Research Article

Noureddine Tamma, Naima Benchikha, Mohammed Messaoudi, Gianluca Caruso, Talha Bin Emran, Ayomide Victor Atoki*, Adekunle Ismahil Adeniyi

Chemical composition and biological properties of *Thymus capitatus* plants from Algerian high plains: A comparative and analytical study

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Abstract: The *Lamiaceae* family contains bioactive medicinal compounds mostly used as ornamental plants and traditional medicine, as well as in the food, cosmetics, and pharmaceutical sectors. Common uses include treating high cholesterol, diabetes, respiratory diseases, heart disease, and food poisoning. These medicinal uses were linked to their components and numerous biological properties, including antimicrobial and antioxidants. The goal of this study was to investigate the phytochemicals and biological activities of the petroleum ether extract of *Thymus capitatus* plant from two different regions of eastern Algeria (Souk ahras and Guelma), as well as to extract volatile oils using a Clevenger device and then analyze by using GC-MS. The results revealed that the total amount of phenolic compounds was better in the phenolic extract of Souk Ahras (3.41 mg GAE g^{-1}), while the amount of flavonoid compounds was higher in the region of Guelma (26.31 mg QE g^{-1}). Following the quantification of phenolic compounds by HPLC, we observed that the phenolic extracts contained most of the standard compounds in variable proportions. Furthermore, we tested the antioxidant activity of the phenolic compounds electrochemically with the cyclic voltammetry method. We concluded that the highest antioxidant content was recorded in the Guelma region extract (3.17 mg GAE g^{-1}). We have also evaluated the antioxidant activity by a chemical method using 2,2-diphenyl-1-picrylhydrazyl, and the results showed that the Guelma extract exhibited a high effectiveness in terms of IC_{50}% values. When extracting the volatile oils, it was found that the highest yield was in the Guelma region

Keywords: *Thymus capitatus*, phenols, flavonoid, volatile oil, biological activity

1 Introduction

With roughly 6,900–7,200 species, Lamiaceae (the mint family) is one of the plant families with the greatest global distribution [1,2]. It is a broad family that includes woody climbers, subshrubs, shrubs, trees, and vast forest forms, in addition to annual, biennial, and perennial herbs [3]. It consists of 242 genera with 200 or more species, including the warm and temperate parts of the world's Salvia (986), Scutellaria (468), Plectranthus L'Hér. (325), Thymus (315), Hyptis Jacq. (295), Teucrium (287), Nepeta (251), and Vitex (223). Furthermore, this family has a worldwide distribution and a high degree of diversity [4]. These are found throughout the world's warm and temperate climates [5]. Furthermore, this family is distributed globally and has a high degree of diversity [6]. They are able to adjust to many habitats and can be easily grown, but they cannot survive in the coldest high-latitude or high-altitude areas [7]. The following seven locations have a high diversity of Lamiaceae worldwide: (1) the Indo-Malesia region (Southeast Asia); (2) Africa, including Madagascar and the southern Sahel; (3) China; (4) Australia; (5) South America; (6) North America and Mexico; and (7) the Mediterranean and southwest Central Asia [3,8].

This research focuses on the genus *Thymus*, which is widely dispersed throughout the Mediterranean

* **Corresponding author: Ayomide Victor Atoki,** Department of Biochemistry, Kampala International University, Ishaka, Uganda, e-mail: atokiav@kiu.ac.ug
Noureddine Tamma, Naima Benchikha: Laboratory of Applied Chemistry and Environment, Department of Chemistry, Faculty of Exact Sciences, University of El Oued, El Oued 3900, Algeria
Mohammed Messaoudi: Nuclear Research Center of Birine, P.O. Box 180, Ain Oussera, Djelfa 17200, Algeria
Gianluca Caruso: Department of Agricultural Sciences, University of Naples Federico II, Naples, Italy
Talha Bin Emran: Department of Pharmacy, Faculty of Allied Health Sciences, Daffodil International University, Dhaka 1207, Bangladesh
Adekunle Ismahil Adeniyi: Department of Physiology, Kampala International University, Ishaka, Uganda

region and has a great deal of endemism [9–12], including plants that thrive in Northwest Africa and are spread out along the entire coast up to the desert zones [13,14]. In Algeria, it is represented by 17 species and subspecies [15–17].

The formation of volatile, phenolic, and flavonoid compounds and odorants distinguishes various aromatic plants [18–20]. The latter is utilized as a source of naturally occurring bioactive compounds that have biologically beneficial properties, particularly antibacterial, antioxidant, antiseptic, and anti-inflammatory properties. Volatile phenolics and flavonoid compounds are a source of bioactive chemicals that are being extensively researched for their potential application as a synthetic product substitute in treating infectious diseases and other pathological problems linked to oxidative stress. One of the most popular families in the world for spices and extracts with potent antioxidant and antibacterial qualities is the Lamiaceae family. The genus Thymus includes many widely dispersed species in this family that are utilized as antibacterial and anti-inflammatory agents in the customary pharmacopoeia of the Mediterranean region.

The most important species in the Lamiaceae family is the *Thymus capitatus*.

Thymus capitatus has antiseptic, antibacterial, antispasmodic, and anticytotoxic properties that are utilized in traditional medicine [1,21–23].

The most important aspect of this study was being able to identify the chemical makeup of leaves, extract flavonoids and volatile oils, and assess their biological activity.

Thymus capitatus is an aromatic plant of the family Lamiaceae spread across the Mediterranean region, where it is known among ancient peoples for its medicinal and protective properties [1–3]. This species has been used in traditional medicine to treat many diseases and health disorders, including infections of the larynx and pharynx, as an analgesic for dental and joint pain, to reduce blood sugar and strengthen the immune system, it is used as a gas-relieving drink and facilitates the digestion process, and strengthens the muscle and reduces sagging [24–29].

The leaves and flowering tops of *Thymus capitatus* contain many phenolic compounds and flavonoids, and their essential oil is the main and biologically active ingredient. It also contains a group of vitamins and minerals [29,30].

In this work, we conducted a comparative study of the properties of *Thymus capitatus* grown in the regions of Guelma and Souk Ahras.

