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GC-MS analysis of Essential oil Components of the Libyan *Thymus capitatus*

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Abstract: Background:

Thymus capitatus is a medicinal and aromatic plant widely distributed in the Mediterranean region, known for its essential oils rich in bioactive compounds. Essential oils are complex mixtures of secondary metabolites with potential applications in food preservation, pharmaceuticals, and cosmetics. Variations in essential oil composition are influenced by geographic, genetic, and environmental factors.

Objective:

This study aimed to perform a comprehensive chemical analysis of the essential oil extracted from *Thymus capitatus* collected in Mesellata region, northwestern Libya, to identify and quantify its main constituents using Gas Chromatography-Mass Spectrometry (GC-MS), and to assess its potential as a source of natural bioactive compounds.

Methods:

Fresh aerial parts (stems and leaves) of *Thymus capitatus* were subjected to hydro-distillation using a Clevenger-type apparatus. The extracted essential oil was analyzed by GC-MS to determine its chemical composition. Components were identified by comparison with NIST mass spectral library data.

Results:

The GC-MS analysis revealed that the essential oil of *Thymus capitatus* was predominantly composed of monoterpene hydrocarbons and oxygenated monoterpenes. Carvacrol was the major constituent (56.27%), followed by p-cymene (9.51%), γ-terpinene (8.39%), and thymol (6.54%). Minor constituents included linalool, terpinen-4-ol, endo-borneol, and caryophyllene. Comparative analysis with other regional studies demonstrated chemotypic variability, classifying the Mesellata oil within the carvacrol-rich chemotype.

Conclusions:

The essential oil of *Thymus capitatus* from Mesellata exhibits a rich composition dominated by bioactive phenolic monoterpenes, supporting its potential use as a natural antimicrobial and antioxidant agent. The findings contribute to the chemotypic characterization of Libyan *T. capitatus* and highlight its possible applications in food, cosmetic, and pharmaceutical industries.

Keywords: *Thymus capitatus*, Medicinal & Aromatic plants, essential oil, GC-MS analysis, phenolic monoterpenoids

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INTRODUCTION

The Mediterranean region is famous for its diverse range of medicinal and aromatic plants, attributable to its favorable climate and distinct geological features. These plants carry significant cultural importance, as herbal treatments are a core aspect of traditional medicine in Mediterranean and North African societies[1]. Indeed, medicinal and aromatic plants (MAPs) are crucial for the economic, social, and ecological well-being of local communities globally. They find extensive use in the pharmaceutical, cosmetic, food, and fragrance industries, highlighting their significance in pharmacology[2]. In North Africa, for instance, numerous wild plants (such as various *Thymus species*) have historically been utilized to address health issues (ranging from infections to chronic conditions) and are commonly used for purposes like food preservation and flavor enhancement, showcasing a robust tradition of natural healing. This rich heritage of ethnobotany underscores the vital function of MAPs in regional healthcare and the population's livelihoods[3].

Essential oils (EOs) are intricate combinations of volatile secondary metabolites that plants primarily produce for protection and communication. They mainly comprise terpenoids (such as monoterpenes and sesquiterpenes) and aromatic phenolics, generated through the plant's terpene biosynthetic pathways 2-C-methyl-D-erythritol 4-phosphate pathway (MEP) and Mevalonate pathway(MVA) and phenylpropanoid pathways[4]. The chemical composition of an EO can differ significantly – factors such as genetic variation, locality, climate, soil type, and timing of harvest can affect the quantity and makeup of oils. For example, the same species may yield different dominant compounds when cultivated in various climates or seasons. Despite these differences, many EOs exhibit remarkable bioactivities. They demonstrate broad-spectrum antimicrobial, antifungal, and antioxidant properties, among others[5]. These biological effects are linked to their distinct multi-component structure, which can disrupt microbial membranes or influence inflammatory pathways. Due to these abilities, EOs are being investigated as natural alternatives for food preservation and in medicine (for instance, as antimicrobials against resistant bacteria)[6]. However, their effectiveness can be influenced by compositional variability, making it essential to characterize their components under varying conditions for optimal use.

