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Total phytochemical analysis of *Thymus munbyanus* subsp. *coloratus* from Algeria by HS-SPME-GC-MS, NMR and HPLC-MS^n studies

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ABSTRACT

In this article, we report a comprehensive characterization of volatile and polar constituents extracted from the aerial parts of *Thymus munbyanus* subsp. *coloratus*, a shrub that is used as culinary ingredient and as traditional medicine in Algeria, mainly to treat respiratory and gastrointestinal disorders and endocrine dysfunctions. Headspace solid phase microextraction (HS-SPME) coupled with gas chromatography-mass spectrometry (GC-MS) was used to assess volatile constituents, whereas the phytochemical composition of solid residues obtained from extraction with solvents at diffrent polarity was obtained by an integrated Nuclear Magnetic Resonance (NMR) and liquid chromatography coupled with tandem mass spectrometry (LC-MS^n) approach. Fourty-five apolar ccompounds were identified, mainly oxygenated monoterpenes (65.8%), sesquiterpene hydrocarbons and nonoterpene hydrocarbons (18.6 and 14.5%, respectively). On the other hand, LC-MS^n and NMR analyses revealed the presence of aglyconic and glycosilated flavonoids, phenylpropanoid derivatives and triterpenoid acids related to oleanolic acid, mainly in the methanol, dichloromethane and hexane extracts. Overall, these data indicate that *Thymus munbyanus* subsp. *coloratus* could be a potential source of antioxidants and bioactive compounds, and our results represent a starting point for further research on this plant species.

1. Introduction

The Thyme (*Thymus* L.) is one of the largest genera of the Lamiaceae family [1], comprising more than 215 species distributed throughout Europe, Asia, and North Africa [2]. In Algeria, 20 species have been reported [3,4], among which *T. munbyanus* Boiss. & Reut. that is considered as endemic in North Africa [2]. Two subspecies of *T. munbyanus* have been registered, namely *T. munbyanus* subsp. *coloratus* (Boiss. & Reut.) Greuter & Burdet (TMC) (also known as *Thymus coloratus* Boiss. & Reut.), and *T. munbyanus* subsp. *munbyanus* (TMM), according to the Plant List database (http://www.theplantlist.org).

The TMC subspecies is widely diffused in the whole Mediterranean region and in northern Algeria [5], where it grows in lawns, rockeries and mountainous regions [3,5]. The aerial part of this plant consists of a small subshrub up to 30 cm tall, with branched and prostrated stems and tender, simple small leaves [6]. It presents flowers whose size does not exceed 7 to 8 mm, with small floral leaves roughly purple-stained at least at the base. The ramified stem is usually tetragonal, and woody in its lower part [3,5].

The leaves and blooming aerial parts of *Thymus* plants are commonly used as aromatic ingredients of culinary preparations and, because of their antioxidant capacity, they are also employed in food preservation [7]. Antitussive and carminative properties have been attributed to *Thymus* species, and the aerial parts are commonly used in the traditional medicine to treat upper respiratory

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infections, acute and chronic bronchitis, cough, and colds [8]. Furthermore, due to tonic and antiseptic properties, *Thymus* plants are used to treat other diseases such as cystitis, insomnia, and indigestion [7]. In Algeria, *T. munbyanus* is used for the treatment of respiratory and gastrointestinal diseases and as a remedy for endocrine dysfunctions [9].

Several studies have been focused on the characterization of the essential oils (EOs) and secondary metabolites from *Thymus* spp. Phenolic monoterpenes such as thymol, carvacrol and p-cymene have been described as the most abundant constituents of the volatile fraction, while among the polar secondary metabolites, phenolic acids (rosmarinic acid and caffeic acid), triterpenes (ursolic and oleanolic acids) and flavonoids (e.g., luteolin, hispidulin and eriodicytol) have been reported as the most representative [8–12]. Recently, EOs and apolar constituents from carbon dioxide supercritical fluid extracts and pressurized liquid extracts of *T. munbyanus* subsp. *coloratus* were characterized and investigated for biological activities, showing antioxidant properties and antimicrobial and cytotoxic activities against several pathogenic bacteria and human melanoma cells, respectively [5,12]. However, to the best of our knowledge, there are no published reports on the aroma profile and the non-volatile polar constituents of this subspecies, which could be of interest as a source of antioxidant and bioactive compounds. Therefore, in the present work we report an extensive characterization of the volatile composition and the non-volatile polar constituents of crude extracts of *T. munbyanus* subsp. *coloratus* aerial parts, obtained using different extractive and analytical approaches. A headspace solid phase microextraction (HS-SPME) coupled with gas chromatography-mass spectrometry (GC-MS) method was used for the characterization of volatiles, while integrated NMR and LC-MS^n approaches allowed the identification and quantification of polar constituents.