This study was conducted to investigate the natural products present in both regions by extracting these products and studying the antioxidant activity by electrochemical and chemical methods.

2 Materials and methods

2.1 Chemicals and reagents

Gallic acid (GA), quercetin, aluminum trichloride solution ($AlCl_3$), sodium carbonate solution (Na_2CO_3), ascorbic acid, methanol (MeOH), ethanol ($C4H_9OH$), petroleum ether (RO-R'), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and the Folin–Ciocâlteu reagent (FCR) were obtained from a laboratory of Applied Chemistry and Environment.

2.2 Plant materials

Aerial parts of *Thymus capitatus* were collected in April 2022 from two different regions of eastern Algeria (Souk ahras and Guelma). Harvested samples were air-dried away from direct sunlight and stored in sealed containers at room temperature until needed for experiments.

2.3 Sample extraction process

2.3.1 Phenolic extract

After soaking 50 g of *Thymus capitatus* powder in petroleum ether for 24 h, the powder was filtered, and the remaining material was extracted using ethanol (80/20). This procedure was done three times, using a fresh solvent each time, until the extract was eventually refined using methanol [31,32].

2.3.2 Volatile oil extract

A glass flask with 600 ml of distilled water at 100°C was filled with 50 g of *Thymus capitatus* powder. To prevent burning the plant or altering its natural state, a cooling device was positioned between two vertical tubes. The extracted oil floats on the water's surface because of the difference in densities between the water and the oil. After reaching the boiling point, the distillation process lasts for roughly 3 h. The method is then repeated for the two varieties of *Thymus capitatus* [33–35].

2.3.3 Determination of total phenolic content (TPC)

The TPC of each extract was determined using the FCR. A diluted solution of GA was mixed in methanol (0.02–0.1 mg ml^{-1}),

taking 0.2 ml of each concentration and adding 0.5 ml of the reagent. FCR was left for 5 min in the dark, and then 0.8 ml of sodium carbonate (7.5% Na_2CO_3) was added and was left in darkness for 30 min. The blue color was obtained, and the absorbance was read at a wavelength of 765 nm. The total phenol concentration in these extracts is expressed as mg EAG g^{-1} per gram of extract. The correlation coefficient for the calibration curve obtained was R^2 = 0.994. The same technique was applied to plant extracts [13,36–38].

2.3.4 Determination of total flavonoid content (TFC)

This procedure was based on the oxidation of flavonoids by aluminum chloride ($AlCl_3$ 2%), leading to the formation of a yellow-brown compound.

Several concentrations of quercetin were prepared, ranging from 0.01 to 0.1 mg ml^{-1}; 1 ml was taken from each concentration and 1 ml of aluminum trichloride solution (2% $AlCl_3$) was added to it and left for 30 min in total darkness at the laboratory temperature, after which the absorbance of each concentration was measured at 420 nm [39,40].

The concentration of total flavonoids in these extracts was measured as mg EQ g^{-1} of the extract. The correlation coefficient obtained for the calibration curve was R^2 = 0.994. This same technique was also applied to plant extracts [41,42].

2.4 Chromatographic HPLC analysis

The reference compound was injected to determine its characteristic retention time. Then, the sample (the extract) to be analyzed was injected under the same conditions as the reference compound with the same volume (20 µl) at a concentration of 5 mg ml^{-1}. Then, the retention times of the compounds in the sample were read on the chromatogram and compared to the reference values, allowing us to identify the phenolic compounds present in the sample [43–46].

2.5 Chromatographic GC-MS analysis

Analysis of the volatile oil samples from the regions of Guelma and Souk Ahras was carried out by gas chromatography, where the main components were identified by comparing their spectra and retention times with standard compounds [33,47,48].

2.6 Antioxidant activity

2.6.1 Cyclic voltammetry test

This method allows for determining the conditions in which the oxidation–reduction reaction takes place and estimating the degree of reversibility of the whole (oxidation–reduction). It also sometimes allows the determination of the reaction mechanism at the electrode, as well as the speed constants for rapid electrochemical reactions [49,50]. Antioxidant activity was measured by the determination of the products resulting from oxidation or the ability to inhibit free radicals, where the antioxidant activity of phenols depends on their reversible property to bind with hydroxyl groups [51]. Electrochemical measurements have advantages for determining antioxidant activity. The oxidation potential of GA measured by cyclic voltammetry was used to compare the antioxidant capacity of flavonoid compounds. This electrochemical technique was applied to analyze the antioxidants present in the plant extracts [52–54].

2.6.2 DPPH test

The DPPH° test was used to measure the antiradical power of pure molecules or plant extracts in a model system (organic solvent, ambient temperature). It is used to measure the ability of an antioxidant (HA, generally phenolic compounds) to reduce the chemical radical DPPH° by hydrogen transfer. DPPH°, initially purple, turns into DPPH-H, pale yellow [6]. First, we prepared a DPPH solution in methanol by dissolving 2 mg of DPPH in 50 ml of methanol to produce a dark violet solution. Then, we prepared different concentrations of ascorbic acid ranging between 0.04 and 0.4 mg m^{-1}. We took a quantity of 1 ml from each ascorbic acid concentration, and we added 1 ml of DPPH solution to it in glass tubes. We left the mixture in the dark for 30 min at laboratory temperature and then read the absorbance at 517 nm [55].

3 Results

3.1 Extraction yield

3.1.1 Phenolic extract

The extraction yield of the total phenol and volatile oils from *Thymus capitatus* for both regions is presented in Table 1.

Table 1: Yield of total phenol and volatile oil extraction

Extracts	Samples	Yield (%)
Phenolic extract	Phenolic extract in the Guelma region (A)	8.6976
	Phenolic extract in the Souk Ahras region (B)	19.5476
Volatile oil extract	Volatile oil extract in the Guelma (A″) region	3.1516
	Volatile oil extract in the Souk Ahras (B″) region	1.3387

3.1.2 Total phenols and flavonoids

The standard curve relationship for each of the phenols and the reference flavonoids was used to calculate the equivalent amount for 1 g of the extract. The results are reported in Table 2.

3.2 Identification by HPLC

The compounds of the phenolic extract of the regions of Guelma and Souk Ahras were identified, and their retention times were compared with those obtained for the same standard compounds.

