



ISSN Print: 2664-6064
 ISSN Online: 2664-6072
 NAAS Rating (2026): 4.69
 IJAN 2026; 8(3): 125-133
www.agriculturejournal.net
 Received: 04-01-2026
 Accepted: 23-02-2026
 Published: 25-02-2026

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***Thymus serpyllum* effects on the production of pro-inflammatory mediators and human peripheral blood mononuclear cells**

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DOI: <https://www.doi.org/10.33545/26646064.2026.v8.i3b.764>

ABSTRACT

Thymus serpyllum L., commonly referred to as Wild thyme, has an ancient history of culinary and medicinal use. Widely used in traditional medicine to treat various diseases. We used (GC-MS) to identify volatile chemicals, biological impacts, and proinflammatory activities of *Thymus serpyllum* L., one of many conventional methods for analysing plant extracts for their chemical makeup. We can mention the presence of the most common kind of chemicals: Thymol, MF:C₁₀H₁₄O, MW: 150.22 g/mol, 1,1,3-Trimethyl-3-cyclohexene-5-one MF:C₉H₁₄O, MW: 138.21 g/mol, Carvacrol methyl ether, MF:C₁₁H₁₆O, MW: 164.24 g/mol, (E,E)-2,4-Decadienal, MF:C₁₀H₁₆O, MW: 152.23 g/mol, Heptanoic acid, MF:C₂₀H₃₁NO₃, MW: 333.5 g/mol, MW: 136.23 g/mol, (-)-Linalool, MF:C₁₀H₁₈O, MW: 154.25 g/mol, 4-isopropyl-1-methylenecyclohexane, MF:C₁₀H₁₈, MW: 138.25 g/mol, D-Limonene, MF:C₁₀H₁₆, MW: 136.23 g/mol, hexadecen-3-ol, MF:C₂₀H₄₀O, MW: 296.5 g/mol, 8-Hydroxy-delta-cadinene, MF:C₁₅H₂₂O₂, MW: 234.33 g/mol, 1,5,5-Trimethylcyclopentadiene, MF:C₈H₁₂, MW: 108.18 g/mol, 1-Terpinen-4-ol acetate, MF:C₁₂H₂₀O₂, MW: 196.29 g/mol, *cis*-Cinnamaldehyde, MF:C₉H₈O, MW: 132.16 g/mol, (E,Z)-2,4-heptadienal, MF:C₇H₁₀O, MW: 110.15 g/mol, alpha-Pinene-oxide, MF:C₁₀H₁₆O, MW: 152.23 g/mol, Tetrahydro-4,6,6-trimethyl-2H-pyran-2-one, MF:C₈H₁₄O₂, MW: 142.2 g/mol, *trans*-Ocimenol, MF:C₁₀H₁₆O, MW: 152.23 g/mol, Methyl Palmitate, MF:C₁₇H₃₄O₂, MW: 270.5 g/mol, and *p*-Cymene, MF:C₁₀H₁₄, MW: 134.22 g/mol. The result showed that the production of pro-inflammatory mediators (TNF- α , Interleukin-1 β and Interleukin-6 Pg/mL respectively) recorded under the effect of *Thymus serpyllum* (0-150 μ g/mL LPS) were (4.92, 9.00, 5.78, 4.50, 33.00, 17.09, 13.72, and 11.00) Pg/mL respectively for TNF- α . While recorded (6.45, 9.00, 4.72, 2.40, 36.00, 17.09, 9.50, and 7.99) Pg/mL respectively for Interleukin-1 β . In the same time the levels of Interleukin-6 were (34.80, 58.00, 55.09, 20.71, 177.09, 172.00, 141.73, and 135.08) Pg/mL respectively.

Keywords: *Thymus serpyllum*, Pro-Inflammatory, Human Blood Mononuclear

Introduction

An interesting ethnobotanical, nutritional and phytotherapeutic case study is the *Thymus serpyllum* L. The genus (Lamiaceae) or wild thyme, as it is more commonly called. This evergreen plant is native to a wide range of Palearctic climes, but it is currently grown all over the globe for its fragrant essential oil and other culinary and medicinal parts. There is little scientific information available about the essential oil of *Thymus serpyllum* (*T. serpyllum*) because of its *anti-inflammatory* properties, and the last monograph of the genus *Thymus* (which encompasses wild thyme) was published 20 years ago. The use of wild thyme for medicine is not as diverse or interesting as its history and culture. *T. serpyllum* is a herb of great significance in various regional cuisines and therapeutic methods that have long been used in herbal teas and for rendering essential oil for use as a fragrant culinary flavouring [1]. The flavonoids, essential oils and phenolic acids present could be combined in complex ways which are responsible for the health benefits of the plant in treating and preventing diseases (Page *et al.*, 2021; Heinrich *et al.*, 2020; Heinrich *et al.*, 2022) [2, 3, 4]. Multiple clinical trials, in-vivo and in-vitro research have studied these substances and the results from these studies indicate that they possess antibacterial and hepatoprotective effects. It is especially important to note the positive impact that the plant has on the gut-brain axis, gut microbiome and the treatment of diseases like bowel disease (Benedum *et al.*, 2006) [5]. Inflammation is an important immune system defense mechanism that helps the body to cope with damaged cells and particles, with harmful infections and with environmental pollution. The starting point of this response is two key elements: monocytes and macrophages (Zaruelo *et al.*, 2002) [6]. A plethora of molecules associated with inflammation are secreted by them. Mediators may be chemokines, cytokines, enzymes, peptides, amines, or lipid molecules. The most powerful pro-inflammatory are (TNF- α), (IL-6), and (IL-11). The superoxide dismutase and (iNOS) are two examples of pro-in

