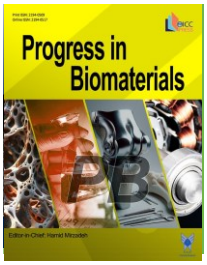

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Unveiling the Multifaced Therapeutic Potential of *Thymus vulgaris* L and its Phytochemical Analysis

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Abstract

The pharmaceutical properties and chemical constituents of *Thymus vulgaris* extract have attracted considerable interest from both researchers and the food industry. In this study, we evaluated the therapeutic potential of *T. vulgaris* extract, focusing on its antioxidant, anti-inflammatory, antidiabetic, antibacterial, and antibiofilm activities. In addition, we characterized its chemical composition and predicted the pharmacokinetic profile of its major compound, thymol, using ADMET analysis, to assess its potential as a natural therapeutic agent. Phytochemical analysis of the methanolic extract revealed high levels of phenolics (225 ± 1.42 mg GAE/g extract), tannins (20 ± 0.57 mg GAE/g extract), and flavonoids (25 ± 0.79 mg RE/g extract). GC–MS analysis identified thymol (28.23%) as the predominant active constituent.The methanolic extract of *T. vulgaris* demonstrated strong antioxidant activity, with IC_{50} values of 27 µg/mL (DPPH assay) and 35 µg/mL (ABTS assay). It also inhibited human erythrocyte hemolysis by 55.60%–74.81% at concentrations between 4 and 50 µg/mL.Furthermore, the extract significantly suppressed carbohydrate-metabolizing enzymes, producing 62.0% inhibition of α-glucosidase and 65.0% inhibition of α-amylase at 100 µg/mL. The extract exhibited notable antibacterial effects against several bacterial strains, with inhibition zones of 11 ± 0.45 mm to 14 ± 0.82 mm and minimum inhibitory concentration (MIC) values ranging from 95 to 450 µg/mL. In addition, it showed potent antibiofilm activity, achieving complete inhibition (100%) at 120 µg/mL. ADMET predictions further confirmed that thymol possesses favorable pharmacokinetic properties.These findings highlight the significant therapeutic potential of *T. vulgaris* extract and suggest that it may serve as a promising natural alternative for managing chronic diseases and combating antibiotic-resistant pathogens.

Keywords: *T. vulgaris*; Antioxidant; Antidiabetic; Anti-inflammatory; **A**ntibacterial; Antibiofilm; Phytochemical analysis

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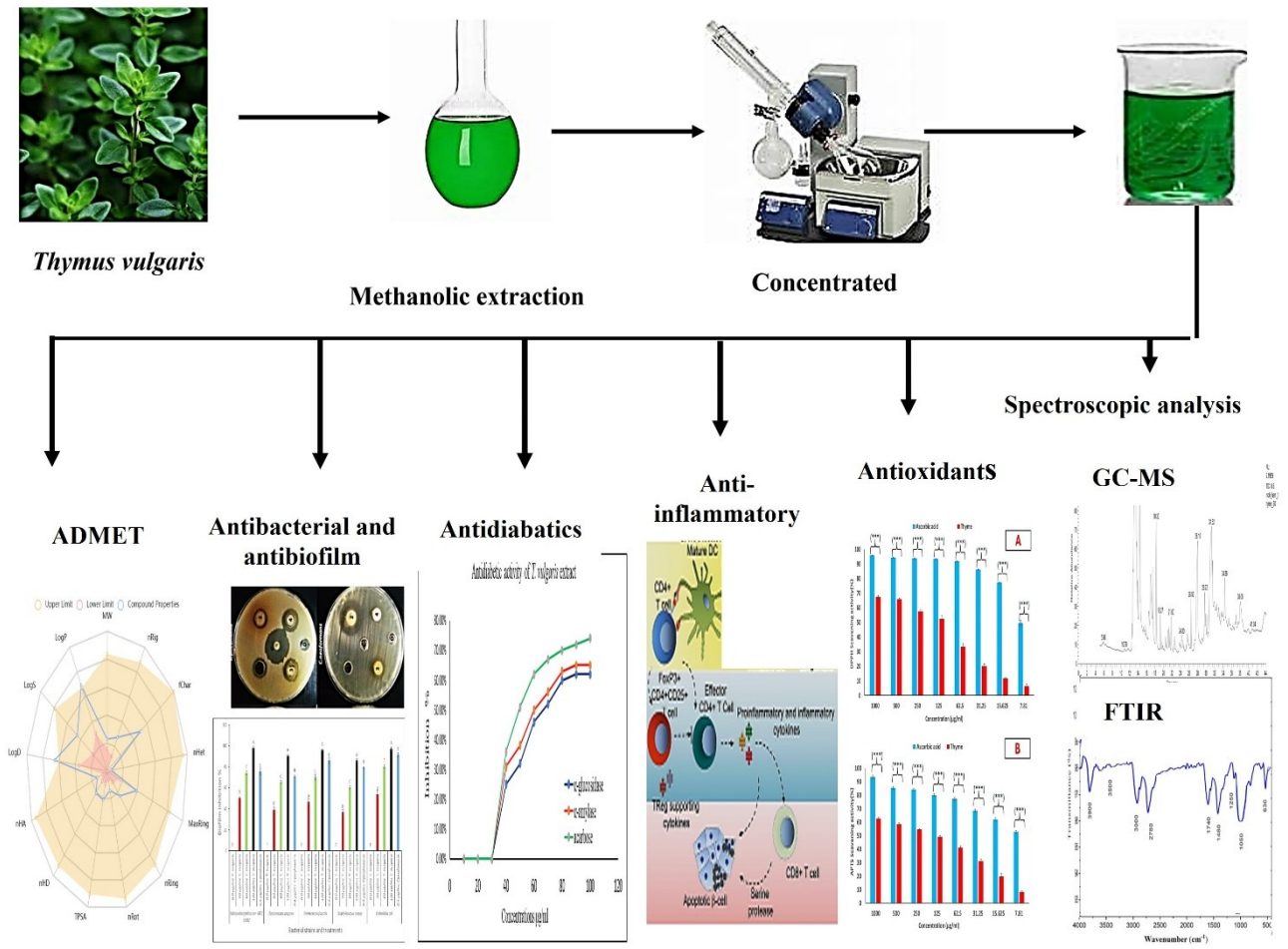
Graphical abstract



