Trop J Nat Prod Res, January 2024; 8(1):5785-5793 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Effect of Temperature and Water Stress on the Antioxidant and Antidiabetic Activities of *Thymus vulgaris* Essential Oil

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ARTICLE INFO

Article history:
Received 18 September 2023
Revised 28 December 2023
Accepted 02 January 2024
Published online 01 February 2024

ABSTRACT

Thymus vulgaris L. (family Lamiaceae) is an aromatic medicinal plant well-known for its essential oil and therapeutic values. It thrives across Europe, Western Asia, the Mediterranean, and Northwestern Africa. This study aims to investigate the effect of climate change on the chemical composition, antioxidant, and antidiabetic activities of *Thymus vulgaris* essential oil. Essential oils were extracted from three distinct *Thymus vulgaris* samples (S1 – S3) cultivated under different climatic conditions. S1 was cultivated under normal seasonal condition; S2 and S3 were cultivated under controlled conditions; 5°C temperature increase and 50% precipitation (S2), 10°C temperature increase and 75% precipitation (S3). Chemical constituents of the essential oils were identified using Gas Chromatography-Mass Spectrometry (GC-MS). The antioxidant activity was assessed using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2'-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging, and Ferric Reducing Antioxidant Power (FRAP) assays. The antidiabetic activity was assessed via the α-glucosidase and α-amylase inhibitory activities. GC-MS analysis identified 23 compounds with varying proportions in the three essential oil samples. The findings showed that S1 had the most potent antioxidant activity with IC_{50} values of 461.83±10.49 µg/mL and 1508.83±7.22 µg/mL in the DPPH and ABTS assays, respectively, while S3 exhibited the highest antioxidant activity in the FRAP assay, with IC_{50} value of 244.64±1.34 µg/mL. For the antidiabetic activity, S1 showed the highest α-amylase inhibitory activity, while S2 exhibited the highest α-glucosidase inhibitory effect. This study sheds light on *Thymus vulgaris'* adaptability and therapeutic potential under changing climatic conditions. These findings underscore the importance of understanding these dynamics for future applications.

***Keywords*:** *Thymus vulgaris*, Essential oil, Bioactive compounds, Antioxidant activity, Antidiabetic activity, Climate change.

Introduction

For thousands of years, plants have been the primary source of remedies for the various diseases that have afflicted humans.[1] Unlike conventional drugs, medicinal plants are well tolerated and rarely cause side effects. As a result, there has been an increasing fascination with botanical extracts in recent years, as scientists are in constant search for bioactive natural products that are free of harmful effects.[2] According to the World Health Organization (WHO), traditional medicinal practices are relied upon by an estimated 80% of the world's population.

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Citation: Laftouhi A, Slimani M, Elrherabi A, Bouhrim M, Mahraz AM, Idrissi AM, Eloutassi N, Rais Z, Taleb A, Taleb M. Effect of Temperature and Water Stress on the Antioxidant and Antidiabetic Activities of *Thymus vulgaris* Essential Oil. Trop J Nat Prod Res. 2024; 8(1):5785-5793. http://www.doi.org/10.26538/tjnpr/v8i1.11

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

There are approximately 3000 essential oils that have been documented, with about 300 of them holding commercial significance and applications in pharmaceuticals, cosmetics, food, and agricultural industries.[3-5] The majority of plants containing essential oils are categorized as "fragrant plants." These essential oils are present in numerous plant parts, including wood, leaves, fruits, barks, seeds, and roots. These oils are intricate mixtures of compounds, composed mainly of terpenes and aromatic compounds. These compounds accumulate within specialized structures in the plant. These structures include the epidermal cells found in the petals of Rosaceae and Oleaceae, the epidermal glands present in Lamiaceae, the secretory pockets of Rutaceae, or the secretory canals within Apiaceae.[6] Their volatility sets them apart from fixed oils, which are lipid-based. Essential oils are blends of more or less diverse components, known as chemotypes, consisting of relatively uncomplicated molecules like terpenes, phenols, oxides, and ketones.[7]

Thymus vulgaris L. commonly called thyme is an aromatic medicinal plant widely distributed across the Mediterranean region, including Morocco, where it holds a prominent place in traditional medicine practices.[8] It is extensively utilized by the rural population of the Tounate region as an alternative to modern medications in several disease conditions due to its rich bioactive constituents.[9] Taounate, a province in northern Morocco has a Mediterranean climate

characterized by two distinct seasons: a chilly and rainy season, and a scorching and arid season.[10] The region's altitude, temperature, and rainfall make it suitable for the growth of various plant species, including *Thymus vulgaris*.[8] The essential oils derived from *Thymus vulgaris* has various biological activities, including antibacterial,[11,12] anti-tumor,[13] antioxidant,[14] and antifungal activities.[15-19] Currently, with the increase in temperature and the decrease in precipitation due to climate change, it has become necessary to predict the impact of these changes on the physiological and morphological processes of plants particularly medicinal plants like *Thymus vulgaris*. The medicinal importance of this plant and its use in diverse areas warrant further investigation into the potential effects of climate change on the chemical composition and biological activities of the plant. Therefore, the main purpose of this study was to comprehensively investigate the effect of changing climatic conditions on the chemical composition and biological activities of *Thymus vulgaris*. This was achieved by analyzing the essential oils obtained from three distinct *Thymus vulgaris* samples. Sample 1 is the essential oil of thyme cultivated under standard seasonal temperature and rainfall conditions, while Samples 2 and 3 are essential oils obtained from thyme cultivated under controlled temperature of 5℃ and 10℃, respectively, accompanied by varying levels of precipitation of 50% and 75%, respectively in a controlled environment.