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flammatory mediators (GPx). Folk medicine has made use of aromatic and medicinal herbs for the whole history of humankind. It is estimated that 70,000 species have played a part in traditional medicine around the world. There are approximately 3000 botanical raw materials in the world which trade on a global scale and the World Health Organisation (WHO) has documented more than 20,000 species of plants yielding pharmaceuticals. Species of the Lamiaceae family are perennial herbaceous MAPs from the Mediterranean area. In Iraq, 39 species of the polymorphic genus *Thymus* are found. The genus *Thymus* has a number of pharmacological effects including expectorant, antiseptic and antitussive. The volatile molecules are one of the most important characteristics of *Thymus* species that significantly affect consumers' acceptance in relation to food products. Wild thyme (*Thymus serpyllum*) contains volatiles such as sesquiterpenes, aldehydes, esters and acids (Brasseur, 1983; Bouyahya *et al.*, 2024; Castro-Sánchez *et al.*, 2016; Savoldi *et al.*, 2019) [7, 8, 9, 10]. The volatile oil composition of various species of *Thymus* has been the subject of numerous studies. The analytical investigations reveal the presence of thymol, *p*-cymene, β -caryophyllene and 1,8-cineole as the major volatiles produced by *Thymus*. Previous published data indicates that the volatile constituents of *Thymus serpyllum* have different compositions in different countries (Benkhaira *et al.*, 2023; Batbat *et al.*, 2024) [11, 12]. To date, more than 90 volatile chemicals have been extracted from several samples of thyme. Although thyme has a great number of volatile compounds, the majority of them do not add to the perfume. The gas chromatography-olfactory method (GC-O) was used to separate the volatile chemicals into odour active and non-odour active, depending on the concentration of each chemical in the sample. In addition, by performing the aroma extract dilution analysis (a serial dilution series of the aromatic extract) the relative fragrance intensity of each individual compound can be determined through the GC-O method. It is well-known that MAPs have a high concentration of phenolic chemicals. Lamiaceae species are well known for their high phenolic compound content, and studies have been conducted on oregano, thyme, and wild thyme. However, very few study of these plants by LC-MSMS was published. The wild thyme extract has eight different phenolic constituents. Among the phenolics identified rosmarinic acid was found to be the most abundant, followed by apigenin, quercetin, eriocitrin and apigenin-7-O-glucoside (Cheminal *et al.*, 2020; Boren *et al.*, 2020) [13, 14].

Medicinal plants have been used to obtain pharmaceutical compounds and to treat diseases. The abundance of substances in these plants is responsible for their anticancer, antidiabetic, antibacterial and antifungal properties. Due to the increasing awareness of side effects and non-specificity of pharmaceutical drugs, investigations on natural antibacterial agents have picked up speed (Ouedrhiri *et al.*, 2022) [15]. At this time, antimicrobial resistance is an issue on a global scale. Pathogenic bacteria have become more virulent while the effectiveness of the antibiotics has waned, and the post-antibiotic era has been declared by many experts! This is yet another testament to the necessity of new drugs to deal with the bacteria. EO derived from plants have diverse biological activities such as antioxidant and antibacterial properties. Essential oils are also valued for their medicinal properties and bioactive chemical compounds, making them a crucial part in the drug discovery process. The study of their antibacterial effects has gained considerable popularity in recent years and will no doubt result in the creation of new medicinal products with an effective action on different diseases. Thyme (*Thymus serpyllum* L.) is an aromatic plant. Wild thyme (Lamiaceae) is native to the Africa and Europe and is found at higher altitudes (Jeddi *et al.*, 2023) [16]. *T. serpyllum* is rich in phenolic acids and essential oils. Essential oils of *Thymus serpyllum* are known for their unique chemical make-up, which includes many bioactive chemicals that could have significant medical applications. These qualities are very important in treatments designed to reduce inflammation. In this investigation we aimed at the identification of the volatile chemicals, the biological effects and the pro-inflammatory activities of *Thymus serpyllum*.

Materials and methods

How to collect plant materials and the parts of plants

Dry samples of the whole *Thymus serpyllum* plants were taken just prior to flowering for this study. Plant material was allowed to dry at ambient temperature (ca. 20-25 °C) before examination. Three grammes of finely ground materials were used in a 15 mL brown-coded flask for representativeness tests. An aromatic strip made of cardboard was coated with an ethanol extract. After 1 minute the ends of the strips were cut off when the solvent has evaporated. Thereafter, they were moved to dark-coded 15 mL flasks where they were exposed to the panel after 15 min. All the samples were tested under neutral conditions at ambient temperature (20 °C).

Analysis by mass spectrometry and gas chromatography

Agilent Technologies of the United States of America manufactured the apparatus that was utilised to conduct the experiment. The procedure was pumping half a millilitre of 99.999 percent pure helium gas into the injector of one millilitre per minute and measurement of the dimensions of the column constructed by the HP-5MS. Inside the evaporator detector read 300 Co. Both the injection and the ion-source were kept at 250 and 280 Co, respectively. After 5 minutes at 50 C, the oven was ramped at 4 C/min to 300 C for 10 minutes isothermal. Identification of components involved about 50 minutes and involved more than 62,000 patterns using a NIST library of mass spectra. The concentrations of components of the final product was measured as part of our regular process. Such components, by chance, gave rise to a probability greater than 90%, were used in this experiment. They were calculated three times each.

Control group selection

On average, 36.4% of the male-participants were male. The participants had to refrain from inflammatory conditions, bacterial or viral infections, autoimmune diseases, and refrain from taking any herbal or immunosuppressive drug. The candidates also had to fulfill other requirements, such as not smoking, not having hypertension or metabolic issues, and not using corticosteroids, immunosuppressants or herbal medications. All participants were provided with information before they entered into the study or had their samples collected.

Separating the PBMCs

Blood was collected from each subject into 5 mls in EDTA vials. Within a few hours following collection, density centrifugation was used to isolate the PBMCs. The blood samples were mixed with a PBS solution and a single part of the sample in a sterile container. The diluted blood was spun with the same volume of cell density gradient medium at 2000 RPM (900xg) for 30 minutes at (18-20 C) without hesitation. The PBMCs were washed twice with cold PBS after the removal of the white layer, and centrifuged at 1200 RPM for 10 minutes at room temperature. One millilitre of ice-cold PBS was used to resuspend the cell pellets. After this, a hemocytometer was used to quantify PBMCs and a trypan blue dye test to measure the viability of the PBMCs. We re-analyzed and evaluated the samples that had a viability rate of over 95%.