2. Experimental Section

2.1. Plant Material

Mixture of inflorescences, stems and leaves of *T. munbyanus* subsp. *coloratus*, growing in Mechta Fatima, Province of Bordj Bou Arreridj (North-East Algeria, N 36°060; E 04°760, 820 m) was harvested in March 2016. Identification of the botanical species was performed by Dr. Miara M.D.J., using available literature [3]. A voucher specimen was deposited in the Herbarium Universitatis Camerinensis (CAME, included in the online edition of Index Herbariorum c/o School of Biosciences and Veterinary Medicine, University of Camerino, Italy), under the codex CAME 27741, and it was also archived in the anArchive System for Botanical Data (http://www.anarchive.it). Before undergoing extraction, plant material was washed in running water and dried in the shadow at r.t. for 7 days.

2.2. HS-SPME-GC-MS analysis

For HS-SPME, the method previously described by Ascrizzi et al. [13] was used. Briefly, a Supelco SPME device coated with polydimethylsiloxane (PDMS, 100 μm) was used. After the equilibration time, the fibre was exposed to the headspace for 30 min, and sampling was accomplished in an air-conditioned room (22 ± 1 °C) to guarantee a stable temperature. Once sampling was finished, the fibre was withdrawn into the needle and transferred to the injection port of the GC-MS system.

GC-MS analyses were performed as reported in [14]. A Varian CP-3800 gas-chromatograph coupled to a Varian Saturn 2000 mass spectrometer equipped with electron impact (EI) ion source and ion trap mass detector was used. An Agilent DB-5 capillary column (30 m × 0.25 mm; coating thickness 0.25 μm) was employed as stationary phase. The chromatographic consitions were as follows: the injector and transfer line temperatures were regulated at 220 and 240 °C, respectively; oven temperature was programmed from 60 to 240 °C at 3 °C/min; carrier gas (helium) flow was 1 ml/min; injection was performed in splitless mode. Identification of volatile compounds was performed comparing their retention times with those of authentic standards, evaluating their linear retention indices (LRI) relative to the C_6 – C_{28} series of *n*-hydrocarbons, and by matching their mass spectra against commercial [15,16] and home-made libraries built up from pure substances.

2.3. Extraction of aerial parts and sample preparation for NMR and HPLC-MS^n analyses

The extraction of *T. munbyanus* subsp. *coloratus* aerial parts was performed as previously reported [14,17]. Briefly, 30 g of dried material were ground with a blender. The obtained powder was suspended in 150 mL of methanol and extracted using an ultrasound bath for 10 min. After centrifugation at 4000 rpm for 10 min, the supernatant was removed and the residue was then re-extracted with an additional 50 ml of methanol, for two more times. After collecting the supernatants in a round bottom flask, the solvent was removed under vacuum at 35 °C to constant weight (2.9 g), in order to obtain a dried concentrated extract that was stored in amber glass vials at −20 °C until analysis. The yield of the extraction was 9.6% w/w.

150 mg of the dried extract were dissolved in deuterated methanol and used for NMR analysis. The remaining extract was dissolved in 50 mL of a methanol/water (1:9) mixture and sonicated for 10 min. The obtained solution was partitioned using 20 mL of solvents at increasing polarity, namely hexane, dichloromethane (DCM) and ethyl acetate (EA). For each solvent, extraction was repeated three times. Solvents were dried under vacuum and the residues were dissolved in deuterated methanol for NMR analysis.

2.4. NMR Analyses

NMR analyses were performed on a Bruker Avance III 400 MHz spectrometer, using standard pulse sequences. ^{1}H-NMR spectra were acquired for methanol, hexane, DCM and EA extracts, and ^{1}H, HSQC-DEPT, HMBC, COSY and TOCSY spectra were acquired for the DCM fraction.

