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Effect of bioclimatic and edaphic condition on metabolite recovery diversity of *Thymus algeriensis* essential oils

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Abstract
To delineate the variability of bioactive compounds extracted from the aerial parts of *Thymus algeriensis* essential oil collected from four mounts in Tunisia, we investigated its amounts. Chemical Variations in the terpenes of the aerial parts essential oils of wild *Thymus algeriensis*, which were grown in four sampling sites, were examined by GC-MS. Our finding suggested that the essential oil of four populations contain high amount of terpenic compounds. Results show that this specie contain a high percentage of eucalyptol (9.36 o 13.37%) and camphor (6.50 o 17.64%). Soil edaphic and bioclimatic condition induce the diversity of bioactive compounds of 4 populations of *Thymus*.

Keywords: *Thymus* EOs, yield, terpenic compounds

1. Introduction
Plants continue to be major source of medicine, as they have throughout human history [1]. The study of endemic plants could support their ethnobotanical use by means of a wide comprehension of the qualitative and quantitative phytochemical profile [2]. The *Lamiaceae* family, one of the most important herbal families, incorporates a wide variety of plants with biological and medical applications. The most known members of this family are a variety of aromatic spices like thyme, mint, oregano, basil, sage, savory, rosemary, self-heal, hyssop, lemon balm, and some others with more limited use [3]. From this family, *Thymus* genus, part of the *Lamiaceae* family, consists of over 350 species of aromatic plants with evergreen leaves, prevalent in the Mediterranean area [4]. Geographically, these plants extend to Asia, North Africa, and Europe. Although more than one species is cultivated for culinary (cheese and liqueur flavor agent) or ornamental use [5]. *Thymus* leaves extracts, despite their frequent use as spice and infusions, are used in traditional medicine as astringent, expectorant, antiseptic, anti-rheumatic, diuretic, analgesic and cicatrizing agents [2]. Leaves of *T. algeriensis* are rich with essential oils contained, such as linalool (17.62%), camphor (13.82%), terpinen-4-ol (6.80%), α-terpineol (6.41%), together with small amount of thymol [6]. Such variation in chemical composition of the essential oil obtained from different populations were described by Guesmi *et al*. [7]. Currently, many authors found that the therapeutic functions and the effectiveness of Tunisian *Thymus* is attributed to their abundant phenolic/terpenic compounds, including thymol, carvacrol, terpinen-4-ol, α-bisabolol and (+)-epi-Bicyclosesquiphellandrene [8]. Several reports have shown the variation in the content and terpenic composition of the *Thymus algeriensis* essential oil originating from different locations of the world [7, 4]. The overall aim of this research is to outline the folk uses of *Thymus* by profiling its major chemical composition through Gas Chromatography-Mass Spectrometry (GC/MS) and evaluating its hydrophobic fractions for total phenolics content.

2. Materials and Methods
2.1 Plant material
Thyme essential oils used in this report extracted from 4 populations of *T. algeriensis* were collected in four Tunisian mountainous area (“Orbata”, “Swinia”, “Berda”, “Asker” mount) (Fig.1, Table 1), which are geographically isolated. Voucher specimens have been deposited at the Herbarium of the National Institute of Agronomic of *Tunisia* (INAT) under registration number 1188.

Table 1: The collected plant parts, collection sites, Ethnobotanical indications of essential oils.

N°	Code	Scientific name	Family	Voucher Specimen	Local name	Localisation	Part used; Extraction	Traditional therapeutic indications	References
1	T	*Thymus hirtus sp.algeriensis*	Lamiaceae	n.1188	Moujecha	Jbel Orbata- Gafsa-Tunisia	Upper parts; hydrodistillation	Ulcers and toxicity *in vitro* and *in vivo*	[7, 8].
						Jbel Berda- Kebelli-Tunisia	Upper parts; hydrodistillation		
						Jbel Asker- Guettar-Tunisia	Upper parts; hydrodistillation		
						Jbel Swinia- Sidi Bouzid-Tunisia	Upper parts; hydrodistillation		

2.2 Essential oil extraction

One hundred grams of air-dried aerial parts of thyme was distilled in 500 ml distilled water for 3 h. Essential oils (EOs) were distilled with a Clevenger apparatus. The yield of essential oil is expressed as a percentage of the mass of the essential oil with respect to the air-dried material (% v/w of dried material) [9]. The thyme essential oils (yields of TJO, TJS, TJB and TJA respectively 11 % (v/w), 6 % (v/w), 17 % (v/w) and 2.4 % (v/w). Purified EO was stored in the dark at 4 °C and freshly diluted as needed *in vitro* experiment. A GC/MS apparatus was used to profile the terpene composition.