Determination of the equations in the plant extracts according to the calibration curve in Figure 1 and Table 3.

3.3 GC-MS identification

The identification of the volatile components was based on comparison of their mass spectra with those described by Adams [56], as well as by comparison of their retention indexes with the literature data [57] and by comparison of their retention times with those of pure authentic samples, as shown in Figure 2 and Tables 4 and 5.

3.4 Antioxidant activity evaluation

3.4.1 Cyclic voltammetry

The antioxidant activity of *Thymus capitatus* extracts was estimated by cyclic voltammetry, which allowed us to draw cyclic voltammetric curves.

From the peaks of the curves in Figure 3a and b and from the relationship between the current density and the concentration of the standard GA compound, we found the value of the anodic current density at these peaks, as well as the concentration of the GA equivalent.

3.4.2 Inhibitory ability of free radical DPPH

Inhibition percentage evaluation allowed plotting of curves of the inhibition percentage versus standard GA concentration, as shown in Figure 4a and b. The appropriate concentrations to inhibit 50% of free radicals (IC_{50}%) for each of the phenolic extracts and ascorbic acid are shown.

4 Discussion

4.1 Extraction yield

4.1.1 Phenolic extract

Based on the results of Table 1, we can clearly state that the richness of the plant in phenolic compounds is 2.24 times higher in the case of the Souk Ahras region than in the Guelma region, which is most certainly due to the variability of soil quality between the two regions, thus affecting the quantum yield.

4.1.2 Volatile oil extract

Table 2 shows the plant's richness in volatile oils, whose yield rate was 1.34% in the Souk Ahras region versus 3.15%

Table 2: Amount of the total phenols and flavonoids in the extracts

Extracts	Sample	Quantity
Total phenol	Total phenol in the Guelma region (A)	3.20381 (mg EAG g^{-1})
	Total phenol in the Souk Ahras region (B)	3.41415 (mg EAG g^{-1})
Total flavonoids	Total flavonoids in the Guelma region (A)	26.3140 (mg EQ g^{-1})
	Total flavonoids in the Souk Ahras region (B)	22.0327 (mg EQ g^{-1})

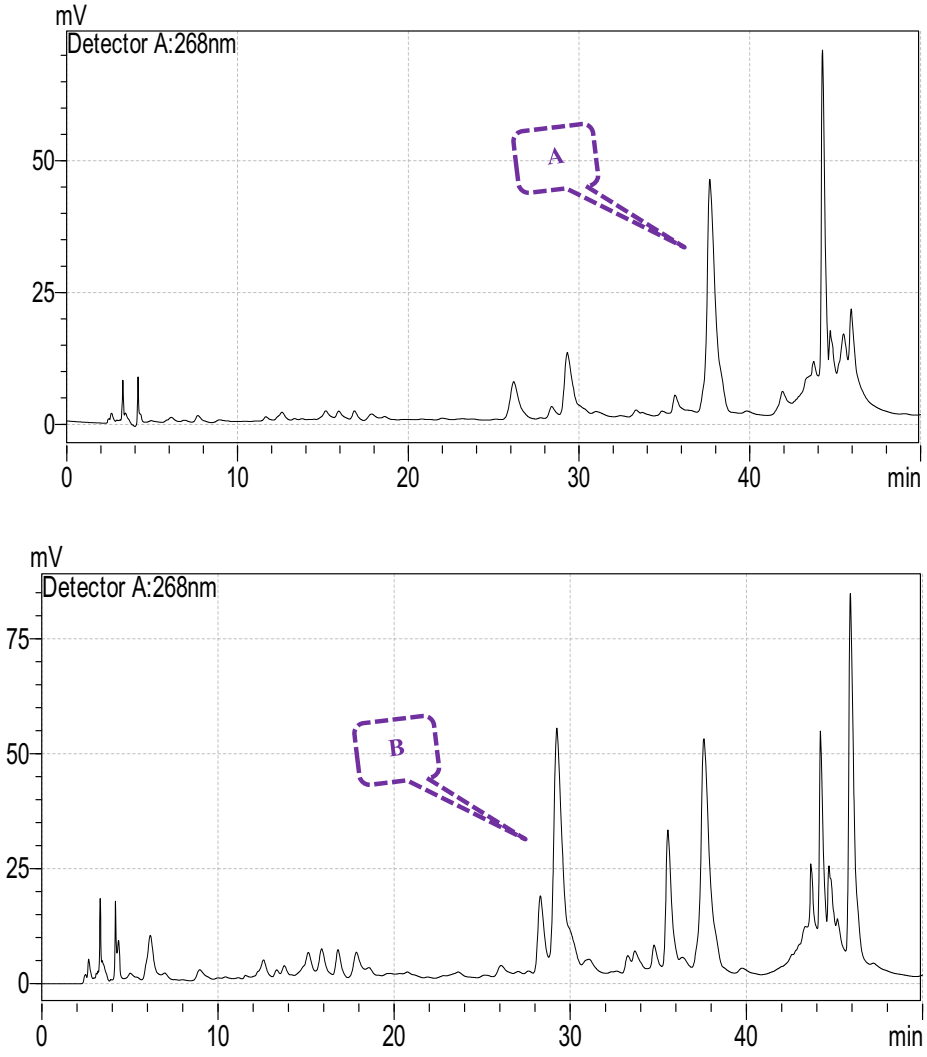

Figure 1: Phenolic compounds in extracts A and B by HPLC analysis. (A: phenolic extract of the Guelma region, and B: phenolic extract of the Souk Ahras region).

Table 3: Quantification of phenolic compounds in samples A and B

Reference phenol	Retention time T_R (min)	Sample A	Sample B
Gallic acid	5.29	5.358	4.936
Chlorogenic acid	13.392	13.331	13.331
Vanillic acid	15.531	15.883	15.923
Caffeic acid	16.277	16.81	16.845
Vanillin	21.46	21.905	21.978
p-Coumaric acid	23.217	23.634	23.752
Rutin	28.37	28.297	28.396
Naringin	34.788	34.756	34.855
Quercetin	45.48	45.166	45.487

Sample A: phenolic extract of the Guelma region. Sample B: phenolic extract of the Souk Ahras region.

in the Guelma region. Also, in this case, we can attribute this difference to the variation in soil quality between the two regions.