2.5. HPLC-MS^n analysis of secondary metabolites in the methanol extract

Polar constituents of the methanol extract of *T. munbyanus* subsp. *coloratus* vegetative parts were tentatively identified by HPLC-MS^n, comparing the fragmentation patterns with literature data and with standard compounds, when available. The dried extract was dissolved in methanol at a concentration of 5 mg/ml, and the solution was filtered through a 0.45 μm Millipore filter. The HPLC-MS^n method used was the same already reported elsewhere [14,18]. Briefly, a Varian 212 binary pump equipped with a Varian Prostar 430 autosampler and coupled to a Varian 500 Ion Trap mass detector (MS) was employed. The mass spectrometer was equipped with an Electrospray Ionisation (ESI) ion source, operating in negative ion mode. Separation of phytocompounds was achieved using an Agilent Eclipse plus C18 column (2.1 × 150 mm, 3.5 μm) as stationary phase and a gradient mixture of acetonitrile (A) and 0.1% formic acid in water (B) as mobile phase. The gradient was set as follows: 0 min, 10% A; 20 min, 54% A; 23 min, 100% A and isocratic up to 32 min. Re-equilibration time was 8 min. Flow rate was 0.2 ml/min. ESI parameters were: needle voltage, 4500 V; capillary voltage, 70 V; RF loading, 100%; nebulizing gas pressure,

20 psi (nitrogen); drying gas pressure, 15 psi; drying gas temperature, 350 °C. *m/z* range was 50 – 2000. The turbo detection data scanning (TDDS) function of the instrument was used to monitor the fragmentation patterns of eluted compounds, setting n = 3 levels of fragmentation.

3. Results and discussion

3.1. HS-SPME-GC-MS analysis

HS-SPME is an easy to automate technique that allows the sampling of volatile and semi-volatile compounds by a solvent-free sample preparation, which are then analysed by chromatographic techniques. In this work, we considered this approach to analyse the volatiles emitted from the aerial parts of *T. munbyanus* subsp. *coloratus,* and findings are reported in Table 1. In a previously published paper, the composition of the EO obtained by hydrodistillation from the aerial parts of the same plant species has been reported [5], and the results were qualitatively and quantitatively different from the ones shown here. Oxygenated monoterpenes comprising the 65.8% of the total composition were the most abundant compounds in the apolar fraction of the powder from the aerial parts, followed by sesquiterpene hydrocarbons and monoterpene hydrocarbons (18.6 and 14.5%, respectively). Oxygenated sesquiterpenes and non-terpene derivatives were considerably less represented (0.2 and 0.1%, respectively). In the EO, the abundance of oxygenated monoterpenes was comparable (59.4%), whereas the precentage of the other constituents was different, with higher abundance of monoterpene hydrocarbons (24.4%) and oxygenated sesquiterpenes (4.9%), and lower sesquiterpene hydrocarbons (8.5%) [5]. Considering the single constituents, a total of 45 volatile components were identified by HS-SPME-GC-MS approach, accounting for 99.2% of the total composition, while the EO analysis allowed the identification of 104 compounds, although 30 of these were present in traces (< 0.1%) [5]. Comparing the most abundant compounds from the two approaches, camphor (26.4%), pulegone (10.6%), camphene (9.4%), terpinen-4-ol (9.1%), 1,8-cineole (6.3%), borneol (6.1%) and germacrene D (5.0%) were the most representative of the HS-SPME extract, constituting almost the 73% of the whole composition, while in the EO, myrcene (4.5%), α-pinene (3.8%), limonene (2.3%) and β-pinene (2.2%) were also among the most characteristic [5]. Nevertheless, it should be considered that the results of HS-SPME and EO analyses are not comparable, due to the completely different sampling methods. In EO extraction the sample is subjected to hydrodistillation, which is a time-consuming process that requires the use of toxic solvents, while HS-SPME allows the direct extraction of volatiles from dried plant material through their adsorption to a fiber, without the need of obtaining the EO. This leads to drastic differences in the extraction times between the two methods and to lower thermal degradation of volatiles in HS-SPME, due to a shorter exposition time of the compounds with high temperatures [19]. Over EO extraction, HS-SPME has the advantages of being significantly less time-consuming and to avoid the use of solvents, hence it could be considered as “ready-to-use” technique that could be easily adopted for the screening of volatiles in plant material.

3.2. NMR and HPLC-MS^n analysis of secondary metabolites

The analysis of the chemical composition of *T. munbyanus* subsp. *coloratus* aerial parts was performed by extraction with solvents at different polarity. The extracts, after solvent removal, were dissolved in deuterated solvent and analyzed by ^{1}H-NMR for obtaining a preliminary phytochemical profile. Methanol extract presents signals that support the extraction of different classes of phytochemicals (Fig. 1). A summary of preliminary assignments of the detected phytoconstituents is reported in Table 2. Starting from the aromatic region of the spectrum, signals ascribable to phenylpropanoid derivatives and flavonoid moieties are observed. Furthermore, signals ascribable to anomeric proton signals as well as to other sugar residues are detected in the range of 4.80-5.10 ppm and 3.00-4-13 ppm, respectively. The aliphatic region shows clear signals ascribable to methyl groups (both secondary and quaternary), as well as superimposed multiplets suggesting the presence of CH and CH_2 that can indicate the presence of triterpenoids. The spectra from hexane (Fig. 2) and DCM (Fig. 3) extracts present mostly signals ascribable to fatty acids and lipids, as well as the signlets that suggest the presence of triterpenoids (Table 2). On the other hand, the use of sequential extraction allowed to obtain another lipophylic fraction of EA that presents purified triter-

Table 1
Aroma profile of *Thymus munbyanus* subsp. *coloratus* aerial parts.