Choices made in the domains of management and investigation

In all cases, unstimulated PBMCs were used. PBMCs were completely immersed in RPMI-1640 in each group as controls. To get a concentration of 0 g/mL of *Thymus serpyllum* extracts, the sample was diluted. The final concentrations of both extracts of *Thymus serpyllum* used for supplementing unstimulated PBMCs was 10 g/mL. The extract of *Thymus serpyllum* (30 μ g/mL) was applied to the PBMCs. The activated PBMCs were cultured for 24 hours in the presence of a *Thymus serpyllum* extract (90 microg/mL) in absence of pericytes. The cells in this well were treated with 1.0 10⁻⁶ g/mL LPS. To get a concentration of 0 g/mL of *Thymus serpyllum* extracts, the sample was diluted. The extracts of *Thymus serpyllum* were pre-incubated with 10-ug/mL of PBMCs. Before exposing the PBMCs to LPS

(1.0 µg/mL). 30 g/mL of *Thymus serpyllum* extracts were added to PBMCs, and then, at 2-hour intervals, 1.0 g/mL of LPS was added. After pre-incubation, 90.5 g/mL of *Thymus serpyllum* extract was added into the PBMC and then 1.5 g/mL of LPS was added.

Culture of cells

The PBMCs were then transferred to a complete culture media (RBM1-1640). The following components were also added to the same medium: 10% FBS, 2 mM L glutamine, 100 IU/mL penicillin and 100 mg/mL streptomycin.

In the periphery of the blood, it is the leading mononuclear cells

Thymus serpyllum dilutions were given to the PBMCs for a preincubation period of 2 hours prior to the addition of LPS (1.0 µg/mL). The samples were added, then swirled gently to mix, and then transferred to the incubator at 37 °C, 95 percent relative humidity and 5 percent CO₂. Pro-inflammatory mediators would be measured by collecting and storing the PBMCs after 24 hours of stimulation with LPS at 70 °C.

The cell viability assay

Thymus serpyllum was found to affect PBMCs as confirmed by MTT assay. When dehydrogenases, enzymes found in living cells, convert this colourless MTT into formazan, which is blue, this is the method used. Incubation conditions comprised of 37 °C, 95% humidity, and 5% CO₂. PBMCs were added to the wells of 96-well plates at 1×10¹ cells/well. Seven groups of cells were used in this experiment: a control group exposed to nothing other than RPMI-1640, and six groups exposed to *Thymus serpyllum* (10 to 360) for 24 hours, 48 hours and 72 hours respectively. The supernatant from the each well was removed after 37 °C further incubation for 4 hours with MTT and 200 ml of DMSO was added to dissolve the formazan crystals produced. Studies were done three times and viability of the untreated PBMCs was used as a control. The PerkinElmer Multimode Microplate Reader was used to measure the colour at an absorbance of 570 nm. The next step was the viability check of PBMCs and its percentages.

The ELISA assay

The levels of TNF-α, IL-1 β and IL-6 in the cell culture supernatants were quantified using the specific ELISA kits following manufacturer's instructions. The optical density of the samples was measured at 450 nm with the ELISA reader and the tests were repeated three times. The maker of the kits can be thanked for the standards they produced to achieve these results.

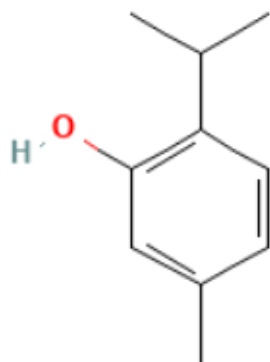
Statistics for data analysis

The statistical tests that were executed on the SPSS software were developed by SPSS Inc. of Chicago, USA (version 20). We used an analysis of variance (ANOVA) and a posthoc Scheffe test to compare the groups' variables. Data are presented as mean ± SEM and significance indicated when *P* < 0.05. A percentage and (SEM) were used to present the MTT assay data.

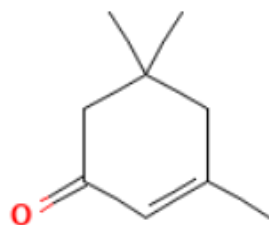
Results and discussion

The *Thymus serpyllum* contained twenty different compounds, according to the GC/MS results. It is possible to examine the isolated phytochemicals and the structure they took on using GC-MS. This method ensures a precise fingerprint by thoroughly treating the compounds, which reduces the margin of error. In addition to this, GC-MS provides quantitative data and a mass spectral database, both of which are very useful for linking the medicinal activity of bioactive