Materials and Methods

Study Area

Taounate is a province located within the pre-Rif and Rif regions in the northern part of the Kingdom of Morocco (34°39'38"N 4°26'00"W, 815 m). It shares borders with the provinces of Elhoceima and Chefchaouen to the north, the Wilaya of Fez to the south, the province of Taza to the east, and the provinces of Sidi Kasem and Ouazane to the west (Figure 1). The province experiences a Mediterranean climate featuring two clearly defined seasons: a damp and cool season, and a warm and arid season. During the summer months, temperatures can soar up to 45°C. The average annual rainfall in Taounate is 790 mm, with occasional recordings of up to 1800 mm in Jbel Outka. The province is located at an altitude of 600 m and enjoys a warm Mediterranean climate characterized by a parched summer, with an annual precipitation of 640 mm.

Plant Collection and Cultivation

Three samples of *Thymus vulgaris* tagged samples 1 – 3 (S1 – S3) were collected from the Taounate region at coordinates 34°39'38"N 4°26'00"W, situated at an altitude of 815 m. The plant was identified by Professor Amina Bari, Laboratory of Biotechnology, Environment, Agrifood, and Health, Faculty of Sciences Dhar El Mahraz, University of Sidi Mohamed Ben Abdellah, Fez, Morocco. An herberium specimen with voucher number RT001270127 was deposited. The cuttings of the three samples were transplanted under different conditions. Sample 1 (S1) was transplanted under normal temperature and precipitation conditions, while samples 2 and 3 (S2 and S3) were transplanted under controlled temperature and precipitation conditions. Under the controlled conditions, climatic conditions were deliberately intensified, involving elevated temperatures and diminished irrigation. Sample 1 was cultivated under usual seasonal mean temperature and precipitation, while samples 2 and 3 were cultivated under elevated temperature of 5°C and 10°C increases above the normal, respectively, and water scarcities of 50% and 75%, respectively within a confined environment. Subsequently, all three samples were harvested on January 5, 2023.

Extraction of Essential Oil

The dried leaves of *Thymus vulgaris* was used for the essential oil extraction. The extraction of essential oils (EOs) was carried out using hydrodistillation in a Clevenger-type apparatus. Three distillation processes were conducted by boiling 100 g each of fresh plant material with 1 L of water for 1 h 30 min in a 2 L flask fitted with a 60 cm column connected to a condenser. The yields of the EOs were determined based on the volume of oil (in milliliters) obtained per 100 grams of dry plant material. The EOs were stored at 4°C in the dark.[21-23]

GC-MS Analysis

The essential oils were subjected to GC-MS analysis to determine their chemical composition. The GC-MS system consist of a multi-mode injector and a 123-BD11 column 15 m x 320 µm x 0.1 µm). The essential oil samples were injected into the column using the split 1/4 mode, Helium was employed as the carrier gas at a flow rate of 2 mL/min. Peak areas in the samples were evaluated and presented as a percentage of the total compounds in each sample. The analysis was conducted using the full scan mode, ranging from 30 to 1000 m/z, with a gain factor of 5 and electron impact ionization. The ion source and quadrupole temperatures were configured at 230 and 150°C, respectively, and the oven temperature program initiated at 30°C and terminated at 360°C.

Assessment of Antioxidant Activity

DPPH Free Radical Scavenging Assay

The antioxidant potential of the EO samples was assessed by measuring their capacity to neutralize DPPH (2,2-diphenyl-1-picrylhydrazyl) free radicals, following the procedure described by Şahin *et al*. (2004).[24] The essential oil sample (50 µL) at different concentrations were mixed with 2 mL of a 60 µM solution of DPPH (Sigma-Aldrich, Steinheim, Germany) in methanol and incubated at ambient temperature in the dark for 20 min. The absorbance of the resulting solution was measured at 517 nm using an Ultraviolet-Visible spectrophotometer (UV Tech LCD UV-VIS Spectrophotometer Double Beam, Model Name/Number: UV 180). A methanol solution of Quercetin (Sigma-Aldrich, China) mixed with 2 mL of the DPPH solution was used as the positive control, while the negative control was the methanol solution of DPPH only. The percentage free radical scavenging activity was calculated using the formula:

$$\text{Antiradical activity (\%)} = \frac{\text{A control} - \text{A sample}}{\text{A control}} \times 100$$

Where; A control is the absorbance of the control and A sample is the absorbance of the test samples. The anti-radical activity was reported in terms of the percentage of free radical scavenging and µg of Quercetin equivalents per mL (µg QE/mL).

ABTS Free Radical Scavenging Assay

The 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) free radical scavenging assay was performed following the procedure described by Pukalskas *et al*. (2002)[25] To initiate the formation of $ABTS^{\bullet+}$ radicals, a mixture of ABTS (Sigma-Aldrich, USA) and potassium persulfate (Sigma-Aldrich, Steinheim, Germany) in water was reacted in the absence of light for 16 h. The resultant solution was diluted with ethanol to attain an absorbance of approximately 0.70 ± 0.02 at 734 nm, ensuring a linear relationship between absorbance and $ABTS^{\bullet+}$ radical concentration up to an absorbance of 2.

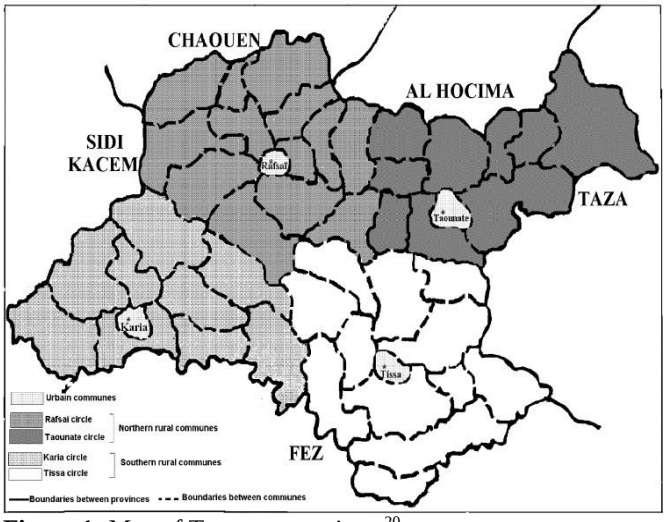

Figure 1: Map of Taounate province.[20]

Thereafter, 100 µL of each essential oil as well as a methanol blank solution (control) were introduced into 2 mL of the prepared ABTS solution and incubated at room temperature for 1 min. the absorbance of the resulting solution was measured at 734 nm. The captured $ABTS^{\bullet+}$ radical was quantified from ascorbic acid standard curve, and the results were expressed as micrograms of ascorbic acid equivalents per milligram of extract (µg AAE/mg extract).