N.	Constituents	*LRI*[a]	*Lit. RI*[b]	[%]
1.	tricyclene	928	926	0.2
2.	α-pinene	941	939	1.3
3	camphene	955	954	9.4
4	β-pinene	982	979	0.6
5	myrcene	993	990	1.9
6	α-terpinene	1020	1017	0.1
7	1,8-cineole	1034	1031	6.3
8	*(E)*-β-ocimene	1052	1050	0.4
9	γ-terpinene	1063	1059	0.3
10	*cis*-sabinene hydrate	1070	1067	0.2
11	terpinolene	1090	1088	0.3
12	linalool	1101	1096	1.1
13	α-campholenal	1128	1126	0.3
14	camphor	1145	1146	26.4
15	pinocarvone	1164	1161	0.1
16	borneol	1168	1165	6.1
17	terpinen-4-ol	1179	1177	9.1
18	α-terpineol	1191	1188	2.8
19	verbenone	1207	1204	0.9
20	isobornyl formate	1232	1235	0.3
21	pulegone	1239	1237	10.6
22	*(E)*-2-decenal	1263	1261	0.1
23	bornyl acetate	1287	1288	1.4
24	piperitenone	1342	1340	0.2
25	α-cubebene	1352	1348	0.2
26	α-copaene	1377	1376	1.2
27	β-bourbonene	1385	1387	1.6
28	β-cubebene	1390	1388	0.5
29	β-elemene	1392	1389	0.4
30	α-gurjunene	1410	1409	2.8
31	β-caryophyllene	1419	1417	1.7
32	β-copaene	1430	1432	0.6
33	aromadendrene	1440	1439	0.3
34	α-humulene	1456	1454	0.3
35	alloaromadendrene	1461	1458	0.5
36	*cis*-muurola-4(14),5-diene	1463	1465	1.1
37	γ-muurolene	1479	1479	0.1
38	germacrene D	1481	1485	5.0
39	*trans*-muurola-4(14),5-diene	1492	1493	0.6
40	bicyclogermacrene	1497	1500	0.3
41	α-muurolene	1499	1500	0.1
42	β-bisabolene	1508	1505	0.1
43	*trans*-γ-cadinene	1514	1513	0.2
44	δ-cadinene	1524	1523	1.0
45	caryophyllene oxide	1582	1583	0.2
	Grouped compounds [%]			
	Monoterpene hydrocarbons			14.5
	Oxygenated monoterpenes			65.8
	Sesquiterpene hydrocarbons			18.6
	Oxygenated sesquiterpenes			0.2
	Non-terpene derivatives			0.1
	Total identified			99.2
	Number of identified compounds			45

[a] LRI relative to C_6-C_{28} n-alkanes on the DB-5 column.
[b] LRI taken from [15].