4.1.3 Total phenol and flavonoid content

The results of Table 3 indicate that the amount of phenolic compounds equivalent to 1 g of the extract is about the same order for both regions and reaches a value of 3.41415 mg GAE g^{-1} for the extract of Souk Ahras compared to 3.20 mg GAE g^{-1} for the Guelma extract. According to Table 4, the quantity of flavonoids equivalent to 1 g of extract is about 26.31 mg QE g^{-1} for the Guelma extract, against a slightly

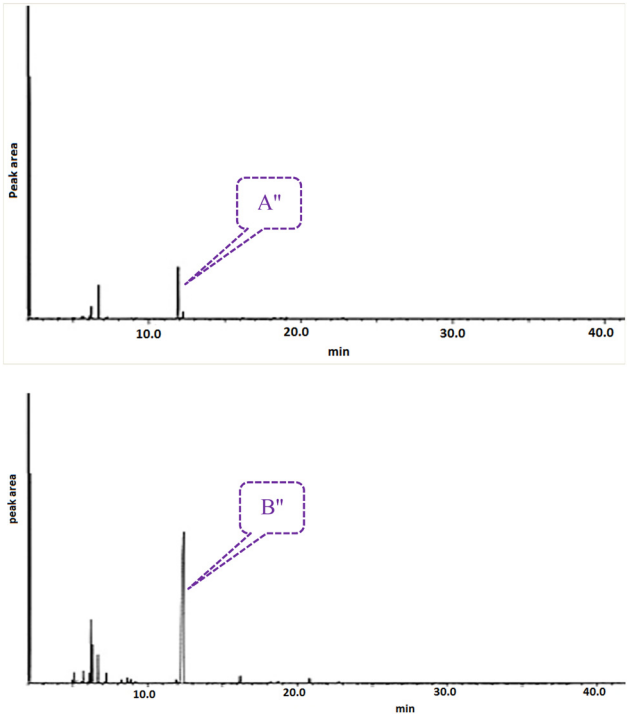

Figure 2: Graphical curves for the chromatography of volatile oils. (A″: volatile oil extract of the Guelma region, and B″: volatile oil extract of the Souk Ahras region).

lower value equal to 22.0327 mg QE g^{-1} for the Souk Ahras. The results show that plants *Thymus capitatus* from both Guelma and Souk Ahras are promising sources rich in phenolic and flavonoid content, which could be the main factor in their antioxidant properties.

4.2 HPLC analysis

The results in Figure 1 and Table 5 clearly show the presence of all nine reference phenolic compounds for both extracts with different proportions. The phenolic compounds such as (chlorogenic acid – vanillic acid – caffeic acid –vanillin – *p*-coumaric acid – rutin – naringin) are present in large amounts in sample A, while the gallic acid and quercetin are present in large amounts in sample B.

The reason for this diversity can be attributed to many biological factors, including genetic and agronomic differences, as well as other environmental factors, such as maturity stages, salinity, temperature, water pressure, and light intensity conditions.

We concluded that the HPLC analyses performed enabled us to better understand the phenolic and flavonoid compounds of *Thymus capitatus* from the Guelma and Souk Ahras regions.

Table 4: Chemical composition of volatile oil from Souk Ahras

RT (min)	IR (cm^{-1})	Sample A”	Ratio%
2.023	661	Methyl cyclopentane	41.04
2.148	969	1-Octen-3-ol	38.44
2.322	952	3-Octanone	0.07
2.559	888	5-Methyl-3-heptanone	0.07
2.639	958	beta-Myrcene	0.1
4.050	1,228	3,7-Dimethylocta-2,6-dien-1-ol	0.16
4.984	919	4-Carene	0.19
5.093	998	1-Methyl-4-(1-methyl ethyl)-1,3-cyclo hexanadiene	0.08
5.130	948	2-Carene	
5.238	1,042	1-Methyl-2-(1-methylethyl) benzene	0.04
5.548	1,042	*o*-Cymene	0.34
5.717	1,042	1-Methyl-4-(1-methyl ethyl)benzène	0.31
5.940	998	gamma-Terpinene	0.03
6.102	998	1-Methyl-4-(1-methyl ethyl)-1,4-cyclo hexa dine	0.52
6.201	1,082	Linalool	1.95
6.679	1,082	3,7-Dimethyl-1,6-octadien-3-ol	5.35
7.236	1,262	Thymol	0.28
8.871	1,262	2-Methyl-5-(1-methyl ethyl)phenol	0.06
9.145	1,262	3-Methyl-4-isopropyl phenol	0.06
11.913	1,447	Tetradecamethyl-cyclo-hepta-siloxane	8.22
12.218	1,648	3-Isopotopoxy-1,1,1,7,7,7-hexamethyl-3,5,5-tris (trimethylsiloxy)tetrasiloxane	1.13
16.168	1,811	3-Butoxy-1,1,1,7,7,7-hexamethyl-3,5Tris(trimethylsiloxy) tetrasiloxane	0.09
18.240	1,068	Dodecamethyl-pentasiloxane	0.15
18.716	1,446	3-(1,5-Dimethyl-4-hexenyl)-6-methylene-cyclohexene	0.1
19.051	1,398	1*H*-Octahydro-3,8,8-trimethyl-6-methylene-7-methanoazulene	0.21
22.770	1,654	Hexadecamethyl-cyclooctasiloxane	0.12
43.401	1,765	2,6-Dihydroxybenzoic acid, 3 TMS	0.60

Sample A”: volatile oil extract of the Guelma region.