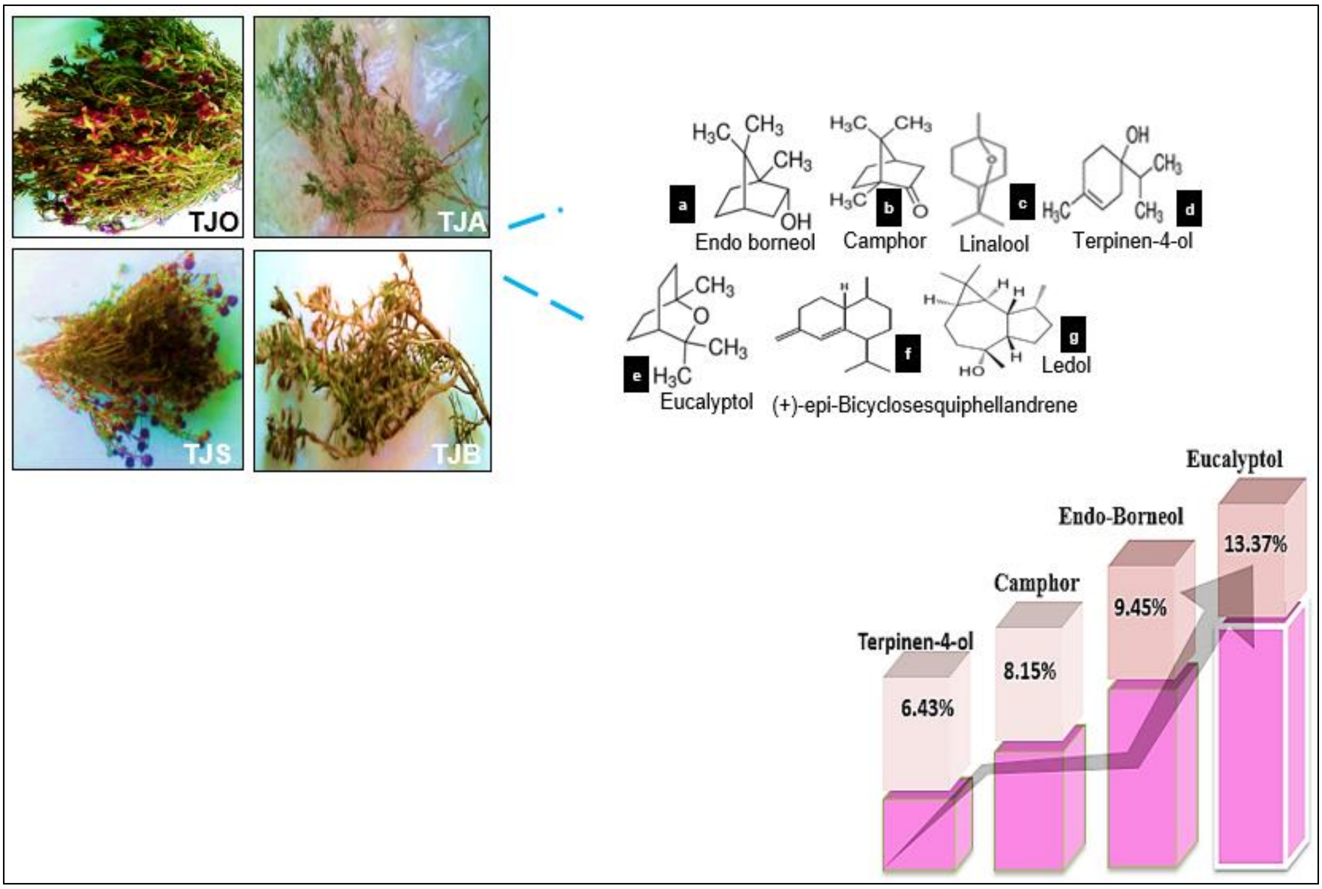

Fig 1: Recoveries of major terpenic components extracted from *Thymus* essential oil