Ferric Ion Reducing Antioxidant Power (FRAP) Assay

The antioxidant activity in terms of Ferric (Fe^{3+}) ions reducing power measures the ability of a sample to reduce Fe^{3+}/ferricyanide complex to Fe^{2+} through single electron transfer.[26] Briefly, 0.5 mL of each essential oil at different concentrations was mixed with 2.5 mL of a 0.2 M phosphate buffer solution at pH 6.6 and 2.5 mL of a 1% potassium ferricyanide $K_3Fe(CN)_6$ solution (Sigma-Aldrich, USA). The mixture was incubated at 50°C for 20 min and then 2.5 mL of 10% trichloroacetic acid was added to stop the reaction. The mixture was then centrifuged at 3000 rpm for 10 min, and 2.5 mL of the supernatant of each concentration was mixed with 2.5 mL of distilled water and 0.5 mL aqueous solution of 0.1% $FeCl_3$. The absorbance of the reaction mixture was measured at 700 nm. Increase in the absorbance of the reaction mixture signifies reduction potential of the test sample. Catechin was used as the reference standard, and the assay was performed in triplicate. The IC_{50} values were reported as means ± SD of triplicates determinations.

Determination of Antidiabetic activity

The *antidiabetic activity* of the essential oils was assessed *in vitro* by measuring the inhibitory effects on α-amylase and α-glucosidase.

Alpha-Amylase Inhibitory Assay

The method described by Bouchmaa *et al*. (2022)[27] was adopted for the α-amylase inhibitory assay. This involved incubating 250 µL of the essential oil sample and 250 µL of 0.02 M sodium phosphate buffer (pH 6.9) with α-amylase enzyme (240 U/mL) (Puerto Rico, (Farco Chemical Supply)) at 37°C for 20 min. Subsequently, 250 µL of a 1% starch solution (Spain, Scharlau Chemie, s.a) in the same buffer was added, and the reaction mixtures were further incubated at 37°C for 15 min. The percentage of inhibition was calculated using the formula:

$$\text{Inhibition (\%)} = \frac{(AC - ACb) - (AS - ASb)}{AC - ACb} \times 100$$

Where; AC is the control, ACb is the control blank, AS is the sample, and ASb is the sample blank.

Alpha-Glucosidase Inhibitory Assay

For the α-glucosidase inhibitory assay, a modified method of Bouchmaa *et al*. (2022)[27] was used. This involved the incubation of a combination of 150 µL of the essential oil and 100 µL of a sodium phosphate buffer solution (pH 6.7) containing α-glucosidase at a concentration of 0.1 U/mL (Puerto Rico, (Farco Chemical Supply) at 37°C for 10 min. After the pre-incubation, 200 µL of the substrate containing 1 mM p-Nitrophenyl-α-D-glucopyranoside (p-NPG) (Sigma Aldrich, Riedel-de Haen, Denmark) in a 0.1 M sodium phosphate buffer at pH 6.7 was introduced into the reaction mixture. The reaction was allowed to proceed at 37°C for 30 min and was terminated by the addition of 1 mL of 1 M Na_2CO_3 (Sigma-Aldrich, Steinheim, Germany). The absorbance was measured at 405 nm with a spectrophotometer (UV Tech LCD UV-VIS Spectrophotometer Double Beam, Model Name/Number: UV 180). The experiments were performed in triplicate with varying concentrations of the essential oil, and Acarbose was employed as the positive control.

Statistical Analysis

Data were presented as means ± standard error of means (SEM), and statistical analysis was conducted using Graph Pad Prism 5 Software (San Diego, CA, USA). The comparison between means was carried out using one-way analysis of variance (ANOVA).

Results and Discussion

Chemical Constituents of Essential Oil Samples of Thymus vulgaris

The chemical constituents of the essential oil extracted from samples of *Thymus vulgaris*, collected from different climatic conditions are presented in Table 1, Figure 2, Figure 3, and Figure 4. The percentage of each compound is listed, allowing for a comparison of the differences between the three samples (Table 1). The composition of essential oils in plants is known to vary widely depending on factors such as climate, soil, and geographic location.[28] The essential oil extracted from *Thymus vulgaris* was composed mainly of thymol and carvacrol. These compounds exhibited noticeable differences in their proportions among the three samples. Carvacrol was more prominent in the three samples with sample 2 having percentage carvacrol content of 83.3%, followed by Sample 1 with percentage carvacrol content of 69.9%, and Sample 3 with a comparatively lower percentage carvacrol content of 26.6%. Thymol was the second most prominent compound in all three samples, with Sample 3 having the highest thymol content of 34.8%, followed by Sample 1 with thymol content of 6.4%, and Sample 2 with thymol content of 4.6%. These findings underscore the significant influence of the climatic conditions at the plants' collection site on the chemical constituents of the extracted essential oil. Other constituents such as α-pinene, p-cymene, and camphor also exhibited noticeable variations in their proportions among the three samples of *Thymus vulgaris* essential oil. For instance, α-pinene has a percentage content of 3.4% in sample 1, 1.9% in sample 2, and 1.2% in sample 3. On the other hand, the percentage of p-cymene was highest in sample 1, accounting for 8.1%, this was followed by sample 3 with 3.4% p-cymene, and lastly sample 2 with p-cymene content of 0.4%. Camphor was highest in sample 2 with percentage content of 2.8%, followed by sample 3 with percentage content of 2.3%, and sample 1 with percentage content of 0.9%. These variations further substantiated the significant impact of climatic conditions on the chemical composition of *Thymus vulgaris* essential oil. The differences in the percentage composition of these compounds may have profound effect on the biological activities and ethnomedicinal value of the essential oil of *Thymus vulgaris*. For example, carvacrol and thymol have been documented to exhibit antimicrobial, antioxidant, and anti-inflammatory activities,[29] and is extensively utilized in traditional medicine for managing various health concerns. Additionally, the differences in the chemical composition may also affect the aroma of the essential which in turn could affect their use in the food and cosmetic industries. In summary, the findings from the present investigation have shown that the chemical composition of *Thymus vulgaris* essential oil can vary significantly contingent upon the prevailing climatic conditions in the location of plant cultivation. Variations in temperature, humidity, rainfall, and other environmental factors can impact plant growth and metabolism, resulting in alterations to their chemical composition.[29,30]