4.3 GC-MS analysis

From Figure 2, it appears that the essential oil of the Souk Ahras region contains 28 compounds whose structures have been determined and which represent a total of 100%. Similarly, 44 compounds were found in the essential oil of the Guelma region. In total, 28 compounds were identified structurally, which constitutes a percentage of

Table 5: Chemical composition of volatile oil from Guelma

RT (min)	IR (cm^{-1})	Sample B″	Ratio%
2.022	902	2-Methyl-5-(1-methylethyl)-bicyclo [3.1.0]hex-2-ene	26.45
2.145	902	3-Thujene	25.47
2.319	948	2-Pinene	0.05
2.636	948	2,6,6-Trimethylbicyclo[3.1.1] hept-2-ene	0.02
3.152	948	alpha-Pinene	0.06
3.203	943	Camphene	0.06
4.277	943	2,2-Dimethyl-3- methylenebicyclo [2.2.1]heptane	0.05
4.971	969	1-Octen-3-ol	0.44
5.080	958	beta-Myrcene	1.31
5.279	1,042	*o*-Cymene	0.41
5.537	1,042	1-Methyl-4-(1 -methyl) Benzene	0.28
5.624	1,059	Eucalyptol	0.18
5.704	998	gamma-Terpinene	1.50
5.936	998	1-Methyl-4-(1-methylethyl)-1,4-Cyclohexadien	0.17
6.021	1,082	Linalool	0.08
6.091	1,082	3,7-Dimethyl-1,6-octadien	1.26
6.195	1,121	2-Bornanone	7.74
6.260	1,121	Camphor	0.36
6.313	1,138	endo-Borneol	4.71
6.451	1,138	1,7,7-Trimethylbicyclo[2.2.1] heptan-2-ol	0.03
6.668	1,137	Terpinen-4-ol	3.48
6.807	1,137	4-Methyl-1-(1-methylethyl) -3-cyclohexene	0.10
7.133	1,262	2-Methyl-5-(1-methylethyl)-phenol	0.16
7.229	1,262	3-Methyl-4-isopropylphenol	1.24
8.232	1,262	Thymol	0.42
8.648	1,494	Caryophyllen	0.67
9.185	2,788	2.2′-Methylenebis-6-(1,1-dimethylethyl)-4-methyl phenol	0.16

Sample B: volatile oil extract of the Souk Ahras region.

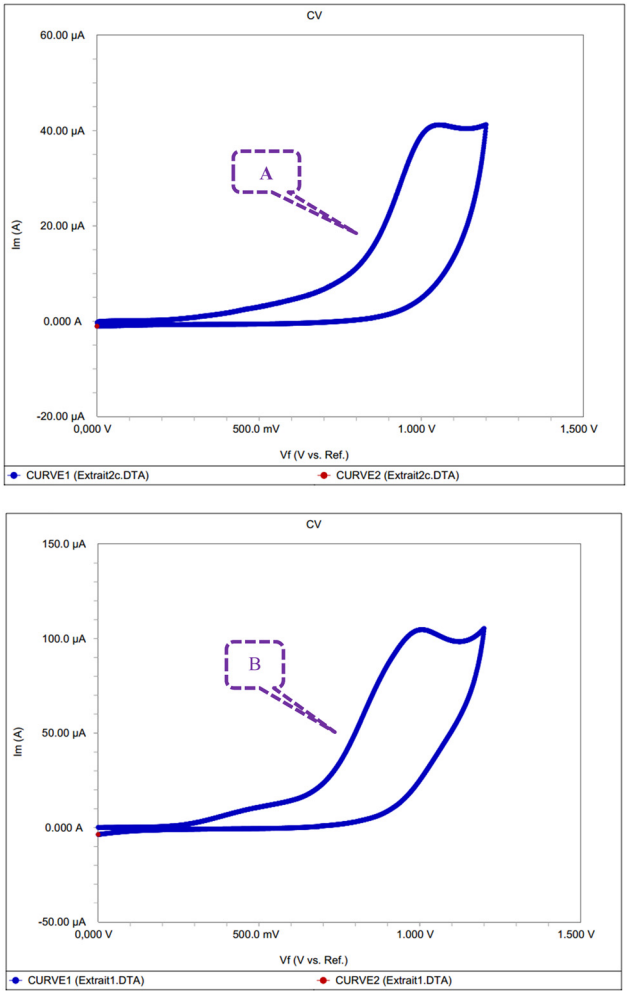

Figure 3: Cyclic voltammetry curves for extracts A and B (A: phenolic extract of the Guelma region and B: Phenolic extract of the Souk Ahras region).

77.34%. The diversity of the chemical composition of the essential oil from one region to another is affected by temperature, humidity, soil quality, harvesting period, and the age of the plants.

4.4 Antioxidant activity

4.4.1 Cyclic voltammetry test

The amount of antioxidant compounds was estimated by the periodic metric method based on the GA curve in Figure 3. The antioxidant activity estimated was about 0.55 mg EGA g^{-1} for the extract of Souk Ahras region; on the other hand, it strongly increased by a factor of 5.79 times to reach a value of 3.17 mg EGA g^{-1} for the Guelma region's extract. The quantities of estimated antioxidants are acceptable.

4.4.2 DPPH test

From Figure 4, it is shown that the phenolic extract of the Guelma region has the highest antioxidant activity at IC_{50}, with a concentration of 0.4 mg ml^{-1}, while the antioxidant activity of the phenolic extract of the Souk Ahras region at IC_{50} has a value of 0.5 mg ml^{-1}.

The phenolic extracts of plants showed the ability to give hydrogen as they showed their ability to inhibit the DPPH root through the IC_{50} value.