2.3 Analysis of hydrophobic fractions of *Thymus* with GC/MS

Thymus EOs was analysed using gas chromatography coupled to mass spectrometry (GC–MS) on an Agilent 6890 gas chromatograph with autosampler coupled to an Agilent 5973 MSD detector (Agilent Technologies, Palo Alto, CA, USA) with an electron impact ionization of 70 eV. At a temperature programmed to rise from 40 °C to 280 °C at a rate of 5 °C/min A, we used Phenomenex ZB-5MSi capillary column (30 m×0.25 mm, 0.5 µm film thickness; Agilent Technologies, Hewlett-Packard, CA, USA). Helium (99.999% purity) was used as gas carrier with a flow rate of 0.7 mL/min, a split ratio of 60:1, scan time and mass range of 1s and 50-550 m/z, respectively. Identification of EOs was realized by matching the recorded mass spectra with those stored in the Wiley 09 NIST 2011 mass spectral library of the GC/MS data system.

2.4 Statistical Analysis

The results were expressed as mean ± SD. Differences between assays were compared by a one-way analysis of variance (ANOVA). A value of $p < 0.05$ was considered statistically significant.

3. Results and Discussion

3.1 The percentage Yield of *T. algeriensis* essential oil

As reported in Fig. 2, the percentage yield were too high and ranged from 2.4% to 17% (v/w). Our results showed that statistically significant differences of yield was observed and the best one was detected in the EO of TJB and TJO (inferior arid bioclimate) ($P < 0.05$). Population location, altitude, soil edaphic condition and bioclimatic stage affects the percentage yield variation. Similar results were previously obtained for the yields of volatiles of *T. algeriensis* collected from 8 locations [4].

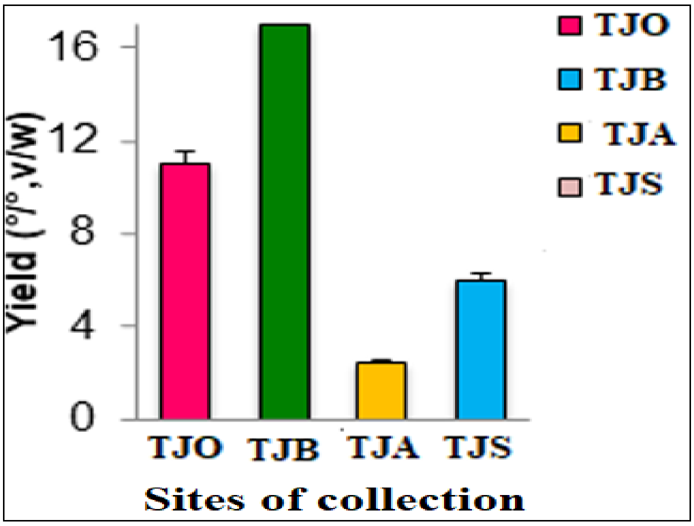

Fig 2: The percentage Yield of thyme essential oil. TJO: *Thymus algeriensis* collected in the Orbata mount; TJB: *Thymus algeriensis* collected in the Berda mount; TJA: *Thymus algeriensis* collected in the Asker mount; TJS: *Thymus algeriensis* collected in the Swinia mount.

3.2 Characterisation of Hydrophobic fractions of *Thymus* with GC/MS

In the present research, we found that amongst all samples, the best yields were obtained with *Thymus* collected in Berda mount (TJB), and this may be attributed to its geographical distribution and climate of this location. Moreover, *Thymus* EOs were analysed qualitatively and quantitatively with the developed GC/MS method for the estimation of terpenic compounds obtained by extraction with Clevenger apparatus. As listed in Figure 1, forty-eight compounds were identified. Monoterpenes are the most abundant bioactive compounds. Table 1 shows that TJS is mainly composed of Camphor, Endo-borneol, Eucalyptol, Ledol and Terpinen-4-ol. Of these terpene compounds, the most terpenic-enriched samples was Camphor (17.64%). Whereas, organic compounds were the most abundant components of TJB. In the literature, it has been shown that thymol [10] are the most abundant bioactive compounds of essential oils extracted from the aerial parts of some species belonging to the genus *Thymus* (*T. vulgaris*).

