Research Article

Comprehensive GC-MS Analysis of Aromatic Profiles in Wild Thyme (*Thymus vulgaris* L.) Volatile Oil

Ali Sabah Al-Hasan

Department of Horticulture, Agricultural College, University of Al-Qadisiyah, Al-Diwaniyah, Iraq.

Abstract | *Thymus vulgaris* L., also known as wild thyme, is a well-known aromatic herb important for its therapeutic uses and is extensively used in the food, pharmaceutical, and cosmetic products. This study focused on exploring the chemical profile of essential oil extracted from the aerial parts of wild thyme collected from naturally grown plants around Diwaniyah city in Iraq, a region previously unexplored for aromatic compounds identification and profiling. The harvesting of plant samples was done at the full flowering season; air dried at room temperature under shaded conditions, and hydrodistillation was carried out for three hours using a Clevenger apparatus to isolate essential oil. The analysis of thyme essential oil was carried out on gas chromatography mass spectrometry (GC-MS) for the detection of vital constituents, for identification and quantification of volatile compounds optimized parameters was followed through consulting the spectral databases and retention index values. Around thirty-four constituents were detected in total, with thymol (8.99%), carvacrol (8.43%), p-cymene (7.55%), linalool (7.47%), γ-terpinene (7.13%), and isolimonene (6.37%) being the major constituents. The current experiment offers the first detailed chemical analysis of Iraqi wild thyme essential oil, emphasizing its potential as it contains several biologically active compounds usable for various industrial applications.

Received | May 16, 2025; **Accepted** | May 26, 2025; **Published** | August 04, 2025
***Correspondence** | Ali Sabah Al-Hasan, Department of Horticulture, Agricultural College, University of Al-Qadisiyah, Al-Diwaniyah, Iraq; **Email:** ali.alhasan@qu.edu.iq
Citation | Al-Hasan, A.S., 2025. Comprehensive GC-MS analysis of aromatic profiles in wild thyme (*Thymus vulgaris* L.) volatile oil. *Sarhad Journal of Agriculture,* 41(3): 1249-1254.
DOI | https://dx.doi.org/10.17582/journal.sja/2025/41.3.1249.1254
Keywords | *Thymus vulgaris*, Essential oil, Volatile compounds, GC-MS analysis, Hydrodistillation

Introduction

Wild thyme (*Thymus vulgaris* L.) is an important member of the Lamiaceae family, it is well-known famous for aromatic and therapeutic uses across the globe. Around 110 species and over 300 cultivars of thyme have been reported (Aksit *et al*., 2022; Saleem *et al*., 2022). It is renowned for its abundant essential oil; thyme has long been valued for its unique fragrance and therapeutic uses. Thyme essential oil is considered one of the most valuable natural essential oil worldwide, commonly used in aromatherapy and for diverse health benefits (Król and Kiełtyka-Dadasiewicz, 2015). Cultivation of thyme, is worldwide and famous for its pleasant flavor in many food items, as well as for cosmetic products and herbal medicines (Raal *et al*., 2024; Jannati *et al*., 2021).

Links Researchers

Thyme essential oil contains several bioactive compounds being used as antioxidants, antispasmodic, antidiabetic, antifungal, anticancer, antibacterial, anti-inflammatory, and pesticidal activities. These therapeutic applications have made oil of thyme an important natural source in treating and preventing various health issues (Boukhatem *et al*., 2020; Almanea *et al*., 2019). Quality and efficacy of thyme oil mainly depend on its chemical composition and oil recovery. Thyme essential oil is rich in secondary metabolites including phenolics, tannins, glycosides, saponins and flavonoids, these compounds contributing to its medicinal value (Mokhtari *et al*., 2023; Guz *et al*., 2025). However, quality and composition of thyme essential oil substantially also depend on environmental factors. Biotic stress such as insect attack and pathogen infestation, along with abiotic stress including salinity, drought, and genetic variation among plant populations, notably affect essential oil composition. Additionally, different extraction techniques such as duration of distillation, choice of technique, time of harvest, and the specific plant parts being used for essential oil extraction also have crucial role in defining the composition of extracted oil and concentration (Alhasan and Hussein, 2022; Al-Ameri *et al*., 2020; Król and Kiełtyka-Dadasiewicz, 2015; Pirbalouti *et al*., 2013).