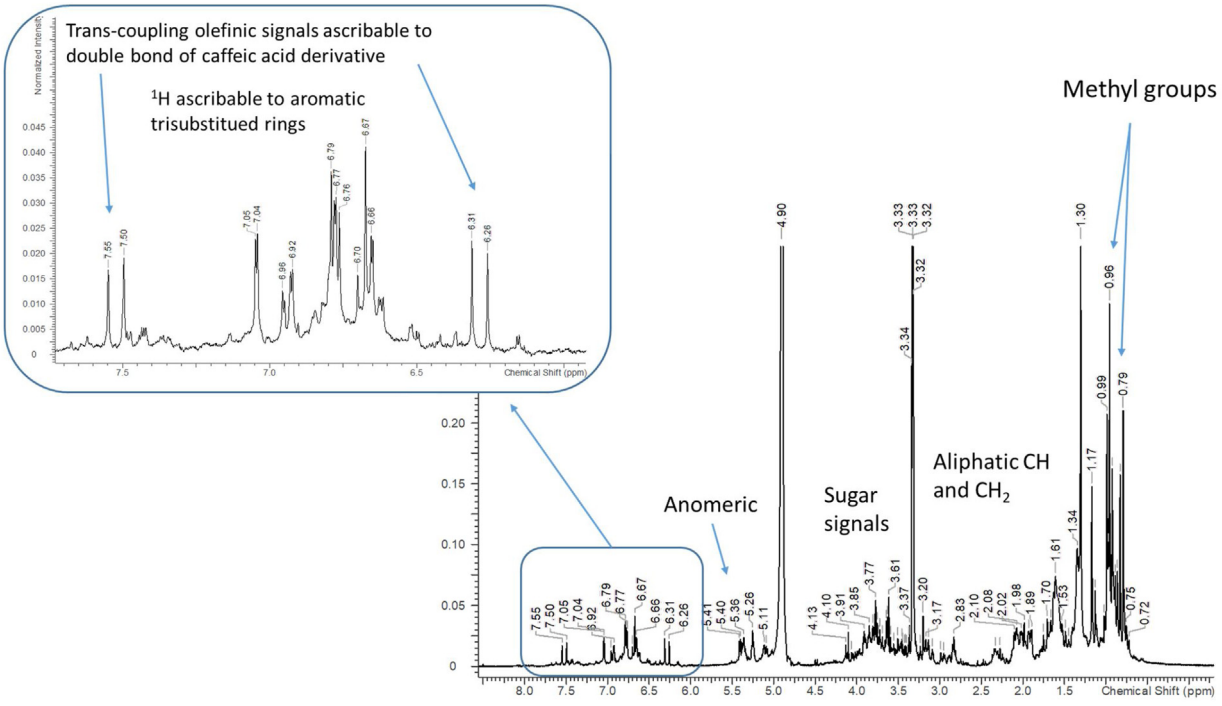

Fig. 1. [1]H-NMR spectrum obtained from the analysis of the methanol extract of *Thymus munbyanus* subsp. *Coloratus.*

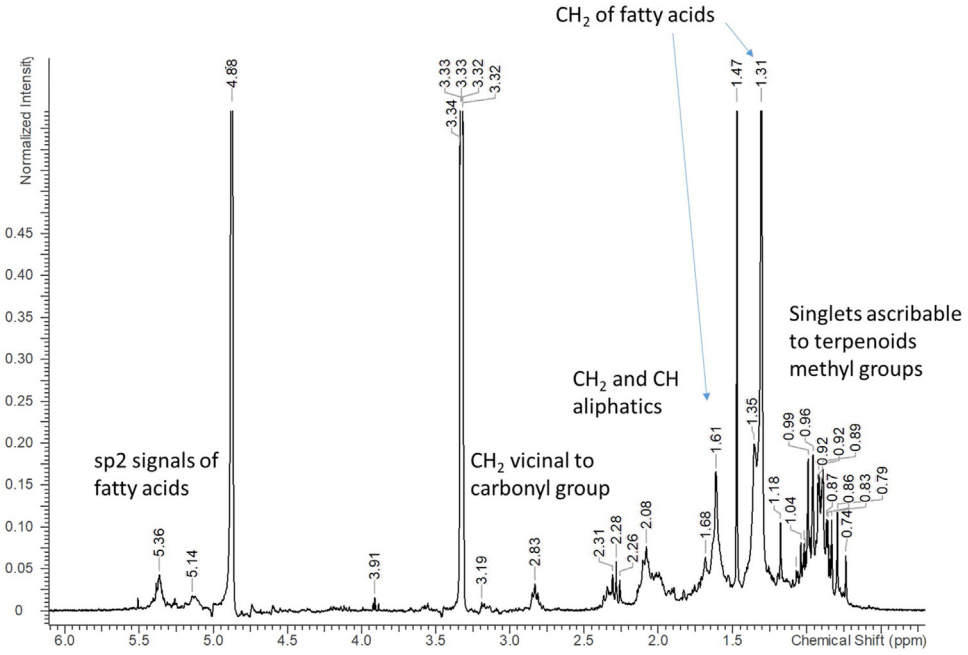

Fig. 2. [1]H-NMR spectrum obtained from the analysis of the hexane extract of *Thymus munbyanus* subsp. *Coloratus.*

penoid fraction, being its spectrum charactherized by almost the absence of signals ascribable to lipids (Fig. 4). Thus, preliminary NMR analysis allowed the observation of the presence of phenolic constituents containing phenylpropanoids and flavonoid units, glycosidic derivatives and triterpenoids. Detailed observation of [1]H-NMR signals in the different extracts suggests the presence of rosmarinic acid as well as triterpenoid acid related to oleanolic acid (Table 2).