Essential oils are frequently categorized based on their primary chemical components, leading to the idea of "chemo types." A chemo type denotes a subset of plants within the same species that exhibit varying chemical profiles in their essential oils[7]. These variations are generally characterized by the major constituents; for instance, within a single species, one chemo type might be high in thymol while another is predominantly composed of carvacrol. Such differences in chemo types usually arise from genetic diversity and environmental factors, resulting in the classification of essential oils by their major compound groups (e.g., terpenoid-dominant oils versus phenylpropanoid-dominant oils). In the *Thymus* genus, for example, different populations of the same species can produce various chemotypes that are identified by key terpenes like thymol, linalool, or geraniol. These chemo type differences have practical significance: the biological efficacy and scent of an essential oil can vary with its chemical composition[8]. Gaining an understanding of and categorizing essential oils based on their constituents allows researchers and industries to focus on specific chemo type for particular applications (such as choosing a carvacrol-rich chemo type for enhanced antimicrobial properties)[9].

Modern metabolomics techniques are essential for deciphering the biosynthetic pathways of secondary metabolites in plants, including the constituents of essential oils. Metabolomics, which involves the thorough profiling of metabolites, enables researchers to correlate specific compounds' presence and concentrations with the associated enzymes and genes responsible for their synthesis[10]. By merging metabolomics information with genomics or transcriptomic, scientists can pinpoint critical genes within pathways that are tasked with the formation of complex terpenes and phenolic found in essential oils. For instance, the integration of metabolite profiles with gene expression evaluations in aromatic plants has revealed particular terpene synthase genes that are linked to the accumulation of monoterpenes such as cineole or geraniol. These investigations illuminate how plants direct outputs from primary metabolism (like isopentenyl diphosphate) into various essential oil components through specialized pathways. In practice, untargeted metabolomic studies indicate that most volatile components of oils originate from a limited number of core pathways, particularly the plastidial MEP pathway for monoterpenes and the cytosolic mevalonate pathway for Sesquiterpene. Consequently, metabolomics not only assists in identifying biosynthetic routes but also enhances the understanding of regulation by revealing potential bottlenecks or changes in response to varying conditions (such as alterations in essential oil profiles due to stress). Ultimately, this systems-wide understanding is beneficial for metabolic engineering and conservation, allowing for the improvement of desirable oil constituents or the sustainable cultivation of medicinal plants by focusing on their biosynthetic frameworks[10].

Thymus capitatus, commonly referred to as cone head thyme or Spanish oregano (Zaatar) is a shrub from the Mediterranean region prized for its strong essential oil. It has a storied history of ethnobotanical use for treating conditions like respiratory infections, digestive problems, diabetes, coughs, and skin irritation. This traditional use is backed by contemporary research showing that the essential oil of *Thymus capitatus* possesses notable antimicrobial and antioxidant properties. Chemically, the essential oil is usually abundant in monoterpene phenols[11]. In many cases, carvacrol is the dominant component, a bioactive phenolic terpene known for its significant antibacterial properties. However, some populations display a chemo type where thymol is the predominant

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compound, indicating the species' chemical variation across different locations. Other prevalent constituents include p-cymene, γ-terpinene, and linalool, which together make up most of the oil's composition. The chemical profile is influenced by geographic and environmental factors: research has identified differences in the composition and bioactivity of *Thymus capitatus* oils from various countries and differing altitudes[12]. For example, oils sourced from North African or Eastern Mediterranean regions often feature a carvacrol-rich profile, while certain Western Mediterranean variants have thymol as a key component. Regardless of these differences, the oil is consistently effective in producing strong biological effects. Studies from Greece and Tunisia have shown the effectiveness of *Thymus capitatus* essential oil against foodborne and clinical pathogens, such as Salmonella, Listeria, and even Candida yeasts. There is an increasing body of evidence supporting its antifungal, ant biofilm, and anti-inflammatory effects, along with preliminary investigations into its anticancer potential[13].

Aims of the study

The main objective of this study is to perform a gas chromatography-mass spectrometry (GC-MS) analysis of thyme essential oil sourced from Mesellata region in northwestern Libya. This analysis focuses on identifying and quantifying the chemical components of the essential oil. By determining its chemical composition, the study to provide a foundation for future research into its potential health benefits, particularly its antimicrobial and antioxidant properties, as well as its applications in the food, cosmetics, and pharmaceutical industries.

Methods and Materials

Plant material:

Aerial parts (stems & leaves) of *Thymus capitatus* were collected in December 2024 from Mesellata region, northwestern Libya. The species was identified by Prof. Mohammad Makhlouf, Department of Botany, Faculty of Science, Tripoli University, Libya, and the voucher specimen were deposited in the herbarium of faculty of science, Tripoli University.

Essential oil extraction:

The extraction method was described by Anh, T. T et al some modification [14]. Fresh plant samples: aerial parts (stems and leaves) of *Thymus capitatus* (about 100 grams) were cut to small pieces and subjected to essential oil extraction by hydro-distillation with Clevenger-type apparatus for 3 h in triplicate. The extracted Essential oil was dried over anhydrous sodium sulfate and stored in well-sealed umber glass vials and kept in refrigeration at 4°C until further uses.