Although, thyme has been explored comprehensively in many regions across the globe, but limited information available about the phytochemical characteristics of wild thyme naturally grown in Iraq particularly Diwaniyah city vicinity. The current study focuses on addressing this gap by analyzing the oil composition of *Thymus vulgaris* from Iraqi habitats, providing comprehensive knowledge of its chemical constituents and potential medicinal uses.

Materials and Methods

Plant material collection

Wild thyme (*Thymus vulgaris* L.) samples were collected from naturally growing populations (Al-Diwaniyah city) in Iraq during the peak flowering season. The aerial plant parts (leaves and flowering tops) were harvested in the early morning to preserve volatile integrity.

Sample preparation

Freshly collected plant material was cleaned of soil and debris, then air-dried in a shaded, well-ventilated area at room temperature (22–25°C) for 10–14 days until a constant weight was achieved. The dried plant material was subsequently ground into coarse powder using a stainless-steel laboratory mill and kept in airtight glass containers at 4°C until extraction.

Essential oil extraction

The oil was extracted through using the hydrodistillation technique using a Clevenger-type apparatus. For this, 100 grams of dried thyme powder were combined with 750 mL of distilled water in a 1-liter round bottom flask and subjected to hydrodistillation for duration of three hours. After distillation, the essential oil was carefully separated from the water layer and then dried using anhydrous sodium sulfate to eliminate any residual moisture. The oil yield was expressed as a volume-to-weight percentage (v/w) based on the dry plant material. The purified essential oil was stored in amber-colored sealed vials and kept refrigerated at 4°C until it was ready for further testing.

Gas chromatography–mass spectrometry (GC-MS) analysis

The analysis of extracted oil was carried out through using GC-MS (Shimadzu GC-17A) gas chromatograph paired with a Shimadzu QP-5000 mass selective detector (Shimadzu Corporation, Japan) for detection chemical profile. The separation process was carried out on an HP-5MS fused silica capillary column, which is 30 meters long, has an internal diameter of 0.25 mm, and a film thickness of 0.25 micrometers.

GC conditions:

- Carrier gas: Helium (purity ≥ 99.999%)
- Flow rate: 1.0 mL/min (constant flow mode)
- Injection volume: 1.0 µL of essential oil diluted 1:100 in n-hexane
- Injector temperature: 250°C
- Split ratio: 1:20
- Oven temperature program: Initial temperature set at 60°C (held for 3 min), increased to 150°C at 4°C/min (held for 2 min), then further ramped to 250°C at 5°C/min and held for 5 min

MS conditions:

- Ionization mode: Electron impact (EI) at 70 eV
- Mass scan range: 40–550 m/z
- Ion source temperature: 230°C
- Interface temperature: 280°C

- Acquisition mode: Full scan

Compound identification

The identification of volatile constituents present in extracted thyme essential oil, was carried out by comparing their recorded mass spectra with reference spectra found in the database of NIST and Wiley. For further confirmation calculated retention indices was compared with already reported values in earlier studies literature. For determination of retention indices series of n-alkanes were used (C8 to C20) in the same gas chromatography conditions. The proportion of each compound was calculated using peak area normalization, without applying any correction factors (Adams, 2017).

Statistical analysis

The experiment was conducted with three replications, with findings presented as the average value plus or minus the standard deviation. Data analysis and visualization were performed using GraphPad Prism version 9.0 (GraphPad Software, USA). Consistency of the chemical profiles was assessed by comparing peak areas from multiple repeated measurements.