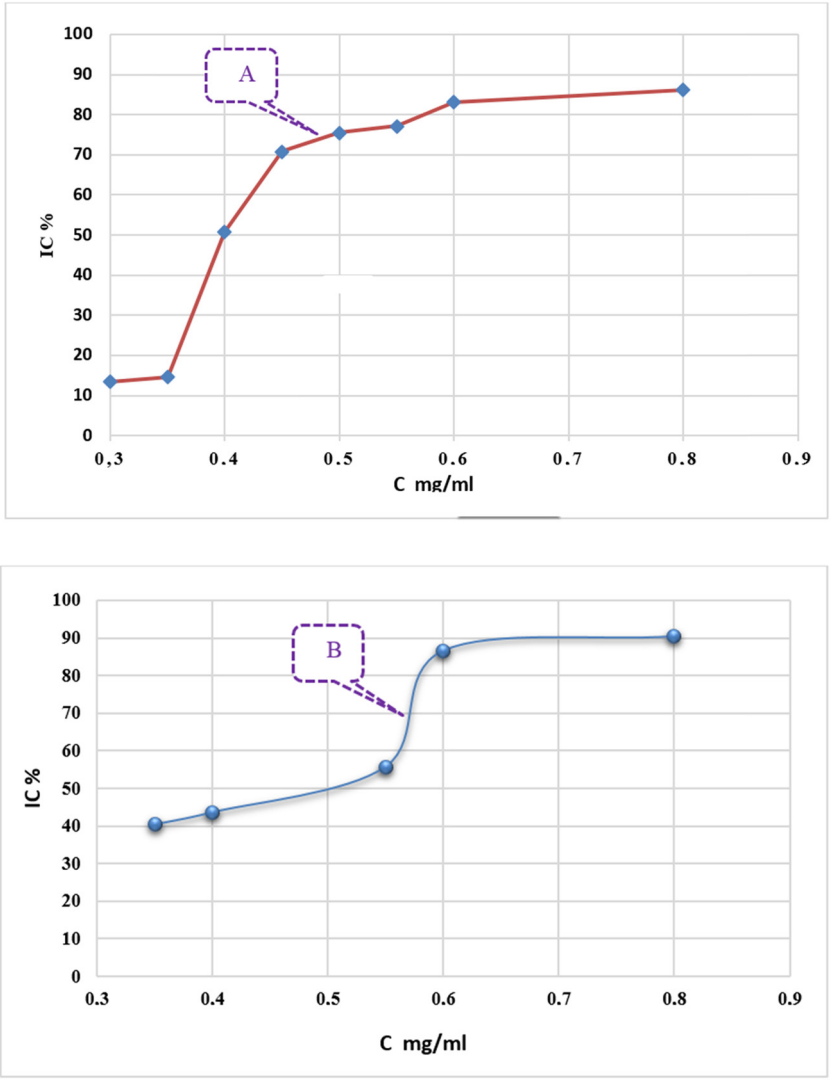

Figure 4: Percentage inhibition curves for extracts A and B (A: phenolic extract of the Guelma region and B: Phenolic extract of the Souk Ahras region).

5 Conclusion

This work has been a continuation of previous research in the field of medicinal plants, aimed to reveal further therapeutic value of the active substances contained in *Thymus capitatus* plants grown in the regions of Guelma and Souk Ahras, as they play an important role in the traditional medicine.

The results clearly indicated that all tested extracts showed significant phenolic and flavonoid contents and remarkable antioxidant activity.

The quantities of phenols are relatively large in *Thymus capitatus* plants grown in the Souk Ahras region, reaching 3.41 mg GAE g^{-1} of extract, while the amount of flavonoids was higher in the Guelma region (26.31 mg QE g^{-1} of extract). The HPLC results also showed that *Thymus capitatus* specifically contained nine phenolic compounds, and the reference amounts of phenols were greater in *Thymus capitatus* extract of the Guelma region. The high percentage of flavonoids in the *Thymus capitatus* plant extract of the Guelma region is responsible for the antioxidant activity, as shown by the cyclic voltammetry test and DPPH test.

Bioassays showed significant activity of the investigated phenolic and flavonoid extracts. The chemical characteristics and antioxidant capacity actions of indigenous species from Algerian flora are all abundantly detailed in these studies. The findings unequivocally show that plant phenolic and flavonoid extracts can offer an intriguing natural substitute that can be helpful for both pharmaceutical treatments and dietary restrictions.

Climate variations and environmental stressors, such as salt and drought, exert an impact on phenolic compounds.

Consequently, it can be inferred that the composition of the soil in the arid Guelma and Souk Ahras regions of the Algerian high plains alters the stresses exerted on the physical and chemical processes governing phenols. This alteration leads to an upsurge in reactive oxygen species, a phenomenon successfully achieved and observed in the obtained results. Consequently, the characteristics of phenolic compounds, operating as a harmonized equilibrium within soil and plant systems, serve as a stimulus for further exploration into these compounds concerning abiotic pressures, commonly referred to as external environmental stresses.

These findings provide a scientific basis that can be further enriched in support of folk medicine, and the extracts mentioned may open up opportunities to develop more efficient and effective food preservatives in terms of antioxidant agents. Future studies regarding *Thymus capitatus* extracts should hopefully focus on their "*in vivo*" antioxidant activity.

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References

[1] Tzima K, Brunton NP, Rai DK. Qualitative and quantitative analysis of polyphenols in lamiaceae plants—a review. Plants. 2018;7:25.
[2] Messaoudi M, Rebiai A, Sawicka B, Atanassova M, Ouakouak H, Larkem I, et al. Effect of extraction methods on polyphenols, flavonoids, mineral elements, and biological activities of essential oil and extracts of *Mentha pulegium* L. Molecules. 2021;27(1):11.
[3] Harley RM, Atkins S, Budantsev AL, Cantino PD, Conn BJ, Grayer R et al. Labiatae. In VII flowering plants dicotyledons, lamiales (Except acanthaceae including avicenniaceae). In: Kadereit J. The families and genera of vascular plants. 6, Berlin/Heidelberg, Germany: Springer; 2004. p. 167–275.
[4] http://www.worldfloraonline.org/taxon/wfo-7000000318 (accessed on 20 June 2023).
[5] Raja RR. Medicinally potential plants of Labiatae (Lamiaceae) family: An overview. Res J Med Plant. 2012;6:203–13.
[6] Stankovic M, editor. Lamiaceae species: Biology, ecology and practical uses. Basel, Switzerland: MDPI; 2020.
[7] Carović-Stanko K, Petek M, Grdiša M, Pintar J, Bedeković D, Ćustić MH, et al. Medicinal plants of the family Lamiaceae as functional foods—A review. Czech J Food Sci. 2016;34:377–90.
[8] Hedge I. A global survey of the biogeography of the Labiatae. In: Harley RM, Reynolds T. Advances in Labiatae Science. London, UK: The Royal Botanical Garden, Kew; 1992. p. 7–17.
[9] Valerio F, Mezzapesa GN, Ghannouchi A, Mondelli D, Logrieco AF, Perrino EV, et al. Characterization and antimicrobial properties of essential oils from four wild taxa of Lamiaceae family growing in Apulia. Agronomy. 2021;11:1431.
[10] Perrino EV, Valerio F, Jallali S, Trani A, Mezzapesa GN. Ecological and biological properties of *Satureja cuneifolia* Ten. and *Thymus spinulosus* Ten.: Two wild officinal species of conservation concern in Apulia (Italy). A preliminary survey. Plants. 2021;10:1952.
[11] Perrino EV, Wagensommer RP. Crop wild relatives (CWRs) threatened and endemic to Italy: Urgent actions for protection and use. Biology. 2022;11:193.
[12] EMPB. The Euro + Med plantbase project, the information resource for euro-mediterranean plant diversity: Lamiaceae occurring in Algeria. https://www.emplantbase.org/home.html (accessed on 21June 2023).
[13] https://ww2.bgbm.org/EuroPlusMed/PTaxonDetail.asp? NameId = 111505&PTRefFk = 8000000 (accessed on 08 June 2023).
[14] African plant database. https://africanplantdatabase.ch/en/nomen/145270 (accessed on 14 June 2023).
[15] WCSPWorld checklist of selected plant of lamiaceae familly. Royal Botanic Gardens, Kew. https://wcsp.science.kew.org/reportbuilder.do (accessed on 11 June 2023).
[16] Fakchich J, Elachouri M. An overview on ethnobotanico-pharmacological studies carried out in Morocco, from 1991 to 2015: Systematic review (part 1). J Ethnopharmacol. 2021;267:113200.
[17] Bouafia M, Amamou F, Gherib M, Benaissa M, Azzi R, Nemmiche S. Ethnobotanical and ethnomedicinal analysis of wild medicinal plants traditionally used in Naâma, Southwest Algeria. Vegetos. 2021;34:654–62.