GC-MS analysis

Analysis of the essential oil was conducted by using a gas chromatography (Agilent 8890GC System),

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coupled to a mass spectrometer (Agilent 5977B GC/MSD) and equipped with a HP-5MS fused silica capillary column (30m, 0.25mm i.e., 0.25mm film thickness). The oven temperature was maintained initially at 50°C, then programmed from 50 to 240°C at a rate of 5°C/min and from 240°C to 280°C at a rate of 10°C/min, then held for 10min at 280°C. Helium was used as the carrier gas; at Flow rate of 1.1mL/ min. The essential oil was dissolved in diethyl ether (30µL essential oil /mL diethyl ether), and then 2µL of this solution wasinjected intothe GC with a split ratio of 1:50. The injection temperature was 240°C. Mass spectra in the electron impact mode (EI) were obtained at 70eV and scan m/z range from 39 to 500amu. The isolated peaks were identified by matching them with data from the mass spectra library (National Institute of Standards and Technology, NIST)

Results and Discussion

The results showed that the group of monoterpene hydrocarbons in the *Thymus capitatus* essential oil from Libya consists of thirteen compounds (figure 1). The highest percentage of the total area was Carvacrol, accounting for 56.27%, while the lowest while the lowest rate was attributed to α-Pinene, which constituted 0.99% of the total area.

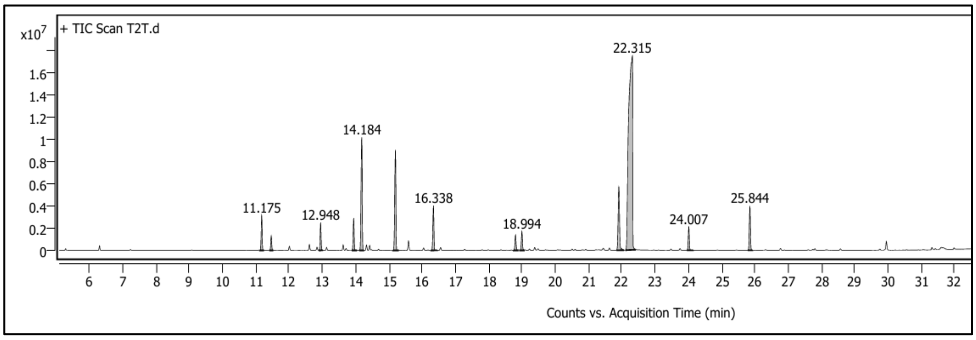

Figure 1 Gas Chromatography-Mass Spectrometry (GC-MS) to identify compounds from the sample of Libyan *Thymus capitatus* essential oil.

1.Monoterpene hydrocarbons

Monoterpenes, which consist of hydrocarbons, represent the largest group of secondary metabolites in plants and are frequently present in essential oils. Monoterpenes and their derivatives play a crucial role in the creation and development of novel biologically active substance. Because of their low molecular weight, many of them are found as essential oils' constituents [15].

As shown in Table (1), the compound β-Thujene was initially identified, showing a retention time of 11.175 minutes and an area of 6,277,782.21, which amounted to 2.43% of the total area. Subsequent to this, α-Pinene was detected at 11.463 minutes, exhibiting a notable area of 2,561,361.36, constituting 0.99% of the overall total. This compound is recognized for its fresh pine aroma and is commonly utilized in the fragrance sector.

Following that was β-Myrcene, which had a retention time of 12.948 minutes and an area of 5,021,062.54, accounting for 1.94% of the sample. This compound is valued for its fragrant and herbal

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scent. The examination proceeded with α-Terpinene, detected at 13.941 minutes, showing an area of 6,122,816.1, equating to 2.37% of the overall area. The key compound was p-Cymene, identified at 14.184 minutes with an area of 24,617, representing 9.51% of the total composition. γ-Terpinene was also significant, demonstrating a retention time of 15.194 minutes and an area of 21,713 adding 8.39% to the overall area.

Table 1: Monoterpene hydrocarbons identified of Libyan *Thymus capitatus* oil expressed as % of constituents by GC-MS.