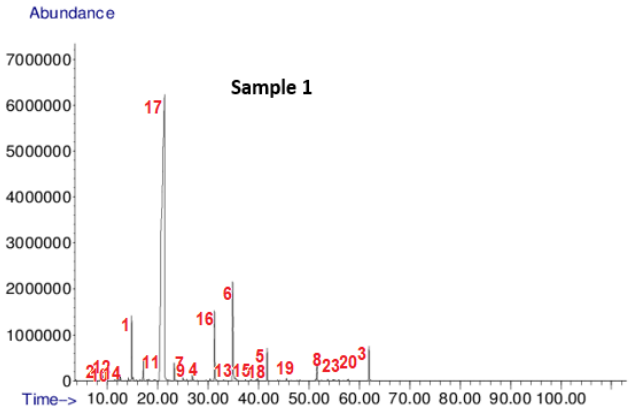

Figure 2: Gas chromatogram of sample 1

Table 1: The chemical composition of essential oils samples of Thymus vulgaris collected under varying climatic conditions

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

S/N	Compound Name	IR	% Content		
			Sample 1	Sample 2	Sample 3
1	α-Pinene	937	3.4	1.9	1.2
2	Sabinene	965	0.7	0.7	0.5
3	β-Pinene	975	2.1	0.1	0.4
4	Myrcene	984	0.5	0.2	0.9
5	α-Terpinene	1009	1.2	0.3	2.1
6	p-Cymene	1013	8.1	0.4	3.4
7	1,8-Cineole	1025	0.8	0.1	0.5
8	Limonene	1032	0.9	1.9	1.3
9	γ-Terpinene	1050	0.4	0.7	22.8
10	Linalol	1086	0.7	0.3	0.4
11	Camphre	1127	0.9	2.8	2.3
12	Trans-Pinocarveol	1127	0.8	0.5	0.1
13	Borneol	1153	0.2	0.3	0.2
14	Terpinen-4-ol	1165	0.7	0.6	1.7
15	Carvacrylmethylether	1231	0.1	0.1	0.1
16	Thymol	1290	6.4	4.6	34.8
17	Carvacrol	1298	69.9	83.3	26.6
18	(E)-Caryophyllene	1420	0.1	0.3	0.1
19	Aromadendrene	1438	0.1	0.1	-
20	Alloaromadendrene	1458	0.1	0.1	-
21	Ledene	1493	-	0.1	0.1
22	Spathulenol	1564	-	0.1	-
23	Caryophyllene oxyde	1571	0.1	0.1	0.1
	Total		98.2	99.6	99.6

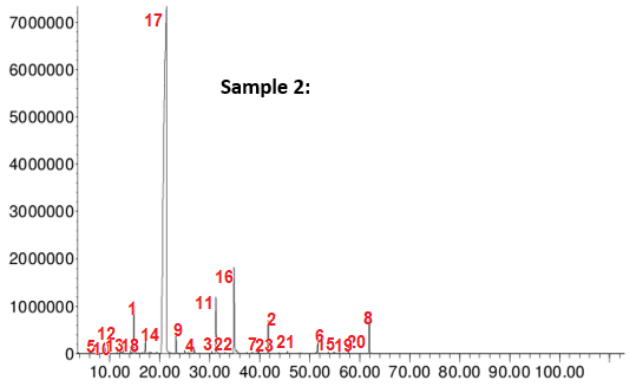

Figure 3: Gas chromatogram of sample 2

Thymus vulgaris essential oil is rich in thymol and carvacrol, collectively constituting more than 85% of the oil. These results agree with previous studies which revealed thymol and carvacrol as the primary constituents of *Thymus vulgaris* essential oil, and were shown to have antimicrobial, antioxidant, and anti-inflammatory properties.[4,31] Given the well-documented antimicrobial and antioxidant properties of thymol and carvacrol, their variations could impact on the therapeutic potential of *Thymus vulgaris* essential oil.

The variation in the chemical composition of *Thymus vulgaris* essential oils under varying weather conditions may also be attributed to changes in the biosynthesis and accumulation of chemical compounds under different environmental stresses, as documented in previous researches.[32,33] Furthermore, findings from a previous study also support the idea that environmental conditions and agricultural practices significantly influence the chemical compositions of essential oils of *Thymus* species.[34] This study underscores the need for further exploration into how climatic conditions influence the therapeutic potentials of *Thymus vulgaris* essential oil, which will shed light on the complex interplay between climate, chemical composition, and medicinal effect of plants.