[18] Hemmami H, Seghir BB, Zeghoud S, Ben Amor I, Kouadri I, Rebiai A, et al. Desert endemic plants in algeria: A review on traditional uses, phytochemistry, polyphenolic compounds and pharmacological activities. Molecules. 2023;28(4):1834.
[19] Ouakouak H, Benarfa A, Messaoudi M, Begaa S, Sawicka B, Benchikha N, et al. Biological properties of essential oils from *Thymus algeriensis* Boiss. Plants. 2021;10(4):786.
[20] Jaouadi R, Cardoso SM, Silva AMS, Ben Hadj Yahia I, Boussaid M, Zaouali Y. Variation of phenolic constituents of tunisian *Thymus capitatus* (L.) hoff. et link. populations. Biochem Syst Ecol. 2018;77:10–5.
[21] Bayer E, Buttler K, Finkenzeller X, Grau J. Guide de la flore m_editerran_eenne: Caract_eristiques, habitats, distribution et particularit_es de 536 esp_eces. ed. Nature Delachaux Et Niestl_e 288 Pages; 2009.
[22] Džamić AM, Nikolić BJ, Giweli AA, Mitić-Ćulafić DS, Soković MD, Ristić MS, et al. Libyan *Thymus capitatus* essential oil: Antioxidant, antimicrobial, cytotoxic and colon pathogen adhesion-inhibition properties. J Appl Microbiology. 2015;119(2):389–99.
[23] Kaileh M, Vanden Berghe W, Boone E, Essawi T, Haegeman G. Screening of indigenous palestinian medicinal plants for potential anti-inflammatory and cytotoxic activity. J Ethnopharmacol. 2007;113:510–6.
[24] Goudjil MB, Zighmi S, Hamada D, Mahcene Z, Bencheikh SE, Ladjel S. Biological activities of essential oils extracted from *Thymus capitatus* (Lamiaceae). South Afr J Botany. 2020;128:274–82.
[25] Casiglia S, Bruno M, Scandolera E, Senatore F, Senatore F. Influence of harvesting time on composition of the essential oil of *Thymus capitatus* (L.) Hoffmanns. & Link. growing wild in northern Sicily and its activity on microorganisms affecting historical art crafts. Arab J Chem. 2019;12(8):2704–12.
[26] Ballester-Costa C, Sendra E, Fernández-López J, Pérez-Álvarez JA, Viuda-Martos M. Assessment of antioxidant and antibacterial properties on meat homogenates of essential oils obtained from four Thymus species achieved from organic growth. Foods. 2017;6(8):59.
[27] Miri YB, Djenane D. Antifungal, anti-aflatoxigenic, antioxidant activity and in vivo efficacy of essential oil of the aerial parts of *Thymus capitatus* (L.) Hoffmann's & link. Phytothérapie. 2018;17:299–309.
[28] Samejo MQ, Sumbul A, Shah S, Memon SB, Chundrigar S. Phytochemical screening of *Tamarix dioica* Roxb. ex Roch. J Pharm Res. 2013;7(2):181–3.
[29] Jaradat N, Hussen F, Al Ali A. Preliminary phytochemical screening, quantitative estimation of total flavonoids, total phenols and antioxidant activity of *Ephedra alata* Decne. J Mater Env Sci. 2015;6(6):1771–8.
[30] Aldughaylibi FS, Raza MA, Naeem S, Rafi H, Alam MW, Souayeh B, et al. Extraction of bioactive compounds for antioxidant, antimicrobial, and antidiabetic applications. Molecules. 2022;27(18):5935.
[31] Munteanu IG, Apetrei C. Analytical methods used in determining antioxidant activity: A review. Int J Mol Sci. 2021;22(7):3380.
[32] Farhan M, Rizvi A, Aatif M, Ahmad A. Current understanding of flavonoids in cancer therapy and prevention. Metabolites. 2023;13(4):481.
[33] Aly MM, Al-Ghamdi M, Bafeel SO, Khedr AM. Antimicrobial qctivities and phytochemical analysis of the essential oil of Lavandula dentata and Plectranthus tenuiflorus, collected from Al Baha region, Saudi Arabia. Life Sci J. 2013;10:4.
[34] Abdelmajeed NA, Danial EN, Ayad HS. The effect of environmental stress on qualitative and quantitative essential oil of aromatic and medicinal plants. Arch Des Sci. 2013;66(4):100–20.
[35] Snoussi M, Noumi E, Punchappady-Devasya R, Trabelsi N, Kanekar S, Nazzaro F, et al. Antioxidant properties and anti-quorum sensing potential of *Carum copticum* essential oil and phenolics against *Chromobacterium violaceum*. J Food Sci Technol. 2018;55(8):2824–32.
[36] Benabdallah FZ, Zellagui A, Demirtas I. Chemical composition of essential oils and antioxidant activities of extracts of two endemic plants from Algeria. Int J Pharm Sci Res. 2017;13:244–50.
[37] Marin M, Novakovic M, Vuckovic I, Te_sevic V, Kolarevic S, Vukovic-Gacic B. Wild *Thymus capitatus* hoff. et link. chemical composition, Antioxidant and antimicrobial activities of the essential oil. J Essent Oil Bear Plants. 2018;21:388–99.
[38] Benmohamed M, Guenane H, Messaoudi M, Zahnit W, Egbuna C, Sharifi-Rad M, et al. Mineral profile, antioxidant, anti-inflammatory, antibacterial, anti-urease and anti-α-amylase activities of the unripe fruit extracts of pistacia atlantica. Molecules. 2023;28(1):349.
[39] Haile M, Kang W. Antioxidant activity, total polyphenol, flavonoid and tannin contents of fermented green coffee beans with selected yeasts. Fermentation. 2019;2019:5. doi: 10.3390/fermentation5010029.
[40] Chandra S, Khan S, Avula B, Lata H, Yang MH, ElSohly MA, et al. Assessment of total phenolic and flavonoid content, antioxidant properties, and yield of aeroponically and conventionally grown leafy vegetables and fruit crops: A comparative study. Evid Based Complement Alternat Med. 2014;2014;253875. doi: 10.1155/2014/253875
[41] Arif H, Rehmani N, Farhan M, Ahmad A, Hadi SM. Mobilization of copper ions by flavonoids in human peripheral lymphocytes leads to oxidative DNA breakage: A structure activity study. Int J Mol Sci. 2015;16:26754–69.
[42] Arif H, Rehmani N, Farhan M, Ahmad A, Hadi SM. Mobilization of copper ions by flavonoids in human peripheral lymphocytes leads to oxidative DNA breakage: A structure activity study. Int J Mol Sci. 2015;16(11):26754–69.
[43] Farhan M, Shamim U, Hadi S. green tea polyphenols: A putative mechanism for cytotoxic action against cancer cells. Nutraceuticals and natural product derivatives: Disease prevention & drug discovery. Hoboken, NY, USA: Wiley; 2019. p. 305–32.
[44] Küçükaydın S, Çayan F, Tel-Çayan G, Duru ME. HPLC-DAD Phytochemical profiles of *Thymus cariensis* and *T. cilicicus* with antioxidant, cytotoxic, anticholinesterase, anti-urease, anti-tyrosinase, and antidiabetic activities. S Afr J Bot. 2021;143:155–63.
[45] Llorent-Martínez EJ, Córdova MLF, Zengin G, Bahadori MB, Aumeeruddy MZ, Rengasamy KRR, et al. Parentucellia latifolia subsp. latifolia: A potential source for loganin iridoids by HPLC-ESI-MSn technique. J Pharm Biomed Anal. 2019;165:374–80.
[46] Hossain H, Rahman SE, Akbar PN, Khan TA, Rahman M, Jahan IA. HPLC profiling, antioxidant and in vivo anti-inflammatory activity of the ethanol extract of Syzygium jambos available in Bangladesh. BMC Res Notes. 2016;9(1):1–8.
[47] Silva AM, Félix LM, Teixeira I, Martins-Gomes C, Schäfer J, Souto EB, et al. Orange thyme: Phytochemical profiling, in vitro bioactivities of extracts and potential health benefits. Food Chem. 2021;12:100171.
[48] Sara M, Yamina B, Ramazan E, Mesut G, Selma A. Dietary risk of BlaESBL producing multidrug resistant enterobacteriaceae and their inhibition by Artemisia herba-alba and Thymus Algeriensis essential oils. J Essent Oil-Bear Plants. 2021;24:658–70.