Table 2: Terpenic profile of *T. algeriensis* aerial part analyzed with GC/MS

No	RT[a]	RI[b]	Compounds[c]	Formula	Pic Area (%)				Identification
					TJS	TJO	TJB	TJA	
1	2.287	939	2-methylpentane	C_6H_{14}	1.97	1.94	6.51	0.48	GC-MS
2	2.293	941	1-Pentene	C_5H_{10}	tr	tr[d]	-	-	GC-MS
3	2.321	947	Sulfurous acid, hexyl pentyl ester	$C_{11}H_{24}OS$	tr	-	-	-	GC-MS
4	2.393	948	3-methylpentane	C_6H_{14}	8.17	8.58	29.81	2.06	GC-MS
5	2.518	952	n-Hexane	C_6H_{14}	7.01	7.28	25.12	1.80	GC-MS-RT
6	2.806	955	Methylcyclopentane	C_6H_{12}	3.32	3.53	12.04	0.84	GC-MS
7	2.907	956	3,3-dimethyl-1-Butene	C_6H_{12}	6.55	6.76	23.58	-	GC-MS
8	3.174	957	Cyclohexane	C_6H_{12}	0.90	0.85	2.94	0.23	GC-MS
9	9.292	959	(1S)-2,6,6 Trimethylbicyclo [3.1.1] hept-2-ene	$C_{10}H_{16}$	1.78	8.13	-	-	GC-MS
10	9.323	960	α-Pinene	$C_{10}H_{16}$	-	-	-	3.99	GC-MS
11	9.773	961	Camphene	$C_{10}H_{16}$	1.60	2.65	-	1.91	GC-MS
12	9.917	962	Thuja-2,4(10)-diene	$C_{10}H_{16}$	1.78	-	-	0.63	GC-MS-RT
13	10.467	976	Sabinene	$C_{10}H_{16}$	0.42	1.46	-	1.33	GC-MS
14	10.629	979	β-Pinene	$C_{10}H_{16}$	0.42	0.53	-	3.11	GC-MS
15	11.785	1022	4-Carene	$C_{10}H_{16}$	0.63	-	-	0.70	GC-MS
16	12.023	1026	*p*-Cymene	$C_{10}H_{14}$	1.65	1.49	-	2.02	GC-MS
17	12.173	1041	Limonene	$C_{10}H_{16}$		0.78	-		GC-MS
18	12.292	1053	Eucalyptol	$C_{10}H_{18}O$	9.64	13.37	-	9.36	GC-MS
19	12.648	1065	(*E*)-β-Ocimene	$C_{10}H_{16}$	-	-	-	0.48	GC-MS
20	13.073	1068	γ-Terpinene	$C_{10}H_{16}$	1.23	0.82	-	1.96	GC-MS
21	13.385	1070	p-Menth-8-en-1-ol	$C_{10}H_{18}O$	-	-	-	1.28	GC-MS
22	13.485	1071	cis-Linalool oxide	$C_{10}H_{18}O_2$	0.58	-	-	-	GC-MS
23	14.016	1072	2-carene	$C_{10}H_{16}$	-	-	-	0.45	GC-MS
24	14.229	1073	Linalool	$C_{10}H_{18}O$	153	1.94	-	0.93	GC-MS-RI
25	14.385	1074	3,7-dimethyl-1,5,7-Octatrien-3-ol	$C_{10}H_{16}O$	-	1.63	-	-	GC-MS
26	15.179	1077	alpha.-Campholenal	$C_{10}H_{16}O$	0.80	0.56	-	0.24	GC-MS
27	15.672	1079	Isopinocarveol	$C_{10}H_{16}O$	-	tr	-	0.70	GC-MS
28	15.797	1082	Verbenol	$C_{10}H_{16}O$	-	tr	-	-	GC-MS
29	15.860	1084	Camphor	$C_{10}H_{16}O$	17.64	6.50	-	8.26	GC-MS
30	16.378	1085	Pinocarvone	$C_{10}H_{14}O$	-	-	-	0.32	GC-MS
31	16.547	1087	Endo-borneol	$C_{10}H_{18}O$	9.02	9.45	-	5.94	GC-MS
32	16.884	1091	Terpinen-4-ol	$C_{10}H_{18}O$	6.49	3.99	-	6.52	GC-MS