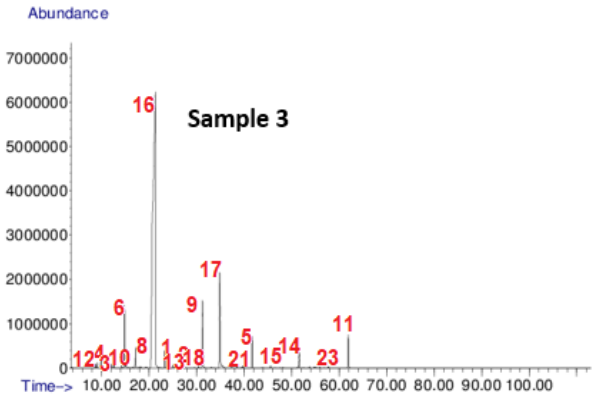

Figure 4: Gas chromatogram of sample 3

Antioxidant Activity of Thymus vulgaris Essential Oil

The antioxidative potential of essential oils has elicited significant interest owing to their possible application in the food, cosmetic, and pharmaceutical industries.[35] For this reason, the present study evaluated the antioxidant activity of three different samples of *Thymus vulgaris* essential oils using DPPH radical scavenging, ABTS radical scavenging, and FRAP assays.

Table 2 presents a summary of the antioxidant activity of *Thymus vulgaris* essential oil for the three samples.

DPPH Free Radical Scavenging Activity of Thymus vulgaris Essential Oil

Figure 5 presents the results of the antioxidant capability using the DPPH assay for the three samples of *Thymus vulgaris* essential oils and the reference standard (Quercetin).

The results show that sample 1 exhibited the most potent DPPH radical scavenging activity with IC_{50} value of 461.83 ± 10.49 µg/mL. This was followed by sample 3 with IC_{50} value of 804.50 ± 3.95 µg/mL, while sample 2 displayed the lowest activity with IC_{50} value of 960.49 ± 7.63 µg/mL. Overall, sample 1 demonstrated a moderate level of antioxidant activity in comparison to quercetin, a known antioxidant compound, while samples 2 and 3 displayed relatively lower antioxidant activity when compared to both sample 1 and quercetin. The chemical examination of the essential oils revealed that carvacrol was the major constituent in all three samples, with the highest percentage found in sample 2 (83.3%), followed by sample 1 (69.9%) and sample 3 (26.6%). Carvacrol is known for its various biological and pharmacological properties, including antioxidant activity. Therefore, it is possible that the higher concentration of this compound in sample 1 contributed to its higher DPPH scavenging activity compared to sample 3. Another primary component found in all three samples was thymol, which is known for its antibacterial and antioxidant properties.[29] Interestingly, sample 3 had the highest percentage of thymol (34.8%), followed by sample 1 (6.4%) and sample 2 (4.6%). This observation could explain the higher DPPH radical scavenging activity of samples 1 and 3 compared to sample 2. γ-Terpinene, another monoterpene was also found in all three samples with sample 3 having the highest percentage of γ-Terpinene (22.8%), followed by sample 2 (0.7%) and sample 1 (0.4%). This compound has a fresh, minty odour and could contribute to the antioxidant and other biological properties of essential oils.[36,37] Other constituents found in the essential oils of *Thymus vulgaris* were p-Cymene, α-Pinene, and α-Terpinene. These compounds could also contribute to the antioxidant potential of *Thymus vulgaris* essential oil.[38] A study conducted by Marchese *et al*. (2017),[39] demonstrated that both *Thymus vulgaris* L. essential oil and its CHO fraction exhibited significant DPPH radical scavenging capabilities. Specifically, Thymus essential oil displayed a scavenging activity with IC_{50} value of 0.30 ± 0.06 mg/mL, while the CHO fraction of thyme exhibited a scavenging activity with IC_{50} value of 0.40 ± 0.05 mg/mL. The results obtained from the study of Kulisic *et al*. (2005),[40] substantiated the DPPH radical scavenging activity of thyme essential oil producing an IC_{50} value of 0.24 µg/mL. Furthermore, a study conducted by Stoilova *et al*. (2008)[41] assessed the antioxidant effect of *Thymus vulgaris* essential oil cultivated in Germany using the DPPH assay. The findings demonstrated moderate antioxidant activity, as indicated by an IC_{50} value of 41.4 µg/mL. In the present study, the DPPH scavenging activity of *Thymus vulgaris* essential oils varied among the different samples and was found to be correlated with their chemical composition. This observation suggests that the high percentage of carvacrol and thymol, and possibly other unidentified compounds, in sample 1 could contribute to its higher antioxidant activity compared to the other two samples. These findings could have potential implications for the development of natural antioxidants from *Thymus vulgaris* essential oils.

Table 2: Antioxidant activity of essential oils samples of Thymus vulgaris collected under different climatic conditions and reference standards

EOs/ reference standards	DPPH IC_{50} (µg/mL)	FRAP IC_{50} (µg/mL)	ABTS IC_{50} (µg/mL)
S1	461.83 ± 10.49	252.36 ± 1.89	1508.83 ± 7.22
S2	960.49 ± 7.63	277.07 ± 1.87	1783.45 ± 6.72
S3	804.50 ± 3.95	244.64 ± 1.34	2381.96 ± 19.19
Quercetin	5.49 ± 0.02	-	-
Ascorbic acid	-	-	2.52 ± 0.02
Catechin	-	13.90 ± 0.03	-

DPPH: DPPH Free Radical-Scavenging Activity; ABTS: ABTS Radical Scavenging Activity; FRAP: Ferric Reducing Antioxidant Power.