[49] Wang. J. Analytical electrochemistry. 2nd edn. New York: Johen Wiley & Sons, Inc, Publication; 2001. p. 1–100.
[50] Tur'yan YI, Gorenbein P, Kohen R. Theory of the oxygen voltammetric electroreduction process in the presence of an antioxidant for estimation of antioxidant activity. J Electroanal Chem. 2004;571(2):183–8.
[51] Parcheta M, Świsłocka R, Orzechowska S, Akimowicz M, Choińska R, Lewandowski W. Recent developments in effective antioxidants: The structure and antioxidant properties. Materials. 2021;14(8):1984.
[52] https://sop4cv.com/index.html (accessed Jun 2023).
[53] Aranzaes JR, Daniel M-C, Astruc D. Metallocenes as references for the determination of redox potentials by cyclic voltammetry—permethylated iron and cobalt sandwich complexes, inhibition by polyamine dendrimers, and the role of hydroxy-containing ferrocenes. Can J Chem. 2006;84(2):288–99.
[54] Chevion S, Roberts MA, Chevion M. The use of cyclic voltammetry for the evaluation of antioxidant capacity. Free Radic Biol Med. 2000;28(6):860–70.
[55] Zahnit W, Smara O, Bechki L, Bensouici C, Messaoudi M, Benchikha N, et al. Phytochemical profiling, mineral elements, and biological activities of *Artemisia campestris* L. grown in Algeria. Horticulturae. 2022;8(10):914.
[56] Goudjil MB, Ladjel S, Bencheikh SE, Zighmi S, Hamada D. Chemical composition, antibacterial and antioxidant activities of the essential oil extracted from the *Mentha piperita* of southern Algeria. Res J Phytochem. 2015;9(2):79–87.
[57] Benali T, Habbadi K, Khabbach A, Marmouzi I, Zengin G, Bouyahya A, et al. GC–MS analysis, antioxidant and antimicrobial activities of *Achillea odorata* subsp. pectinata and *Ruta montana* essential oils and their potential use as food preservatives. Foods. 2020;9(5):668.