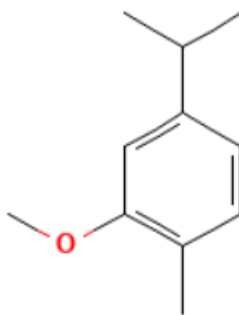
molecules to their specific constituents. Isolating organic compounds is the usual usage of gas chromatography. Analytes are pushed through a gaseous solvent called carrier gas in gas chromatography (GC) by means of a column. By observing the various peaks at the sample and comparing them with the mass spectra in the databases, one can ascertain the compound's composition. When only pure samples of a given chemical are needed, GC-FID can separate mixtures of chemicals while simultaneously identifying them. Using the typical analytical curve, this procedure can help you determine the quantity of the chemical components that matter to you. There is an effect on living systems from any bioactive chemical (Abebe, 2020; Bakkali *et al.*, 2008) [17, 18]. It all depends on the bioactive compound's qualities, dosage, and route of administration; some may have beneficial effects, while others may be harmful. Ingesting the bioactive substances at sufficient amounts has the potential to prevent and treat a wide range of ailments, according to the majority of studies. The food industry is currently focused on creating functional foods to prevent health problems like obesity and cancer, which are becoming more common in people's lives. Due to the presence of functional foods in bioactive chemicals, researchers have shown that consuming them can be beneficial. Incorporating bioactive substances into food not only improves the food's nutritional status, but it also protects against chronic diseases, boosts the immune system, and improves brain function. Antioxidants, which mainly serve to protect human cells and lower the risk of cardiovascular disorders, are one such substance. Essential fatty acids (omega-3, -6, and -9) are another class of bioactive compounds. Because of their positive effects on immunity, cardiovascular health, and cancer prevention, these fatty acids are known as polyunsaturated fatty acids. Wild thyme, for example, has bioactive compounds that include up to 1% essential oil, and the metabolites that have been discovered thus far only represent a fraction of these compounds. In contrast to the non-volatiles, the essential oil's composition and bioactivity have been the subject of substantially more research. A growing number of metabolites have been identified in recent decades (Abu-Darwish, 2009) [19]. In the early 1990s, a significant use of this phytochemical abundance was detected using state-of-the-art analytical equipment, the UPLC-ESI-Q coupling joint, and accurate data interpretation. This was the most up-to-date method for species authentication and differentiation from other thymus species. Some of its contemporaries failed to do so, instead reporting plant components that were clearly pollutants or artefacts, such as silicon derivatives and others. Even though they only make up around 5% of the dry raw material, essential oils have been the subject of a great deal of writing in various publications. More than 20 chemicals are found as a result of the research. When discussing the most common type of compounds, we may mention the presence of: Thymol, MF: C₁₀H₁₄O, MW: 150.22 g/mol, 1,1,3-Trimethyl-3-cyclohexene-5-one MF: C₉H₁₄O, MW: 138.21 g/mol, Carvacrol methyl ether, MF: C₁₁H₁₆O, MW:164.24 g/mol, Heptanoic acid, MF: C₂₀H₃₁NO₃, MW: 333.5 g/mol, (E,E)-2,6-Dimethyl-2,4,6-octatriene, MF: C₁₀H₁₆, MW:136.23 g/mol, (-)-Linalool, MF: C₁₀H₁₈O, MW: 154.25 g/mol, 4-isopropyl-1-methylenecyclohexane, MF: C₁₀H₁₈, MW: 138.25 g/mol, D-Limonene, MF: C₁₀H₁₆, MW:136.23 g/mol, 8-Hydroxy-delta-cadinene, MF: C₁₅H₂₂O₂, MW:234.33 g/mol, 1,5,5-Trimethylcyclopentadiene, MF: C₈H₁₂, MW:108.18 g/mol, 1-Terpinen-4-ol acetate, MF: C₁₂H₂₀O₂, MW:196.29 g/mol, *cis*-Cinnamaldehyde, MF: C₉H₈O, MW:132.16 g/mol, (E,Z)-2,4-heptadienal, MF: C₇H₁₀O, MW:110.15 g/mol, alpha-Pinene-oxide, MF: C₁₀H₁₆O, MW:152.23 g/mol, Tetrahydro-4,6,6-trimethyl-2H-pyran-2-one, MF: C₈H₁₄O₂, MW:142.2 g/mol, *trans*-Ocimenol, MF: C₁₀H₁₆O, MW:152.23 g/mol, Methyl Palmitate, and *p*-Cymene, MF: C₁₀H₁₄, MW:134.22 g/mol.



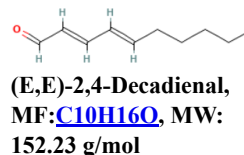
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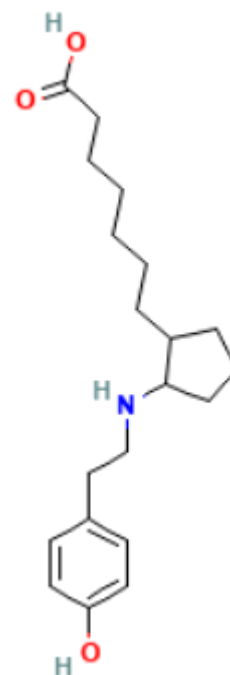
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MF:[C₉H₁₄O](#), MW:
138.21 g/mol



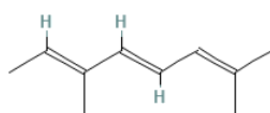
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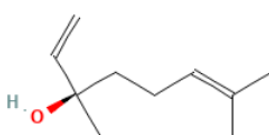
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MF:[C₁₀H₁₆O](#), MW:
152.23 g/mol



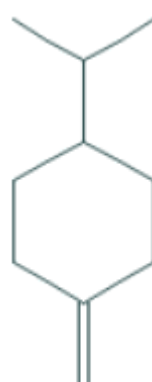
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[C₂₀H₃₁NO₃](#), MW:
333.5 g/mol



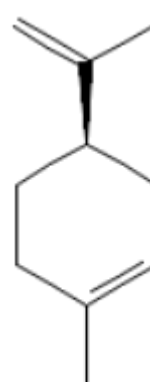
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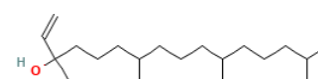
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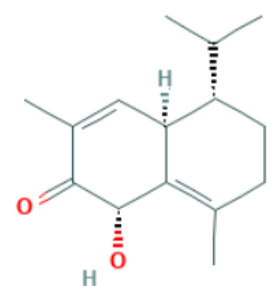
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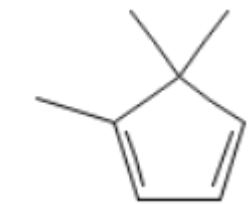
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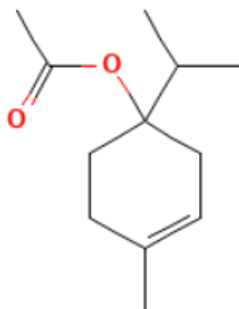
3,7,11,15-Tetramethyl-1-hexadecen-3-ol, MF:[C₂₀H₄₀O](#),
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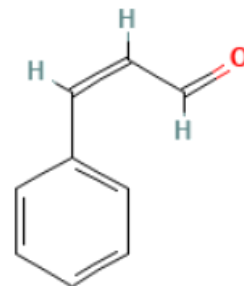
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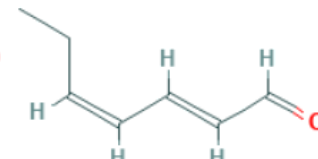
1,5,5-Trimethylcyclopentadiene,
MF:[C₈H₁₂](#),
MW:108.18 g/mol