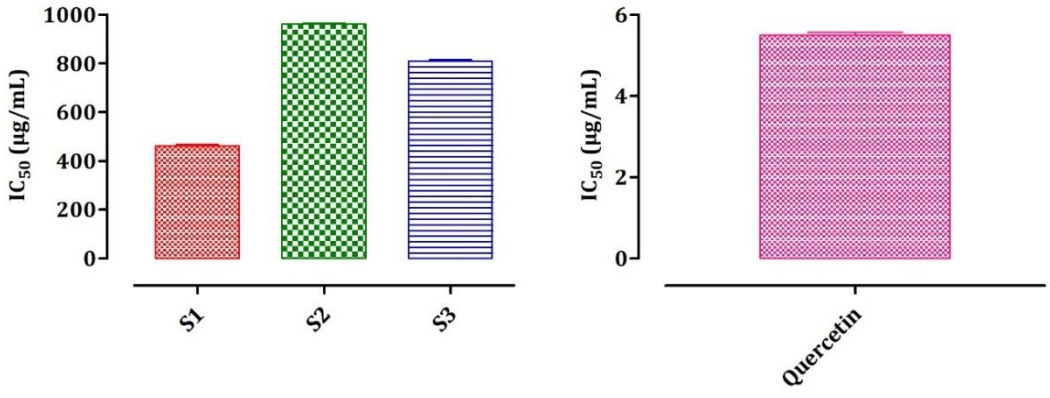

Figure 5: DPPH free radical scavenging activity of three distinct samples of Thymus vulgaris essential oils and quercetin.

ABTS Free Radical Scavenging Activity of Thymus vulgaris Essential Oil

Figure 6 and Table 2 presents the ABTS *radical scavenging activity* of the different samples *of Thymus vulgaris essential oil*. Sample 1 displayed an IC_{50} value of 1508.83 ± 7.22 µg/mL, indicating moderate antioxidant activity. Sample 2 had a slightly higher IC_{50} value (1783.45 ± 6.72 µg/mL), implying a slightly lower antioxidant activity than sample 1. Sample 3 exhibited the highest IC_{50} value (2381.96 ± 19.19 µg/mL), signifying the least antioxidant activity among the three *Thymus vulgaris* samples. All three *Thymus vulgaris* essential oil samples had significantly higher IC_{50} values than Ascorbic acid, suggesting lower antioxidant activity than Ascorbic acid. As earlier stated, thymol and carvacrol are the dominant compounds in the three essential oil samples, with sample 3 having the highest thymol content (34.8%) and sample 2 having the highest carvacrol content (83.3%). These phenolic compounds are likely to be substantial contributors to the observed ABTS scavenging activity of *Thymus vulgaris* essential oil. Minor compounds like limonene, p-Cymene, α-Pinene, and α-Terpinene may also contribute to the observed differences in the radical scavenging activity. Generally, antioxidant activity of plants results from a complex interactions among compounds in the plants. The study conducted by Punya *et al*. (2019),[42] explored the antioxidant potential of *Thymus vulgaris* essential oil using the 2,2-azinobis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) assay, and the results showed that the radical scavenging capability of the essential oil ranged from 6.00% to 67.75%. This implies that *Thymus vulgaris* essential oil exhibits notable antioxidant properties, effectively neutralizing ABTS free radicals. In a comprehensive research carried out by Chbel *et al*. (2022),[43] a comparison was made between the radical scavenging activity of thyme cultivated in France and that cultivated in Morocco using the ABTS assay. The findings revealed that the thyme cultivated in France showed a higher radical scavenging activity, with an impressive percentage scavenging of 99.10% against the ABTS radical compared to that sourced from Morocco which demonstrated a lower ABTS radical scavenging activity of 74.85%. Examination of the chemical constituents found in both essential oils indicated that French thyme primarily consisted of thymol (35.77%), ortho-cymene (17.23%), and γ-terpinene (8.05%), while Moroccan thyme contained significant quantities of borneol (31.04%), α-terpineol (15.16%), and carvacrol (7.13%). These findings suggest that the geographical location of thyme cultivation can influence its antioxidant potential. In summary, results from the ABTS radical scavenging assay indicate that all three samples of *Thymus vulgaris* essential oil exhibit antioxidant activity with Sample 1 showing the lowest IC_{50} value and thus the highest antioxidant activity. A study conducted by Aljabeili *et al*., (2018)[44] investigated the antioxidant activity of *Thymus vulgaris* essential oil using the ABTS assay. The results showed that the oil had a high antioxidant activity, with an IC_{50} value of 192.4 ± 3.9 µmol of TE/g, which is higher than the IC_{50} values observed in the current study for all three samples. The variation in antioxidant activity observed between different studies may be attributed to various factors, such as differences in plant growth conditions, extraction methods, and analytical techniques. Nevertheless, high antioxidant activity of *Thymus vulgaris* essential oil has been consistently documented in existing literature.

Ferric ion (Fe^{3+}) Reducing Antioxidant Power (FRAP) of Thymus vulgaris Essential Oil

The FRAP method is widely utilized to measure the antioxidant capacity of various samples. It quantifies the capacity of the sample to convert ferric ions into ferrous ions and is therefore an indicator of the sample's ability to donate electrons and scavenge free radicals. The results of the FRAP assay (Figure 7) suggest that all three samples of *Thymus vulgaris* possess significant antioxidant activity, with sample 3 exhibiting the highest activity. Among the three *Thymus vulgaris* essential oil samples, sample 3 displayed the lowest IC_{50} value (244.64 ± 1.34 µg/mL), indicating its superior reducing power, and consequently highest antioxidant capacity, as it required a lower concentration to achieve a 50% reduction in ferric ions. Sample 1 exhibited a slightly higher IC_{50} value (252.36 ± 1.89 µg/mL) than sample 3, indicating a comparatively lower reducing power and antioxidant capacity. Similarly, sample 2 demonstrated an IC_{50} value of 277.07 ± 1.87 µg/mL suggesting a relatively lower reducing power and antioxidant capacity than sample 1. The study of Gedikoğlu *et al*. (2019)[45] evaluated the antioxidant activity of *Thymus vulgaris* essential oil cultivated in Turkey using the FRAP assay. Their results revealed a high antioxidant activity, with an IC_{50} value of 3.25 ± 0.017 µM Fe^{2+}/g. However, these values were lower than the ones observed in the current study. It is important to note that the reference standard catechin exhibited a more potent antioxidant capacity than the three samples of *Thymus vulgaris* essential oil, evident from its significantly lower IC_{50} value (5.49 ± 0.02 µg/mL) compared to the *Thymus vulgaris* essential oil samples. Nevertheless, all three *Thymus vulgaris* essential oil samples still demonstrated antioxidant activity, albeit at higher concentrations when compared to the standard Catechin. The antioxidant effect of essential oils derived from *Thymus vulgaris* has been attributed to the presence of various constituents, including carvacrol, thymol and γ-Terpinene. These constituents have been demonstrated to have strong free radical scavenging, and antioxidant capabilities, making *Thymus vulgaris* essential oils a potential natural source of antioxidants.[46,47]