33	16.953	1094	Benzenemethanol.alpha.alpha,trimethyl	$C_{10}H_{14}O$	-	-	-	0.20	GC-MS
34	17.203	1165	α-terpineol	$C_{10}H_{18}O$	0.88	2.24	-	2.74	GC-MS
35	17.384	1226	(1R)-(-)-Myrtenol	$C_{10}H_{16}O$	1.11	1.00	-	tr.	GC-MS
36	18.578	1265	Pulegone	$C_{10}H_{16}O$	030	-	-	tr.	GC-MS
37	18.697	1287	D-carvone	$C_{10}H_{14}O$	-	-	-	0.17	GC-MS
38	19.884	1290	Thymol	$C_{10}H_{14}O$	0.51	-	-	2.69	GC-MS
39	23.733	1297	Caryophyllene oxide	$C_{15}H_{24}$	1.24	0.99	-	1.01	GC-MS
40	24.802	1305	Alloaromadendrene	$C_{15}H_{24}$	-	-	-	0.63	GC-MS
41	25.502	1328	Longifolene	$C_{15}H_{24}$	-	-	-	0.16	GC-MS
42	25.664	1335	γ-elemene	$C_{15}H_{24}$	-	-	-	tr.	GC-MS
43	27.689	1342	(-)-Spathulenol	$C_{15}H_{24}O$	-	-	-	1.01	GC-MS
44	28.50	1378	Ledol	$C_{15}H_{16}O$	4.47	0.92	-	8.49	GC-MS
45	28.901	1427	γ-eudesmol	C15H26O	-	-	-	0.15	GC-MS
46	29.051	1482	(+)-epi-Bicyclosesquiphellandrene	$C_{15}H_{24}$	-	1.16	-	2.64	GC-MS
4B	29.620	1577	Patchoulene	$C_{15}H_{24}$	-	1.21	-	-	GC-MS

[a]Retention time; [b]Retention index relative to C_5-C_{15} n-alkanes on the capillary column phenomenex ZB-5MSi; [c]Compound numbers in the order of retention time and index, [d]tr = trace (<0.05%). In our work, several terpenic compounds were identified in the Orbata mount, whose composition is reported in Table 1, which contained a complex mixture of plant secondary metabolites belonging to the chemical classes of mono-and sesquiterpenes, both compounds are known for their pharmacological effects. In this site, α-pinene and thymol occurred in the moderate mean content (3.99 and 2.69%, respectively).