1-Terpinen-4-ol acetate,
MF:[C₁₂H₂₀O₂](#),
MW:196.29 g/mol



cis-Cinnamaldehyde,
MF:
[C₉H₈O](#),
MW:132.16 g/mol



(E,Z)-2,4-heptadienal,
MF:[C₇H₁₀O](#),
MW:110.15 g/mol

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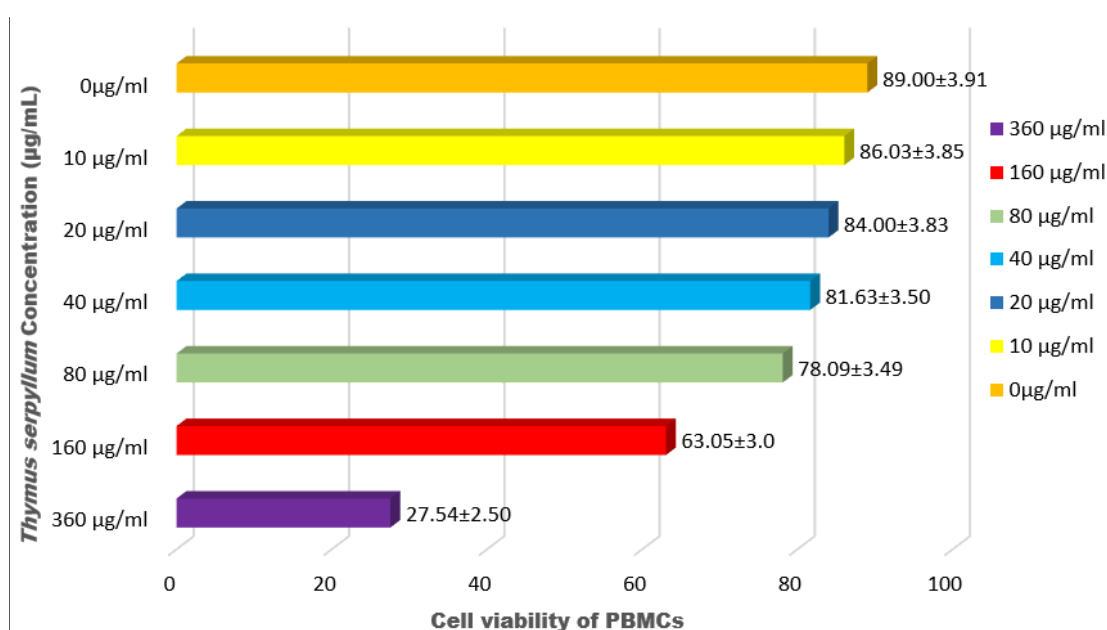
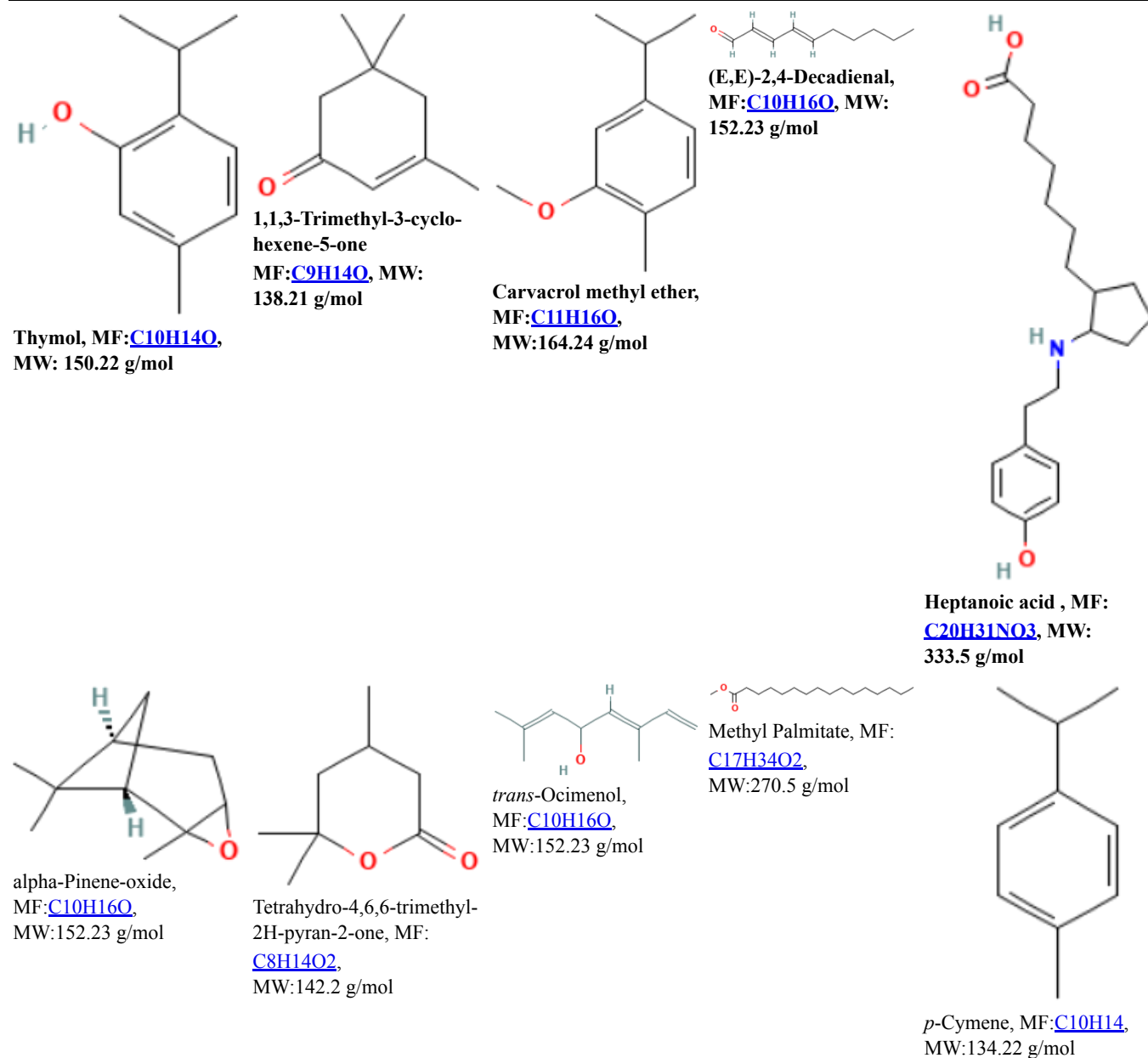


Figure 1. Cell viability of PBMCs [MTT reduction assay] treated with different concentrations of *Thymus serpyllum* during (24h)