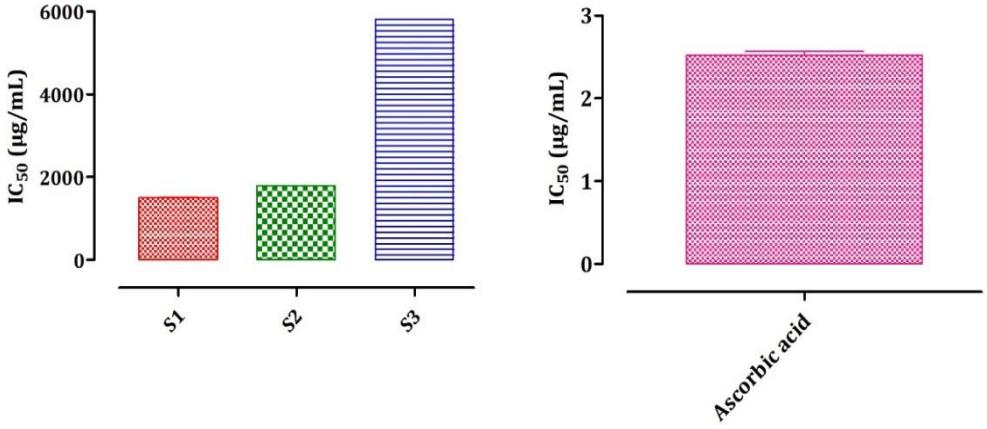

Figure 6: ABTS radical scavenging activity of three distinct samples of Thymus vulgaris essential oils and ascorbic acid.

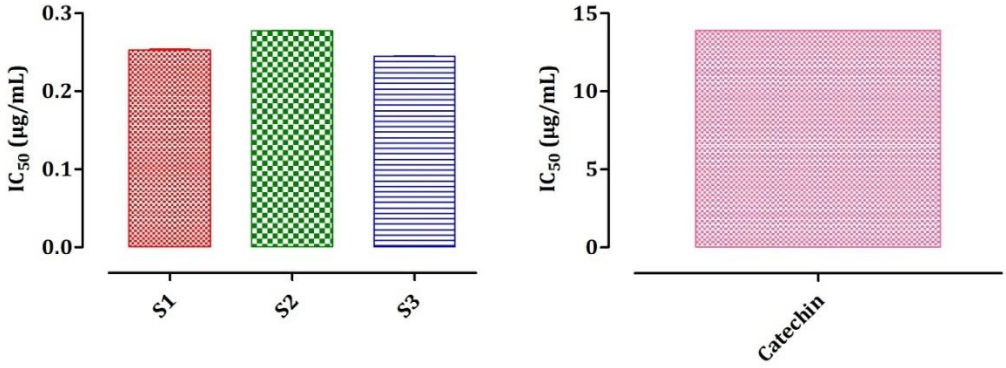

Figure 7: Ferric reducing antioxidant power (FRAP) of three distinct samples of Thymus vulgaris essential oils and catechin.

Table 3: Antidiabetic activity of essential oils samples of Thymus vulgaris collected under different climatic conditions

	α-Amylase inhibition	α-Glucosidase inhibition
Sample	**IC_{50} (mg/mL)**	**IC_{50} (mg/mL)**
S1	37.67 ± 0.12	48.97 ± 0.97
S2	57.88 ± 0.62	25.61 ± 1.58
S3	55.07 ± 0.12	4.,66 ± 0.42
Acarbose	0.396.42 ± 0.03	0.199 ± 0.014

It is important to note that the reference standard catechin exhibited a more potent antioxidant capacity than the three samples of *Thymus vulgaris* essential oil, evident from its significantly lower IC_{50} value (5.49 ± 0.02 µg/mL) compared to the *Thymus vulgaris* essential oil samples. Nevertheless, all three *Thymus vulgaris* essential oil samples still demonstrated antioxidant activity, albeit at higher concentrations when compared to the standard Catechin. The antioxidant effect of essential oils derived from *Thymus vulgaris* has been attributed to the presence of various constituents, including carvacrol, thymol and γ-Terpinene. These constituents have been demonstrated to have strong free radical scavenging, and antioxidant capabilities, making *Thymus vulgaris* essential oils a potential natural source of antioxidants.[46,47]