For the analysis of the secondary metabolites from the aerial parts of *T. munbyanus* subsp. *coloratus*, the methanolic extract was analyzed by HPLC-MS^n in negative ionization mode. Only the negative mode was used due to higher sensitivity in the detection of phenolic compounds and terpenes. The method allowed the detection of 34 constituents, among which 24 where tentatively identified on the basis of their fragmentation patterns and by comparison with literature data (Table 3). Overall, the identified phenolic compounds of the methanolic extract from *T. munbyanus* subsp. *coloratus* include rosmarinic acid (in accordance with the identification data from NMR) and other common phenolic acids, namely ferulic, quinic and caffeic acids. Several derivatives of common flavonoids were also identified, including derivatives of luteolin bonded to differ-

Table 2
^{1}H-NMR signals obtained from the analysis of the MeOH, ethyl acetate, DCM and hexane fractions of the *T. munbyanus* subsp. *coloratus* extract

Signal ^{1}H-NMR Resonance	Assignment	Extract
7.51 d (J = 16.8)	H-7 of caffeic acid unit	MeOH
6.29 d (J = 16.8)	H-8 of caffeic acid unit	MeOH
7.05 d (J = 1.8)	H-2, H-7 of caffeic acid unit	MeOH
6.94 dd (J = 1.8, 7.8)	H-6 of caffeic acid unit	MeOH
6.78 d (J = 7.8)	H-5 of caffeic acid unit	MeOH
6.79 d (J = 1.9)	Aromatic signal of trisubstitued ring	MeOH
6.67-6.66 dd and d partially overlapped	Aromatic signal of trisubstitued ring	MeOH
6.16 d (J = 2.0)-6.48 d (J = 1.8)	Signals ascribable to protons 6-8 of flavonol glycosides	MeOH
5.41-5.25-5.11-5.09	Anomeric signal of glycosidic sugar residues	MeOH
3.00-4.13 multiplets	Signals ascribable to sugar residues	MeOH
3.17 dd	CHOH of position 3 of triterpenoids	DCM, EA
2.27-2.33-2.10-1.93-1.16 multiplets	Signals ascribable to CH_2 and aliphatic CH	MeOH, DCM, EA
1.17-0.96-0.90-0.92-0.82-0.70 singlets	Quaternary methyl groups	MeOH, Hexane, DCM, EA
0.88 d	Secondary methyl group	MeOH, Hexane, DCM, EA
0.92 m	Terminal methyl unit of fatty acid chains	Hexane, DCM
1.31-1.35	Aliphatic CH_2 of fatty acids	Hexane, DCM
1.43-1.61-2.08-2.28-2.83	Deshielded aliphatic CH_2 of fatty acid vicinal to sp2 carbon or to carbonyl function	Hexane, DCM

ent sugar moieties, eriodictyol-7-O-hexoside, gallocatechin and derivatives of quercetin and isorhamnetin (Table 3). Many glycosidic derivatives of eriodictyol have been previously described in the genus *Thymus*, namely eriodictyol-7-O-glucoside [20], eriodictyol-7-O-rutinoside [21], eriodictyol-7-O-glucuronide [22], and eriodictyol-di-O-hexoside [23]. Also rosmarinic acid and luteolin have been already reported as main phenolic compounds in thyme plants. Rosmarinic acid has been identified as the main phenolic acid in several spp., among which *T. pulegioides* [24], *T. mastichina* [25] and *T. x. citriodorus* [26], while luteolin and its glucoside and glucuronide derivatives have been identified as abundant flavonoids in *T. x. citriodorus* [23] and *T. herba* [20].

Finally, the two triterpenes oleanolic and ursolic acids were also detected, thus confirming the preliminary NMR observation that showed signals ascribable to their methyl groups and unsaturations. Previous works on *Thymus* spp. have reported the identification and isolation of different triterpenes related to oleanolic and ursolic acids, as for example in *T. alternans* from Slovakia, from which six triterpenoids were isolated after extracxtion of aerial parts with DCM and were further assayed on a panel of human cancer cell lines, showing a potent citotoxic activity [26].