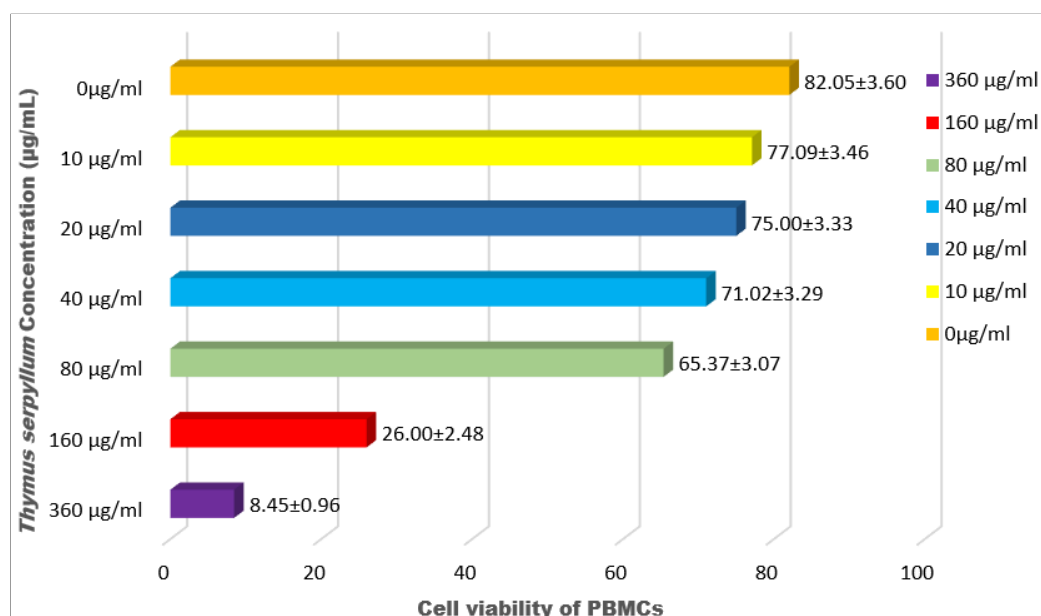


Figure 2. Cell viability of PBMCs [MTT reduction assay] treated with different concentrations of *Thymus serpyllum* during (48h)

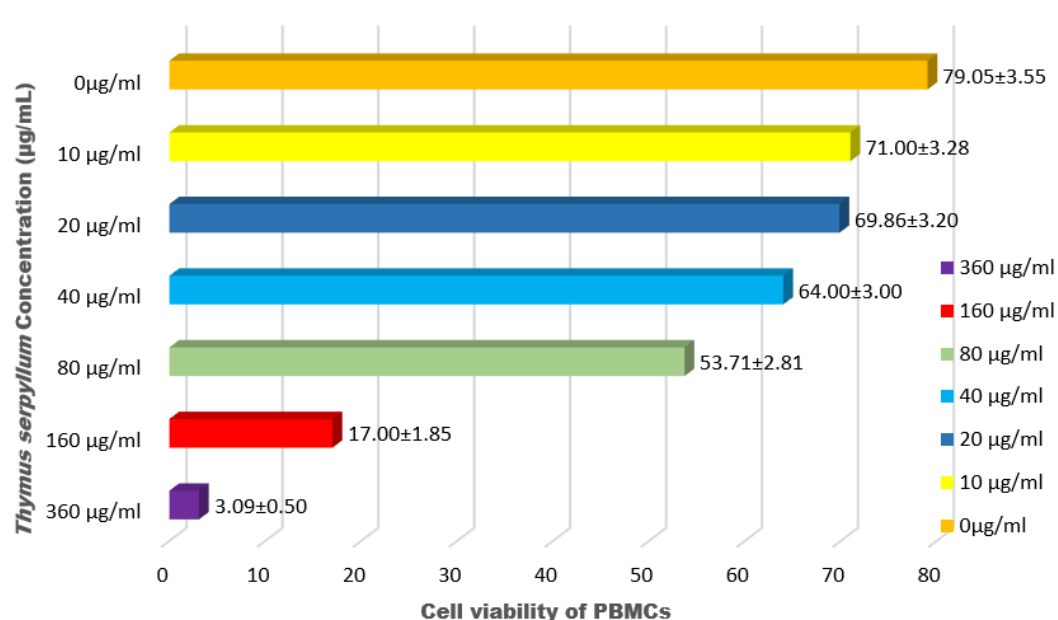


Figure 3. Cell viability of PBMCs [MTT reduction assay] treated with different concentrations of *Thymus serpyllum* during (72h)

Thymus serpyllum Concentration (µg/mL) (0 to 320 µg/mL). Cell viability of PBMCs recorded (89.00 ± 3.91 , 86.03 ± 3.85 , 84.00 ± 3.83 , 81.63 ± 3.50 , 78.09 ± 3.49 , 63.05 ± 3.0 , and 27.54 ± 2.50 in the 24 h); (82.05 ± 3.60 , 77.09 ± 3.46 , 75.00 ± 3.33 , 71.02 ± 3.29 , 65.37 ± 3.07 , 26.00 ± 2.48 , and 8.45 ± 0.96 in the 48 h), and (79.05 ± 3.55 , 71.00 ± 3.28 , 69.86 ± 3.20 , 64.00 ± 3.00 , 53.71 ± 2.81 , 17.00 ± 1.85 and 3.09 ± 0.50 in the 72 h), respectively (Figure 1-3). Effects of *Thymus serpyllum* (0 to LPS+150 µg/mL) on the production of pro-inflammatory mediators (TNF- α , Interleukin-1 β and Interleukin-6 Pg/mL respectively) recorded (4.92, 9.00, 5.78, 4.50, 33.00, 17.09, 13.72, and 11.00) Pg/mL respectively for TNF- α . While recorded (6.45, 9.00, 4.72, 2.40, 36.00, 17.09, 9.50, and 7.99) Pg/mL respectively for Interleukin-1 β . In the same time the levels of Interleukin-6 were (34.80, 58.00, 55.09, 20.71, 177.09, 172.00, 141.73, and 135.08)

Pg/mL respectively (Figure 4-6). The results revealed that there were no statistically significant differences ($P > 0.05$) between the levels of the pro-inflammatory cytokines after being treated with 10, 30 and 90 µg/mL of *Thymus serpyllum* compared to the control group. However, levels of TNF- α , IL-6 and IL-1 β were significantly elevated in the culture media of LPS stimulated PBMCs alone ($P < 0.001$). The selected concentrations of *Thymus serpyllum* affected the levels of TNF- α , IL-1 β and TLR-4 and the differences were statistically significant ($P < 0.001$, $P < 0.01$ and $P < 0.001$, respectively). *Thymus serpyllum* does not significantly effect the production of IL-6 by altering the protein levels ($P > 0.05$). Experiments are presented as the mean $\pm$ SEM for each of the bars in the figure. The significance between the groups was determined by ANOVA when *Thymus serpyllum*.