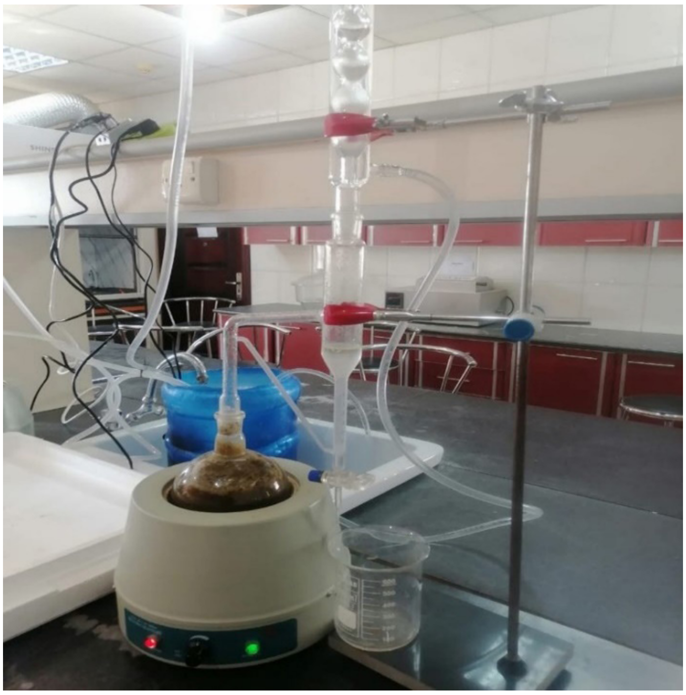

Figure 1: *Hydrodistillation apparatus used to thyme EO extraction.*

Results and Discussion

Using a clevenger-type apparatus, the aerial plant parts of *Thymus vulgaris* L. were subjected to hydrodistillation for three hours, resulting in a clear yellow essential oil with a characteristic aromatic scent. The total essential oil recovery from dried weight of the plant samples was approximately 1.2% (v/w). Subsequent, the constituents of extracted oil was done by gas chromatography-mass spectrometry (GC-MS) in which comprehensive detection of individual constituents was done.

Table 1: *Chemical composition of thyme (Thymus vulgaris) volatile oils.*

Pic	RT	%	Compounds
1	11.59	1.2	Eucalyptol
2	14.00	3.61	Pulegone
3	14.59	3.06	Linolool
4	15.26	1.48	Fenchol
5	17.72	1.47	Lavandulol
6	21.19	3.55	α-terpinene
7	21.54	1.43	Anethole
8	21.87	7.47	Linalool
9	23.45	4.13	Dihydrocarveol
10	26.19	6.37	Isolimonene
11	26.46	3.19	Caryophyllic acid
12	26.75	0.92	Nerol acetate
13	27.51	0.57	α-Copaene
14	27.60	1.86	cis-Geranyl acetate
15	27.71	0.72	Lavandulol acetate
16	29.53	8.43	Carvacrol
17	30.13	4.17	Caryophyllene
18	30.39	0.60	β-Sesquiphellandrene
19	30.98	7.55	P-Cymene
20	32.12	7.13	γ-Terpinene
21	32.21	0.62	Limonene
22	32.59	2.01	Camphene
23	33.16	1.27	β-Bisabolene
24	33.62	0.72	Teresantalol
25	33.78	1.12	Isoledene
26	34.64	8.99	Thymol
27	35.34	1.18	α-Nerolidol
28	36.29	5.57	Caryophyllene oxide
29	38.52	2.93	τ-Cadinol
30	38.91	0.58	β-Selinenol
31	39.70	1.06	(-)-Isolongifolol
32	40.10	0.99	α-Bisabolol
33	45.87	0.77	Cis-Sabinene hydrate
34	54.34	1.18	α-Linolenic acid
35	54.79	2.06	Isomenthol
Total		99.96	

Approximately, 34 bioactive compounds were identified, representing 99.9% of volatile substance present in oil extracted from thyme. The bioactive compounds having major share in oil were thymol

(8.99%), carvacrol (8.43%), p-cymene (7.55%), linalool (7.47%), γ-terpinene (7.13%), and isolimonene (6.37%) (Table 1 and Figure 2). These major constituents quite correspond with those generally found in thyme essential oil, although their proportions can be differ based on factors such as geographical origin, climatic conditions, stage of harvest, and genetic variation in thyme plant population. Among the detected bioactive compounds Thymol and carvacrol, both phenolic monoterpenes having close resembled structures, are known for their potent antimicrobial, antioxidant and anti-inflammatory activities. Their high concentrations in the thyme samples indicates substantial therapeutic potential, these consistent are also being used in traditional medicines. Moreover, the substantial amounts of p-cymene and γ-terpinene, which plays a key role as precursor in biosynthetic to thymol and carvacrol, which confirms that wild thyme belongs to the thymol-carvacrol chemotype.