4. Conclusions

Our study represents, to the best of our knowledge, the first comprehensive characterization of both apolar and polar constituents from *Thymus munbyanus* subsp. *coloratus,* a species widely used as culinary ingredient and in the traditional medicine of Algeria, that could be an evaluable natural source of antioxidants and bioactive compounds. A multi-technique approach was used to cover the analysis of a broad spectrum of compounds. HS-SPME coupled to GC-MS allowed to identify 45 components of the apolar fraction of *T. munbyanus* subsp. *coloratus*, among which camphor, pulegone, camphene, terpinen-4-ol, 1,8-cineole, borneol and germacrene D were the most abundant, representing almost the 73% of the fraction. These results show that studies on the volatile composition of plant material extracted by hydrodistillation and by HS-SPME are not comparable, being the quali- and quantitative composition of *T. munbyanus* subsp. *coloratus* aerial parts reported here considerably different from the EO previously obtained by hydrodistillation and analysed using the same GC-MS method [5]. Furthermore, NMR analysis of methanol, hexane, DCM and EA extracts allowed the rapid determination of phenolic constituents containing phenylpropanoids and flavonoid units,

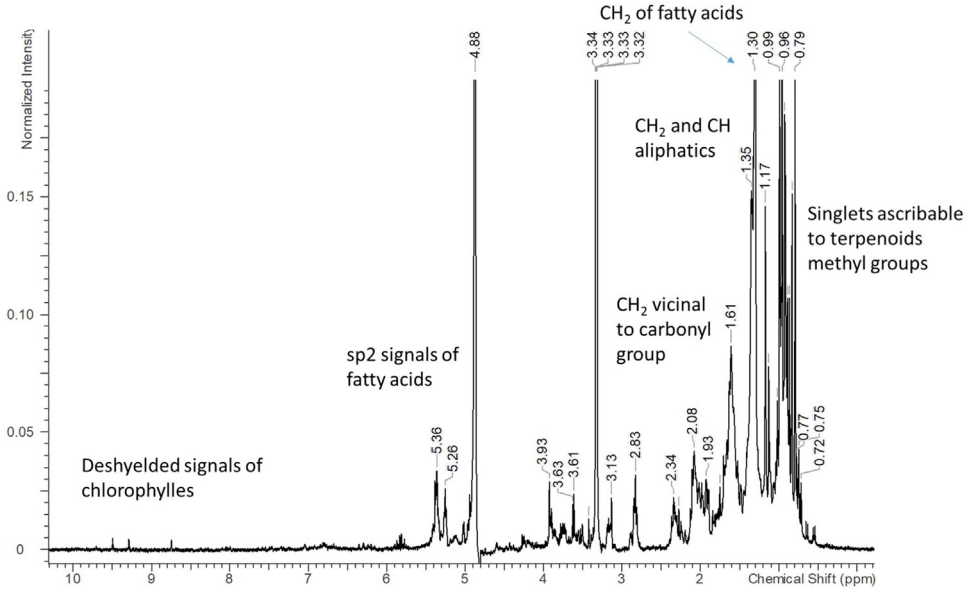

Fig. 3. ^{1}H-NMR spectrum obtained from the analysis of the dichloromethane extract of *Thymus munbyanus* subsp. *Coloratus.*

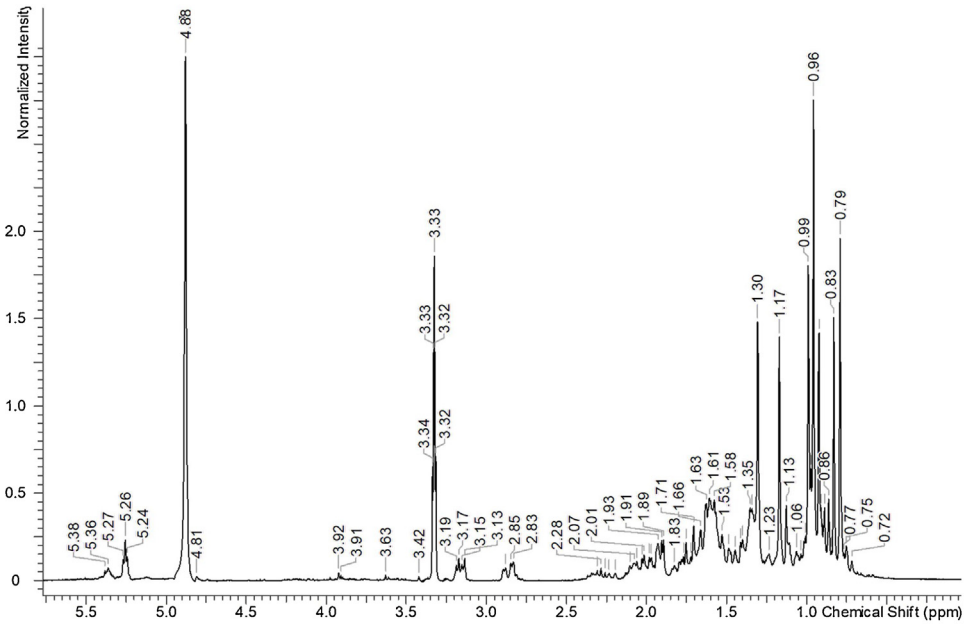

Fig. 4. ^{1}H-NMR spectrum obtained from the analysis of the ethyl acetate extract of *Thymus munbyanus* subsp. *Coloratus.*

Table 3
Identification of secondary metabolites from the methanol extract of *Thymus munbyanus* subsp. *coloratus* by HPLC-MS^{n} in negative ion mode. Compounds are sorted by m/z.