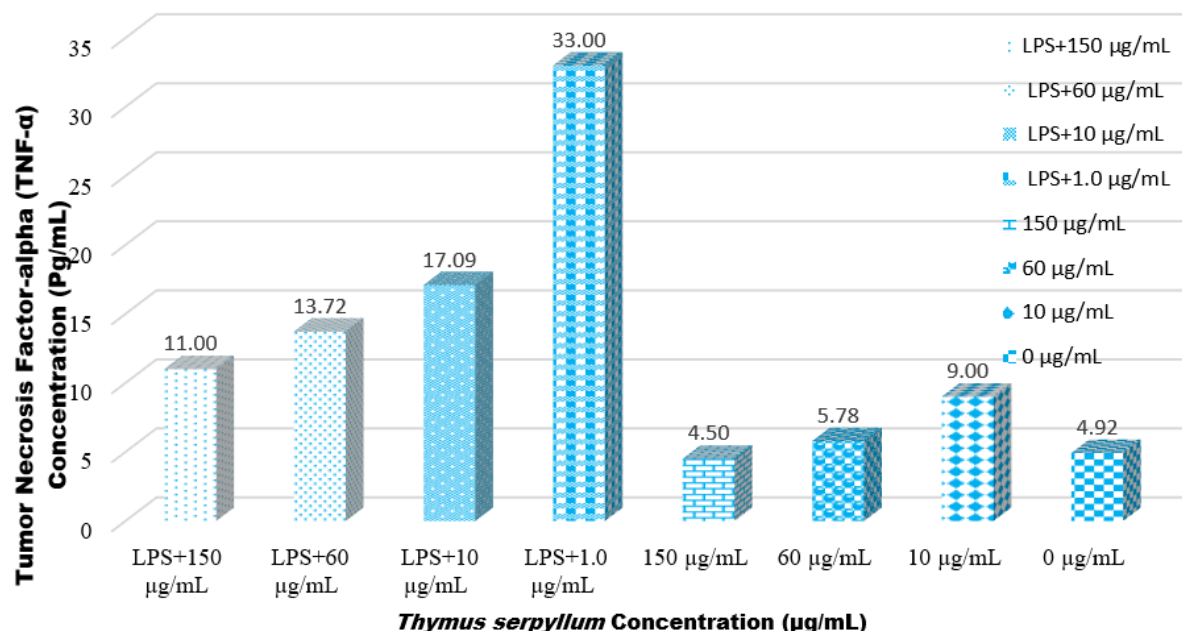


Figure 4. Effects of *Thymus serpyllum* on the production of pro-inflammatory mediators (Tumor Necrosis Factor-alpha (TNF-α)) Pg/mL

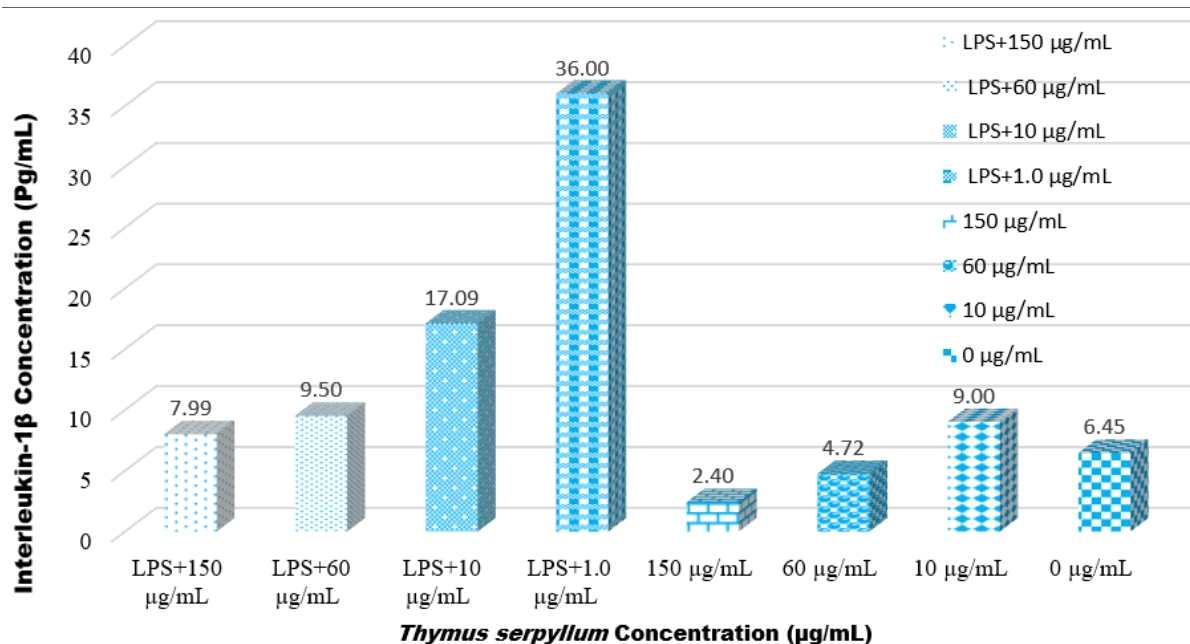


Figure 5. Effects of *Thymus serpyllum* on the production of pro-inflammatory mediators (Interleukin-1β) Pg/mL

The results of MTT were shown in the figures (1-3) to show the effect of *T. serpyllum* on cell. The percentage cell viability of the peripheral blood mononuclear cells (PBMCs) after exposure to 6 concentrations of *Thymus serpyllum* at three different time points (24 to 72 hours). Overall, the concentration of TNF-α, IL-6 and IL-1β were significantly higher in the LPS stimulated PBMCs. The amount of *Thymus serpyllum* had a great effect on the levels of TNF-α, IL-1β and TLR-4. At any rate, if *Thymus serpyllum* is used, there are no changes in the levels of IL-6 and PGE₂ compared to their starting levels. *Thymus serpyllum*, however, decreased the amount of pro-inflammatory chemicals in the cell culture media prior to activation of the PBMCs by the pro-inflammatory chemical LPS. The nutritional composition of *Thymus serpyllum herba* varies between literature sources and studies, fat (5.5g/100g), starch (6.5g/100g) and amino acids (2g/100g). The neutral detergent fiber (NDF) content of wild thyme herba is 15.1%, acid detergent fiber (10.6%), and cellulose (7.1%).