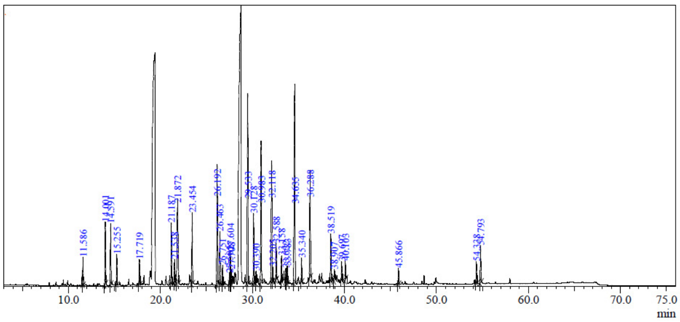

Figure 2: *GC-MS chromatogram of the chemical constituents of thyme herb.*

It was observed that Linalool which is a terpene alcohol, imparts the oil's typical fresh and floral aroma and is recognized for its calming and anxiolytic properties. Furthermore, isolimonene having a fragrance line citrus and possesses antimicrobial and insect repellent properties. Chemical structures of the major constituents present in thyme essential oil, illustrated in Figure 3, highlighting functional groups that mainly determine the bioactivity and volatility of essential oil. These results validate that wild thyme from Iraq contains an essential oil rich in bioactive compounds, with thymol and carvacrol as the major components. While correspond with previous studies, this work also proposes a possibly unique chemotype specific to the Iraqi wild thyme populations.

Thyme essential oil has been extensively explored as it has largely biological activities and uses in medicine, cosmetic and food industry. Numerous studies have consistently detected thymol as the major volatile constituent, particularly in naturally grown wild thyme (Pruteanu *et al*., 2018; Galovičová *et al*., 2021; Shahar *et al*., 2023). In the line of these reports, thymol accounted for 8.99% of essential oil in this study, highlighting its substantial role in the plant's chemical composition.

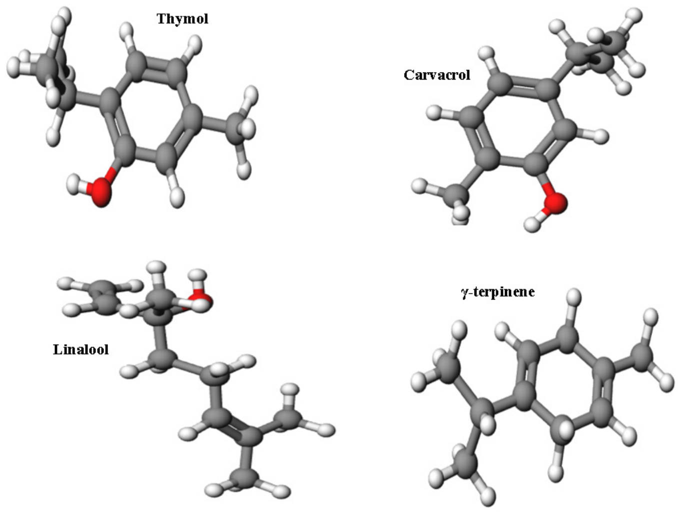

Figure 3: *Molecular structure of identified compounds from thyme (Thymus vulgaris).*

The current study revealed that carvacrol (8.43%), p-cymene (7.55%), linalool (7.47%) and γ-terpinene (7.13%) as major components of thyme essential oil. These findings are in line with previously study conducted by Lebaschi *et al*. (2018) furthermore, similar findings wer also confirmed by Kosakowska *et al*. (2021), and Golparvar *et al*. (2015), those who identified these bioactive compounds as major constituents in thyme essential oil from diverse ecological settings. Biological relevancy of these bioactive compounds stems from their combined antimicrobial, antioxidant and anti-inflammatory characteristics. It is also notable that the biochemical profile of thyme essential oil differs across the globe, influenced by geographic, genetic and environmental factors. For instance, Şener *et al*. (2021) found that different vital compounds could arise depending on these biotic and abiotic variables reflecting the existence of multiple thyme chemotypes. Consequently, thymol is not universally the abundant compound in thyme essential oil extracted from plant samples harvested from varied geographic locations worldwide. The current results align closely with those of Alizadeh *et al*. (2011), who reported p-cymene, linalool, carvacrol and thymol as key components in wild thyme oil, proposing a shared chemotype between Iraqi and other regional thyme plant population. The detection of p-cymene and γ-terpinene, as precursors in pathway of biosynthetic thymol and carvacrol, depicts active monoterpene synthesis effected by enzymatic mechanisms and environmental factors.

