, particularly alcohols, are known to enhance free radical scavenging and reduce lipid peroxidation, primarily due to the presence of hydroxyl groups in their chemical structure (De Moraes et *al.*, 2022). In a study conducted by Jaradat et *al.* (2018), it was demonstrated that *T. pruinosum* essential oil with a high content of sesquiterpenoid alcohols (50.79%) exhibited significant antioxidant activity. It is probable that these characteristics contribute to the antioxidant activity observed in the essential oil derived from *Teucrium polium* subsp. *helichrysoides* (Diels) Maire against DPPH• radicals.

It is expected that a lower (TEC_{50}) and an EC_{50} lower will result in an increased ARE. Table 2 provides an illustrative example of the significance of the ARE. Despite comparable EC_{50} values for BHT and ascorbic acid, the ARE for BHT was 50 times higher than that for ascorbic acid. This evidence substantiates the assertion that the ARE is a more appropriate parameter than the EC_{50}, which is a widely used measure, for the selection of antioxidant compounds. The ARE indicates that *Teucrium polium* subsp. *helichrysoides* (Diels) Maire essential has low antioxidant efficiency, comparable with that of BHT and BHA.

The reducing power of the examined essential oil from *Teucrium polium* subsp. *helichrysoides* closely corresponds to the IC_{50} of 980 µg/mL reported by Touaibia et *al.* (2021) for the essential oil of *Teucrium polium* L. from Djelfa, Algeria. Consequently, reducing power serves as a pivotal indicator of a compound's potential antioxidant capacity, as antioxidants are recognised for their role as reductants and inactivators of oxidants (Bougandoura and Bendimerad, 2012).

It can thus be posited that the antioxidant activity observed in the essential oil of *Teucrium polium* subsp. *helichrysoides* (Diels) Maire is likely due to its major compounds, notably beta-eudesmol, which contains hydroxyl groups. As demonstrated by Kim et *al.* (2018), the antioxidant efficacy of this compound reaches 49% at a concentration of 20 µM (4.4 µg/mL), which is comparable to that BHT and BHA in this study. Wang et *al.* (2019) indicate that the terpenes α-pinene, limonene, myrcene, linalool, and terpineol, which are present in minor quantities in the studied essential oil, were identified as effective DPPH scavengers and reducing agents. The free radical scavenging activity and reducing power of α-pinene were the most pronounced, while the sesquiterpene hydrocarbon β-caryophyllene demonstrated consistently low levels of activity in line with the findings of Ruberto and Baratta (2000).

The linoleic acid free radical, generated by the abstraction of a hydrogen atom from one of its diallylic methylene groups, initiates an attack on the highly unsaturated β-carotene molecule.

This interaction results in the oxidation of β-carotene, leading to a loss of its characteristic orange colour. This change is then quantified spectrophotometrically (Okoh et *al.*, 2011). This phenomenon can be explained by the paradox of polar phenomena described by Laguerre et *al.* (2015). This is because the β-carotene bleaching test resembles a lipid emulsion system in water, were linoleic acid forms micelles in aqueous systems.

The structural features of antioxidants, such as the specific arrangement of hydroxyl groups, can at times play a more pivotal role than their polarity in determining antioxidant effectiveness (Anurak and Kesara, 2021; Gadoum et *al.*, 2024). Moreover, the complex composition of essential oils can result in a range of interactions, including synergistic, additive, or antagonistic effects, between their constituents and the surrounding medium. These interactions can influence the distribution of antioxidants within different phases of an emulsion (Anurak and Kesara, 2021; Koleva et *al.*, 2002).

5 CONCLUSION

The essential oil *Teucrium polium* L. subspecies *helichrysoides* (Diels) Maire from Hoggar contains a high content of oxygenated sesquiterpenes, particularly β-eudesmol, a sesquiterpenoid alcohol, which contribute to its antioxidant properties. This highlights the potential of this molecule as a natural food preservative in the agro-food industry. To the best of our knowledge, this β-eudesmol chemotype has not been previously identified in the literature. It is recommended that future research focus on developing strategies to address the challenges of using this oil as a sustainable food preservative, such as exploring nano-encapsulation, which offers a potential solution by enhancing the oil's efficacy through controlled release. In addition, a prerequisite for the advancement of this field is the thorough evaluation of the safety, environmental impact, and scalability of this technology.

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