Antidiabetic Activity of Thymus vulgaris Essential Oil

The results of the antidiabetic activity of essential oils derived from three distinct samples of *Thymus vulgaris* as measured by the inhibition of α-Amylase and α-Glucosidase enzymes are presented in Table 3. Upon the consumption of carbohydrates, polysaccharides undergo initial breakdown into oligosaccharides or disaccharides through the action of amylase present in saliva and digestive enzymes originating from the pancreas. Alpha-amylase (EC 3.2.1.1) functions as an enzymatic catalyst, facilitating the cleavage of α-bonds within extensive α-linked polysaccharide structures. Although, amylase is present in various tissues, its highest concentrations are observed in pancreatic juice and saliva.[48] Within the small intestine, oligosaccharides undergo hydrolysis into monosaccharides like glucose and fructose. This process is facilitated by α-glucosidase (EC 3.2.1.20, α-D-glucoside glucohydrolase), an enzyme secreted by the epithelial cells of the intestine. Only monosaccharides have the capability to enter the bloodstream and be utilized by the human body. Alpha-glucosidase performs a crucial role in the final stage of carbohydrate digestion. Compounds that inhibit α-glucosidase can effectively slow down the absorption of dietary carbohydrates, thus help to control postprandial hyperglycemia. Such inhibitors hold potential for the treatment of individuals with diabetes and/or obesity.[49] A considerable number of α-glucosidase inhibitors have been identified through bioactivity screening of plants, and a subset of these inhibitors holds promising clinical significance. Although, several medications targeting carbohydrate-hydrolyzing enzymes are currently employed in clinical settings, the need for a large reservoir of inhibitors still remain crucial due to the potential development of resistance among diabetic patients to existing treatment protocols. Substantial endeavors have been geared towards the discovery of potent α-amylase and α-glucosidase inhibitors derived from natural sources. This pursuit aims to pave the way for the development of physiologically functional foods or the introduction of natural antidiabetic agents.[50] In the present study, the outcomes of the antidiabetic activity assessment, specifically the inhibition of α-amylase and α-galactosidase, are presented for three distinct samples of *Thymus vulgaris* essential oil. The results are reported as IC_{50} values in mg/mL. These values indicate the concentration of the essential oil or standard required to inhibit 50% of the enzyme's activity. In the α-amylase inhibition assay, Sample 1 exhibited an IC_{50} value of 37.67 ± 0.12 mg/mL, Sample 2 had an IC_{50} value of 57.88 ± 0.62 mg/mL, and Sample 3 had an IC_{50} value of 55.07 ± 0.12 mg/mL. On comparison, it was observe that Sample 1 had the lowest IC_{50} value, indicating a relatively higher inhibitory activity against the α-amylase enzyme. Sample 3 showed a slightly higher IC_{50} value, indicating a slightly lower inhibitory activity, while Sample 2 exhibited the highest IC_{50} value among the three samples, suggesting the lowest inhibitory activity against the α-amylase enzyme. For the α-galactosidase inhibition assay, Sample 1 displayed an IC_{50} value of 48.97 ± 0.97 mg/mL, Sample 2 had an IC_{50} value of 25.61 ± 0.43 mg/mL, and Sample 3 showed an IC_{50} value of 41.66 ± 0.42 mg/mL. Comparing these values, it was observed that Sample 2 exhibited the lowest IC_{50} value, indicating a relatively higher inhibitory activity against the α-galactosidase enzyme. Sample 3 had a slightly higher IC_{50} value, suggesting a slightly lower inhibitory activity, while Sample 1 showed the highest IC_{50} value among the three samples, indicating the lowest inhibitory activity against the α-galactosidase enzyme. Based on these results, it can be conclude that the three samples possess varying levels of antidiabetic activity in terms of their α-amylase and α-galactosidase inhibitory effects. Sample 1 showed the most potent inhibitory effect against α-amylase but the least effect against α-galactosidase. Sample 2 on the other hand exhibited the highest activity against α-galactosidase but the least activity against α-amylase. Sample 3 displayed moderate inhibitory activity against both enzymes. These observations suggest that the test samples of thyme essential oil may have potential antidiabetic activity, but they are not as potent as the standard compound Acarbose. Thymol and carvacrol are phenolic substances recognized for their antimicrobial and antioxidant activities. They have also been reported to exhibit inhibitory effect against α-Amylase and α-Glucosidase enzymes. The higher concentrations of thymol and carvacrol in Sample 2 (in contrast to Sample 1 and Sample 3) could potentially explain the stronger inhibitory activity observed against α-Glucosidase. Additionally, p-Cymene, another compound present in *Thymus vulgaris*, has also shown inhibitory effect on α-Amylase and α-Glucosidase enzymes. A study conducted by Ali (2021),[50] validated the antidiabetic activity of *Thymus vulgaris* essential oil, demonstrating its potent inhibition of the α-glucosidase enzyme with an IC_{50} value of 125.1 ± 4.25 µg/mL. In summary, the results indicate that the three samples have different

levels of antidiabetic effect, as demonstrated by their α-amylase and α-glucosidase inhibition. These findings provide valuable insights for potential therapeutic applications or further investigations into the antidiabetic properties of these samples.

Conclusion

The chemical constituents of the essential oils exhibited remarkable variability, with Thymol and carvacrol emerging as the predominant compounds. The proportions of these compounds were influenced by the climatic conditions under which the plants were cultivated, underscoring the profound impact of environmental factors on the intricate biochemical makeup of these oils. *Thymus vulgaris* essential oils exhibited substantial antioxidant activity, as evident from their DPPH radical scavenging, ABTS radical scavenging, and ferric reducing antioxidant power (FRAP). The essential oil samples exhibited varied degree of antidiabetic activity as revealed by the intriguing variations in their inhibitory activities against α-amylase and α-galactosidase enzymes. The antioxidant and antidiabetic activities across all three essential oil samples may be attributed to the presence of key constituents like carvacrol, thymol, and γ-terpinene. These compounds have been found to possess antioxidant and antidiabetic activities, lending credence to the potential of *Thymus vulgaris* essential oils as natural sources of antioxidant and antidiabetic agents. The observed differences in the antioxidant and antidiabetic activities among the three essential oil samples of *Thymus vulgaris* underscore the complex interactions between climatic/environmental conditions, the chemical composition and the biological activity of thyme essential oil. The study did not only contribute to our understanding of the impact of climatic conditions on the chemical composition and biological activity of thyme essential oil, but also lends credence to the versatile applications of these oils in the food, cosmetic, and pharmaceutical industries.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgments

The authors extend their appreciation to the researchers who provided support for this project.

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