The current study contributes significant data to the global information *Thymus vulgaris* chemotypes, emphasizing the possible of Iraqi wild thyme as a major source for bioactive compounds with uses in medicinal treatments, natural preservatives and nutraceutical formulations.

Conclusions and Recommendations

Essential oil extracted from wild thyme dried parts (*Thymus vulgaris*) by hydrodistillation technique was comprehensively observed using GC-MS analysis, depicting a complex mixture of 34 different bioactive compounds. The major components of extracted essential oil were thymol (8.99%), carvacrol (8.43%), p-cymene (7.55%), linalool (7.47%), γ-terpinene (7.13%) and isolimonene (6.37%), there all are recognized for their biological activities and characteristic fragrances. These outcomes highlight the rich phytochemical profile of wild thyme found in Iraq and underline its promising uses in pharmaceutical, culinary and cosmetic industries. Additional, study is necessary to explore the chemical and aromatic diversity of various thyme cultivars across Iraq's different ecological zones, which could reveal chemotypic variations with vital commercial and therapeutic implications.

Acknowledgments

The author sincerely thanks the University of Al-Qadisiyah for its valuable support and contribution to this research.

Novelty Statement

The research and experimental work on the subject title is new (first ever study) in the field of entomology science in Iraq.

Conflict of interest
The authors have declared no conflict of interest.

References

Adams, R.P., 2017. Identification of essential oil components by gas chromatography/mass spectrometry. 5 online ed. Gruver, TX USA: Texensis Publishing, pp. 46-52.

Aksit, H., Y. Bayar, S. Simsek and Y. Ulutas. 2022. Chemical composition and antifungal activities of the essential oils of Thymus species (*Thymus pectinatus, Thymus convolutus, Thymus vulgaris*) against plant pathogens. J. Essent. Oil Bear. Plants, 25(1): 200-207. https://doi.org/10.1080/0972060X.2022.2043189

Al-Ameri, D.T., A.K. Hamza, B.H. Hassan and A.S. Alhasan. 2020. Effect of essential oil of colocynth, Citrullus colocynthis and spearmint, Mentha spicata against the khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae). IOP Conf. Ser. Earth Environ. Sci., 553(1): 012045. https://doi.org/10.1088/1755-1315/553/1/012045

Alhasan, A.S. and H.A. Hussein. 2022. Effect of applying different levels of nitrogen fertilizer on growth and essential oil of spearmint (*Mentha spicata* L.). Int. J. Agric. Stat. Sci., 18(1): 137-140. https://connectjournals.com/03899.2022.18.137

Alizadeh, A., O. Alizadeh, S. Sharafzadeh and S. Mansoori. 2011. Effect of different ecological environments on growth and active substances of garden thyme. Adv. Environ. Bio., 5: 780-783. https://www.aensiweb.com//old/aeb/2011/699-703.pdf

Almanea, A., G.S.A. El-Aziz and M.M.M. Ahmed. 2019. The potential gastrointestinal health benefits of Thymus vulgaris essential oil: A review. Biomed. Pharmacol. J., 12(4): 1793-1799. https://doi.org/10.13005/bpj/1810

Boukhatem, M.N., N.H. Darwish, T. Sudha, S. Bahlouli, D. Kellou, A.B. Benelmouffok, H. Chader, M. Rajabi, Y. Benali and S.A. Mousa. 2020. *In vitro* antifungal and topical anti-inflammatory properties of essential oil from wild-growing thymus vulgaris (Lamiaceae) used for medicinal purposes in Algeria: A new source of carvacrol. Sci. Pharm., 88(3): 33. https://doi.org/10.3390/scipharm88030033

Galovičová, L., P. Borotová, V. Valková, N.L. Vukovic, M. Vukic, M. Terentjeva and M. Kačániová. 2021. *Thymus serpyllum* essential oil and its biological activity as a modern food preserver. Plants, 10(7): 1416. https://doi.org/10.3390/plants10071416