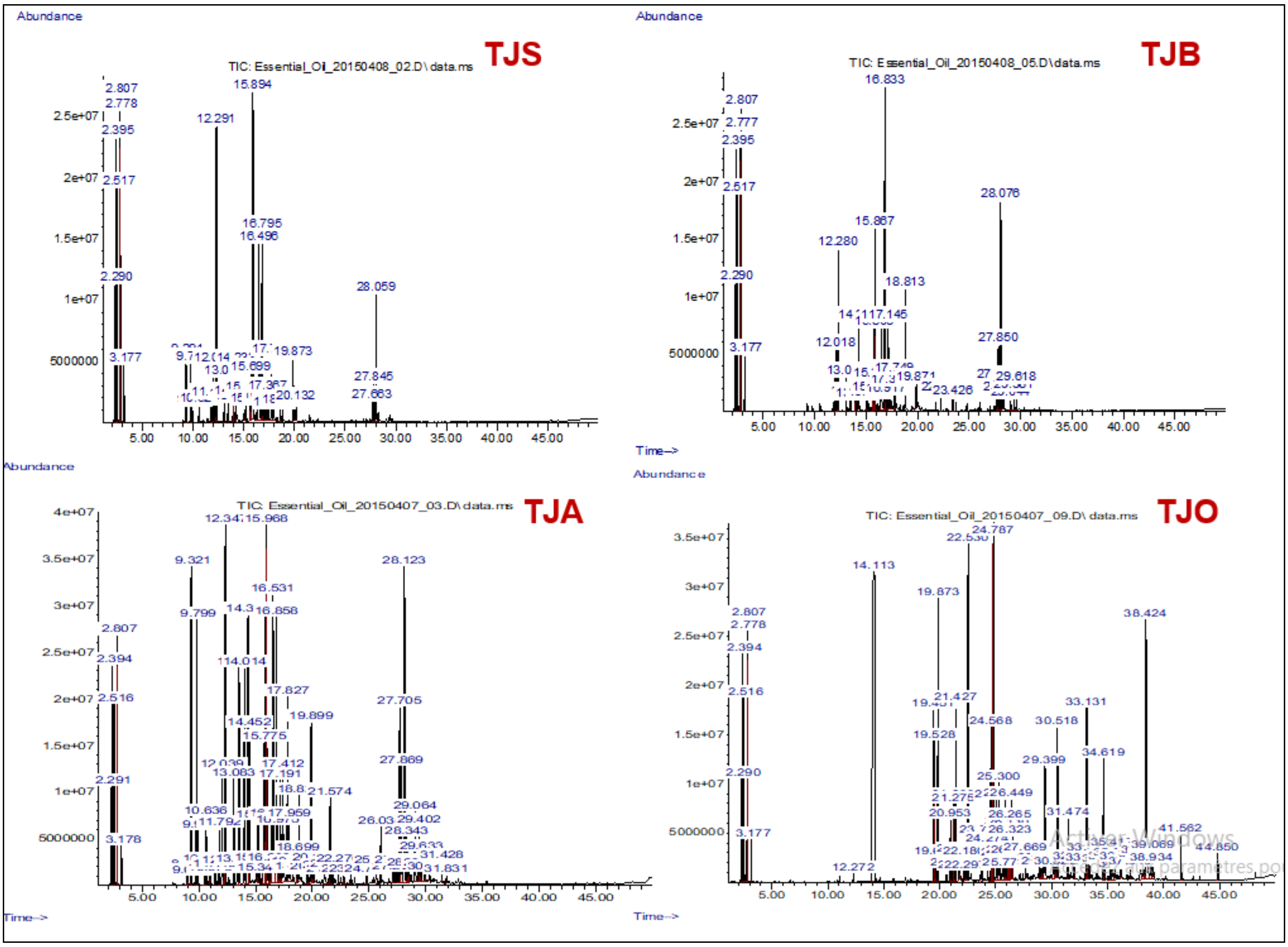

Fig 3: GC/MS chromatogram of terpenic compounds of *Thymus* EOs

3.3 Terpenic variation

These results clearly show that the variation of quantity and quality based on the region of collect and phenological stages ensured its unequivocal recommendation for use in the pharmaceutical and nutraceutical sector [2]. It has been reported that the presence of a significant amount of respective bioactive components in *Thymus* plant and the identification of terpenes lead to high degree of variability in the quantity and composition of aerial parts essential oils from four wild *Thymus algeriensis*, each one growing on different sites. Such chemical variations clearly suggest, apart from a geographical influence, genetic differences between individuals in the populations [11]. Previously, variations in *Thymus algeriensis* essential oil composition was reported for samples originating from different populations in Tunisia [7]. Previous reports on the composition of *Thymus algeriensis* essential oil growing in Gafsa, Tunisia, showed monoterpenes, mainly terpinen-4-ol (33.34%), 1,8-cineole (19.96%), and camphor (19.20%), as its major components [7].There is also data in the literature about the most abundant terpenes, where Camphor, 4-terpineol, 1, 8-Cineole, *Cis*-sabinene hydrate, α-pinene, terpenyl acetate, and viridiflorol, together, made up the major bioactive compounds of *T. algeriensis* collected from different locations and ecological factors in Tunisia [4].

4. Conclusions

As for the vast majority of *Lamiaceae* plants, *Thymus* are aromatic and widely used as spices and antioxidant plants. *Thymus algeriensis* is rich in bioactive phytochemicals, including phenolic acids, tannins and flavonoids. Diversity of terpenic compounds extracted from different populations of *Thymus* collected from various locations may be due to bioclimatic and soil edaphic conditions.

5. Conflicts of Interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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