Peak	RT	Area	Area Sum %	Chemical Compound	Molecular Formula	Matching %
1	11.175	6277782.21	2.43	β-Thujene	$C_{10}H_{16}$	75.55
2	11.463	2561361.36	0.99	α-Pinene	$C_{10}H_{16}$	77.39
3	12.948	5021062.54	1.94	β-Myrcene	$C_{10}H_{16}$	77.36
4	13.941	6122816.1	2.37	α-Terpinene	$C_{10}H_{16}$	84.58
5	14.184	24617782.77	9.51	p-Cymene	$C_{10}H_{14}$	72.49
6	15.194	21713000.21	8.39	γ-Terpinene	$C_{10}H_{16}$	80.76
Percentage of the total composition			25.63 %			

2.Oxygenated monoterpenes

As shown in Table (2), the analysis of essential oil from Libyan *Thymus capitatus* showed the identification of five notable oxygenated monoterpenes as determined by GC-MS. The main compound, Carvacrol (Peak 5), made up 56.27% of the total area, highlighting its significance in the oil. After Carvacrol, Thymol (Peak 4) was the second most plentiful, accounting for 6.54%. The additional compounds comprised Linalool (Peak 1) at 3.43%, Terpinen-4-ol (Peak 3) at 1.49%, and endo-Borneol (Peak 2) at 1.27%. In general, the information emphasizes the oil's abundant presence of oxygenated monoterpenes, recognized for their possible health advantages and fragrant characteristics.

Table 2: Oxygenated Monoterpene identified in Libyan *Thymus capitatus* oil expressed as % of constituents by GC-MS.

Peak	RT	Area	Area Sum %	Chemical Compound	Molecular Formula	Matching %
1	16.338	8874270.25	3.43	Linalool	$C_{10}H_{18}O$	66.74
2	18.804	3278088.73	1.27	endo-Borneol	$C_{10}H_{18}O$	78.23
3	18.994	3855001.03	1.49	Terpinen-4-ol	$C_{10}H_{18}O$	85.34

4	21.905	16923698.21	6.54	Thymol	$C_{10}H_{14}O$	71.78
5	22.315	145610151.2	56.27	Carvacrol	$C_{10}H_{14}O$	71.80
Percentage of the total composition			69%			

3.Sesquiterpene hydrocarbons

A GC-MS analysis showed Caryophyllene to be a noteworthy Sesquiterpene hydrocarbon, representing 3.57% of the oil's overall composition. Caryophyllene, having the molecular formula $C_{15}H_{24}$, is recognized for its spicy, woody scent and possible therapeutic benefits, including anti-inflammatory properties.

Table 3: Sesquiterpene hydrocarbons identified by GC-MS analysis of Libyan *Thymus capitatus* oil

Peak	RT	Area	Area Sum %	Chemical Compound	Molecular Formula	Matching %
1	25.844	9242879.52	3.57	Caryophyllene	$C_{15}H_{24}$	77.18
Percentage of the total composition				3.57		

4.Oxygenated heterogeneous hydrocarbons

The analysis revealed the presence of intricate hydrocarbon compounds. Carvacryl acetate was identified as a major component, accounting for 1.81% of the overall oil composition, with the molecular formula $C_{12}H_{16}O$. Carvacryl acetate is a complex substance that includes a Carvacryl group, functioning as an ester derived from carvacrol (or Carvacryl acetone).

Table 4: Oxygenated heterogeneous hydrocarbons identified by GC-MS analysis of Libyan *Thymus capitatus* oil

Peak	**RT**	**Area**	**Area Sum %**	**Chemical Compound**	**Molecular Formula**	**Matching %**
1	24.007	4683130.05	1.81	Carvacryl acetate	C12H16O	73.49
Percentage of the total composition			1.81			

The GC-MS analysis of *Thymus capitatus* essential oil collected from Mesellata revealed a chemical profile dominated by carvacrol (56.27%), with significant contributions from p-cymene (9.51%), γ-terpinene (8.39%), and thymol (6.54%), alongside minor constituents such as linalool, terpinen-4-ol, and caryophyllene. The presence of high precursor levels (p-cymene and γ-terpinene) alongside moderate carvacrol concentration suggests an active biosynthetic conversion stage, potentially influenced by the plant’s collection during the vegetative phase in winter. Since essential oil composition in *Thymus capitatus* is known to vary with phenological development, environmental factors, and regional differences, it becomes essential to compare these findings with oils from other Libyan and

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Mediterranean populations. Therefore, Table 5 presents a comparative overview of chemical compositions reported from different geographic origins, providing a basis for discussing chemotypic variability and the influence of harvest timing and local ecological conditions.