Golparvar, A.R., A. Hadipanah and A.M. Mehrabi. 2015. Effect of phenological stage on yield, essential oil and thymol percentage of *Thymus daenensis* grown in Iran. Indian J. Fundam. Appl. Life Sci., 5(1): 2903-2910. www.cibtech.org/sp.ed/jls/2015/01/jls.htm

Guz, L., K. Puk and A. Pastuszka. 2025. GC/MS analysis and antibacterial activity of garden thyme (*Thymus vulgaris* L.) extracts against non-tuberculous mycobacteria strains isolated from diseased ornamental fish. Med. Weter, 81(3): 137-143. https://doi.org/10.21521/mw.6987

Jannati, N., M. Gharachorloo and M. Honarvar. 2021. Extraction of thymol compound from *Thymus vulgaris* L. oil. J. Med. Plants By Prod., 10(1): 81-84.

Kosakowska, O., Z. Węglarz and K. Bączek. 2021. The Effect of open field and foil tunnel on yield and quality of the common thyme (*Thymus vulgaris* L.), in organic farming. Agronomy, 11(2): 197. https://doi.org/10.3390/agronomy11020197

Krol, B. and A. Kıełtyka-Dadasıewıcz. 2015. Yield and herb quality of thyme (*Thymus vulgaris* L.) depending on harvest time. Turk. J. Field Crops, 20(1): 78-84. https://doi.org/10.17557/.89347

Lebaschi, M.H., E. Sharifi Ashoorabadi, M. Makizadeh Tafti, S. Asadi-Sanam and K.H. Karimzadeh Asl. 2018. Effects of plant density on quantitative and qualitative yield of three Thymus species in dry farming conditions of three provinces of Iran. J. Med. Aromat. Plants Res., 33(6): 897-914.

Mokhtari, R., M. Kazemi Fard, M. Rezaei, S.A. Moftakharzadeh and A. Mohseni. 2023. Antioxidant, antimicrobial activities, and characterization of phenolic compounds of thyme (*Thymus vulgaris* L.), sage (*Salvia officinalis* L.), and thyme–sage mixture extracts. J. Food Qual., 2023(1): 2602454. https://doi.org/10.1155/2023/2602454

Pirbalouti, A.G., M. Hashemi and F.T. Ghahfarokhi. 2013. Essential oil and chemical compositions of wild and cultivated *Thymus daenensis* Celak and *Thymus vulgaris* L. Ind. Crops Prod., 48: 43-48. https://doi.org/10.1016/j.indcrop.2013.04.004

Pruteanu, A., C. Popescu, V. Vladut and G. Gageanu. 2018. Biochemical analysis of some vegetal extracts obtained from indigenous spontaneous species of (*Thymus serpyllum* L.). Roman. Biotechnol. Lett., 23(5): 14013-14024.

Raal, A., T. Gontova, M. Palmeos, A. Orav, G. Sayakova and O. Koshovyi. 2024. Comparative analysis of content and composition of essential oils of *Thymus vulgaris* L. from different regions of Europe. Proc. Eston. Acad. Sci., 73(4): 332-344. https://doi.org/10.3176/proc.2024.4.02

Saleem, A., M. Afzal, M. Naveed, S.I. Makhdoom, M. Mazhar, T. Aziz, A.A. Khan, Z. Kamal, M. Shahzad, M. Alharbi and A. Alshammari. 2022. HPLC, FTIR and GC-MS analyses of thymus vulgaris phytochemicals executing *in vitro* and *in vivo* biological activities and effects on COX-1, COX-2 and gastric cancer genes computationally. Molecules, 27(23): 8512. https://doi.org/10.3390/molecules27238512

Şener, İ., F. Tekelioğlu, M. Zurnacı, P. Baloglu, M. Gür and K. Güney 2021. Chemical composition, antibacterial and antioxidant activities of *Thymus praecox*. Kastamonu Univ. J. For. Fac., 21(1): 65-73. https://doi.org/10.17475/kastorman.908659

Shahar, B., A. Indira, O. Santosh, N. Dolma and N. Chongtham. 2023. Nutritional composition, antioxidant activity and characterization of bioactive compounds from *Thymus serpyllum* L.: An underexploited wild aromatic plant. Measur. Food, 10: 100092. https://doi.org/10.1016/j.meafoo.2023.100092