N.	R.T. (min)	[M-H]$^{-}$ (*m/z*)	MS^{2} (*m/z*)	MS^{3} (*m/z*)	Tentative compound identification	References
1	11.42	191	173 127 111 85		Quinic acid*	[27]
2	29.30	194	179 149		Ferulic acid*	[28]
3	12.59	305	225	224 207 182 181 165 163 135 133	Gallocatechin	[29]
4	26.87	311	293 275 235 133		15,16-dihydroxy-9,12-Octadecadienoic acid	[30]
5	27.00	325.5	185 183 170		Unknown	–
6	20.10	327	291 229 211 193 171	211	Hydroxy-trimethoxyflavone	[31]
7	21.40	329	229 211	211 209	Trihydroxyoctadecenoic acid	[30]
8	25.43	343	328 313	285 298	Dihydroxy trimethoxy flavonol isomer	[32]
9	12.02	355	263 225 197		Unknown	–
10	14.90	359	161	133 115 105	Rosmarinic acid#	[33]
11	13.90	371	249		Caffeic acid derivative	[34]
12	12.72	387	207 163		Medioresinol	[35]
13	12.80	434	359 387 313 271 227		Unknown	–
14	13.68	447	285	241 199 175	Luteolin-7-O-hexoside	[34,35]
15	11.95	449	287		Eriodictyol-7-O-hexoside	[27]
16	32.00	455.2	407 391 377 363		Ursolic acid#	[36]
17	32.30	455.3	407 391 377 363		Oleanolic acid#	[36]
18	14.73	461	285	267 257 243 241 217 213 199 197 175 151 133	Luteolin 7-O-glucuronide	[37]
19	29.46	471.5	452 424 361 293		Corosolic acid	[38]
20	13.59	477	301	283 255 229 211 201 165 135	Quercetin 3-O-glucuronide	[37]
21	18.50	493	313 295		Salvianolic acid A	[39]
22	14.17	537	313 295		Lithospermic acid isomer	[39]
23	16.05	555	509 493 359		Unknown	–
24	14.25	597.5	329 311		Unknown	–
25	18.71	607	299 285		Methylkaempferol-O-rutinoside	[40]
26	13.51	623	337 285	161	Luteolin-O- hexoside-O-glucuronide	[37]
27	15.75	639	315 301		Isorhamnetin dihexoside	[41]
28	30.16	648	601 568 559 513 419		Unknown	–
29	16.97	658	616 551 548 432		Unknown	–
30	19.43	705	525 507	463	Unknown	–
31	14.21	717	519 359	339 295 267	Pinoresinol dihexoside	[42]
32	27.36	721.5	675 662		Unknown	–
33	18.67	726	627 519 359		Unknown	–
34	15.34	735	537 519		Salvianolic acid isomer*	[43]

Confirmed by NMR data.
* Compounds identified on the basis of comparison with standard available in the lab.

glycosidic derivatives and triterpenoids. Finally, the qualitative HPLC-MS^{n} analysis of the methanol extract allowed to detect 35 secondary metabolites, among which several ones were identified as derivatives of common flavonoids (luteolin, eriodictyol, quercetin and isorhamnetin), typically encountered in *Thymus* spp., and as the triterpenes oleanolic and ursolic acids, as a confirmation of NMR data. Overall, the results here presented represent a starting point for further research on this plant species.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

CRediT authorship contribution statement

Hamdi Bendif: Data curation, Software, Writing - original draft. **Gregorio Peron:** Data curation, Software, Writing - original draft, Writing - review & editing. **Mohamed Djamel Miara:** Visualization, Investigation. **Stefania Sut:** Visualization, Investigation. **Stefano Dall'Acqua:** Supervising, Data curation, Software, Writing - original draft, Writing - review & editing. **Guido Flamini:** Supervising, Data curation, Writing - original draft. **Filippo Maggi:** Supervising, Data curation, Software, Writing - original draft, Writing - review & editing.

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