Table 5: Chemical composition of *Thymus capitatus* oil in other world regions

No.	Country	Instrument	Chemical composition	Ref
1	Zintan, Northwest Libya	GC and GC-MS	Carvacrol (68.19 %), thymol (12.29 %) , p-cymene (3.25 %)	[17]
2	Aljabal Akhdar ,Northeast Libya	GC-MS	Thymol (90.15%), Carvacrol (0.18%), Terpinolene (1.74%), Isoborneol(1.38%)	[18]
3	Algeria	GC–MS	Thymol (51.22%), carvacrol (12.59%) , γ-terpinene (10.3%)	[16]
4	Morocco	GC–MS	Carvacrol (75%) and p-cymene (10.58%)	[19]
5	Tunisia		Thymol(89.06%),p-cimene(5.04%), g-terpinene (3.19%)	[20]
6	Morocco	GC–MS	P-cymene(18.9%),carvacrol (13.4%), geranyl acetate (12.2%) , borneol (10.2%).	[21]
7	Greece	GC–MS	Thymol (39.8%), P-cymene (31%), carvacrol (5.7)	[22]

The essential oil composition of *Thymus capitatus* from Mesellata, Libya, characterized by the dominance of carvacrol (56.27%), p-cymene (9.51%), and γ-terpinene (8.39%), reflects a carvacrol-predominant chemotype, yet displays notable compositional differences when compared to profiles reported from other regions (Table 5). In Zintan, Libya, the essential oil was also carvacrol-dominant (68.19%), but with a significantly higher carvacrol content [17]. A key factor that may account for this difference is the developmental stage and season of plant collection: while the Zintan samples were collected during the summer flowering stage, the Mesellata samples were harvested in winter (December) during the vegetative stage. It is well-documented that essential oil biosynthesis, particularly the accumulation of phenolic monoterpenes like carvacrol, peaks during flowering due to enhanced secondary metabolic activities [5,12]. Thus, the lower carvacrol concentration in the Mesellata oil may reflect the earlier phenological stage, where precursor monoterpenes such as p-cymene and γ-terpinene are still abundant and undergoing biosynthetic conversion.

In contrast, the essential oil from Al Jabal Al Akhdar (northeastern Libya) demonstrated a thymol-dominant chemotype (90.15% thymol and only 0.18% carvacrol) [18], illustrating a distinct metabolic pathway orientation, possibly influenced by the region’s cooler climate and higher altitude. Such sharp chemical divergence even within Libya highlights the significant impact of microclimatic, edaphic, and genetic factors on essential oil composition [5,7].

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Comparisons with neighboring countries further emphasize regional chemotypic variability. In Algeria, a mixed chemotype was observed with thymol (51.22%) and carvacrol (12.59%) [16], while Tunisian populations exhibited strong thymol dominance (89.06%) [20], aligning more closely with the northeastern Libyan profile. Moroccan samples showed greater variability: some were carvacrol-rich (75% carvacrol) [19], while others exhibited high p-cymene (18.9%) and borneol (10.2%) content [21], similar to the relatively high precursor levels detected in the Mesellata oil.

Meanwhile, Greek *Thymus capitatus* oils displayed a balanced composition, with thymol (39.8%) and p-cymene (31%) [22], suggesting an intermediate chemotype shaped by island microclimates.

Taken together, the Mesellata *Thymus capitatus* essential oil represents a carvacrol-predominant chemotype with elevated levels of biosynthetic precursors, influenced by seasonal timing and developmental stage at harvest. These results reinforce the dynamic nature of essential oil biosynthesis and highlight the necessity for considering both phenological stage and environmental conditions when evaluating and standardizing essential oils for commercial and therapeutic applications.

Conclusions

The present study provides an in-depth characterization of the essential oil extracted from *Thymus capitatus* sourced from Mesellata region of northwestern Libya, employing GC-MS (Gas Chromatography-Mass Spectrometry) analysis. The major finding is the predominant presence of carvacrol (56.27%), p-cymene (9.51%), and γ-terpinene (8.39%). Comparative analysis with other regional studies confirmed both similarities and distinctive compositional traits, highlighting the influence of geographic, environmental, and possibly genetic factors on essential oil biosynthesis. The relatively high levels of precursor compounds such as p-cymene and γ-terpinene suggest active oxidative pathways contributing to carvacrol and thymol production, possibly shaped by local ecological conditions. These findings reinforce the chemotypic plasticity of *Thymus capitatus* across different Mediterranean regions and emphasize the importance of region-specific characterization to optimize its potential applications. Given the high concentration of bioactive monoterpenes, particularly carvacrol, the Mesellata chemotype presents a valuable natural source for antimicrobial, antioxidant, and preservative applications in the food, cosmetic, and pharmaceutical industries.

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