Vitamins (mg/100g) are for Vitamin A 4.9. Other bioactive metabolites include (Poly)Phenols (86.6 mg/g), and Phytosterols (45.3 mg/g). Although inflammation is an essential component of the immune system's defence mechanism, chronic inflammation can contribute to the development of various diseases such as cancer, autoimmune conditions, and more. Lipoygenases are enzymes that are involved in the production of lipid mediators known as leukotrienes, one of the major characteristics of inflammation (Figas *et al.*, 2021) [20]. There are leukotrienes that play a role in many inflammatory processes such as recruitment of immune cells and tissue damage. LEUKOTRIENES are made by LOX enzymes which oxidize polyunsaturated fatty acids, including arachidonic acid. These lipid mediators are signaling molecules that activate immune cells and other target cells to initiate an inflammatory response, through binding with specific receptors on these cells. This knowledge of the role of LOX in inflammation could provide insights into the disease mechanisms of inflammatory disease

and potentially help develop new therapies focusing on the LOX enzymes. Inhibitors of LOX activity are investigated as *anti-inflammatory* agents, to minimize tissue damage due to chronic inflammation. Although *T. serpyllum* EO is mainly renowned for its antioxidant properties, emerging evidence suggests its potential *anti-inflammatory*

tory effects (Vasil'eva *et al.*, 2022) [21]. In fact, *Thymus serpyllum* EO is rich in sabinene, thymol and carvacrol, both of which are found to be effective compounds. These compounds have been demonstrated to inhibit pathways like MAPK signalling, which are important regulators of the expression of pro-inflammatory genes.

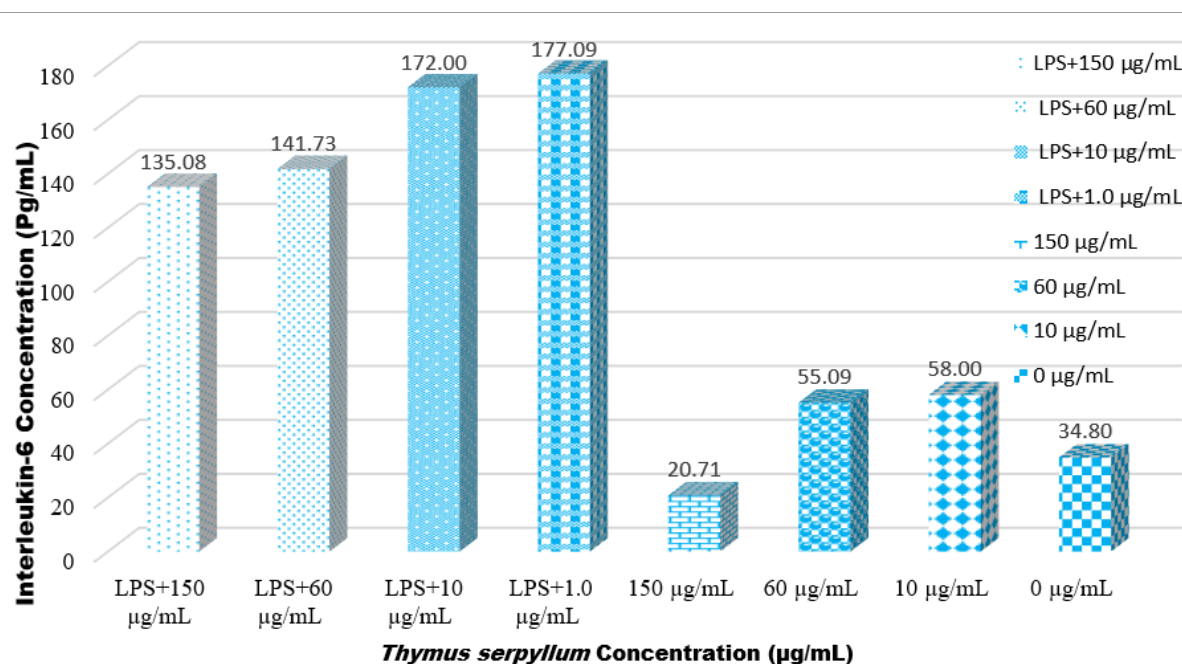


Figure 6. Effects of *Thymus serpyllum* on the production of pro-inflammatory mediators (Interleukin-6) Pg/mL

Conclusion

Essential oils and extracts of *Thymus serpyllum* (wild thyme) exhibit a very high intraspecific variability and chemical diversity in terms of chemotypes. The most common dominant volatiles found by GC-MS are thymol, *p*-cymene, and γ -terpinene; however, caryophyllene oxide- or linalool-rich non-phenol chemotypes are also common. The analysis of chemical composition and biological activity of *Thymus serpyllum* suggests that the oil has a wide range of chemical constituents and possesses high biological activities. Furthermore, even though the initial studies of *Thymus serpyllum* seem to have some positive effects, additional preclinical and clinical research is required to further elucidate its *anti-inflammatory* and potential application in humans. Finally, it was clear that the pro-inflammatory mediators were suppressed in their release and expression by our drug.

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How to cite this article: Al-Shalah ZM. *Thymus serpyllum* effects on the production of pro-inflammatory mediators and human peripheral blood mononuclear cells. *International Journal of Agriculture and Nutrition*. 2026;8(3):125-133. doi: <https://www.doi.org/10.33545/26646064.2026.v8.i3b.764>