Introduction

Chronic diseases and antibiotic-resistant bacteria pose major threats to global health, contributing substantially to morbidity, mortality, and escalating healthcare costs worldwide [1]. Chronic diseases are long-term conditions that rarely resolve spontaneously and are seldom completely cured. These illnesses-including cardiovascular disease, cancer, respiratory disorders, cerebrovascular disease, and diabetes-account for approximately 70% of all deaths in the United States and nearly half of the years of potential life lost before the age of sixty-five [2]. The prevention and management of chronic diseases are shaped by multiple factors within a complex and dynamic system. Interventions that appear beneficial may sometimes lead to unintended consequences due to policy resistance, where preventive measures yield outcomes opposite to those expected. For instance, while antibiotics remain essential for treating bacterial infections, their overuse has accelerated the emergence of multidrug-resistant pathogens [3]. Antimicrobial resistance currently accounts for an estimated 700,000 deaths annually worldwide, a number projected by the World Health Organization (WHO) to reach 10 million by 2050 without effective interventions. In response, the WHO published a list of priority pathogens in 2017, identifying the ESKAPE group (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter* species) as the most critical threat to human health [4-6]. This highlights the urgent need for alternative therapeutic agents that are both safe and effective. Natural plant-derived compounds have long been recognized as valuable sources of bioactive molecules, many of which possess antimicrobial, antioxidant, anti-inflammatory, and antidiabetic activities [7-10]. Compared with synthetic drugs, natural compounds frequently exhibit lower toxicity and fewer adverse effects, making them attractive candidates for drug discovery and development [1]. *Thymus vulgaris* (common thyme), a member of the Lamiaceae family, has been widely used in traditional medicine since antiquity due to its broad

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therapeutic potential. It is also consumed as a culinary herb valued for its health-promoting properties. *T. vulgaris* is rich in phytonutrients, vitamins, minerals, flavonoids, and antioxidants [11]. Notably, it is an excellent source of vitamin A, a potent antioxidant essential for maintaining vision, mucous membranes, and skin health, as well as vitamin C, which strengthens the immune system and protects against oxidative damage. It also provides B-complex vitamins, particularly vitamin B6 (pyridoxine), which regulates γ-aminobutyric acid (GABA) levels in the brain and functions as a natural stress reliever. Additional vitamins present include K, E, and folic acid [12]. According to the USDA National Nutrient Database, thyme has an oxygen radical absorbance capacity (ORAC) value of 27,426 µmol Trolox equivalents per 100 g (µmol TE/100 g), highlighting its strong antioxidant potential [13]. In addition to vitamins, thyme leaves are a rich source of essential minerals such as potassium, calcium, iron, manganese, magnesium, and selenium. Potassium helps regulate fluid balance and cardiovascular function, iron is vital for red blood cell production, and manganese serves as a cofactor for the antioxidant enzyme superoxide dismutase [12-14]. Several studies have demonstrated the therapeutic potential of thyme and its essential oils, particularly thymol and carvacrol, in the management of various diseases. These benefits are attributed to its diverse pharmacological properties, including antioxidant, anti-inflammatory, and antineoplastic activities. Thyme has also been recognized for its antiviral, antibacterial, antifungal, and antiseptic effects [13-16]. Thymol, the principal essential oil constituent, is especially known for its antioxidant, anti-inflammatory, and antimicrobial properties. In the food industry, thymol and dried thyme are widely used in meat products as natural preservatives and flavoring agents, providing a safer alternative to synthetic additives [16-20]. According to Bakó et al. [19], bacterial biofilm formation not only facilitates infection transmission in humans but also promotes colonization of inanimate surfaces such as medical devices. *T. vulgaris* extracts and essential oils have shown strong antibiofilm activity against pathogens such as *Staphylococcus aureus, Pseudomonas aeruginosa,* and *Haemophilus* species, underscoring their potential as natural antimicrobial agents [19-21]. Furthermore, *T. vulgaris* has been reported to inhibit carbohydrate-hydrolyzing enzymes, including α-amylase and α-glucosidase, thereby demonstrating promising antidiabetic activity [12-24]. Despite these promising findings, the therapeutic potential of *T. vulgaris* remains underexplored, particularly regarding pharmacological validation and standardization. Variability in chemical composition caused by environmental factors, harvest conditions, and extraction methods complicates efforts to ensure consistent efficacy [25-27]. Computational approaches, including ADMET prediction and molecular docking, now provide powerful tools to evaluate the pharmacokinetic behavior of plant-derived compounds, bridging this gap and accelerating drug development [28-30]. Although previous studies have investigated the antimicrobial and therapeutic properties of *T. vulgaris*, the present study is distinct in that it is the first to comprehensively evaluate its effectiveness against foodborne pathogens and chronic diseases while also considering associated risks. This novel approach may open new avenues for developing natural antimicrobial agents and reducing reliance on synthetic alternatives. In the context of increasing antibiotic resistance, the findings of this research could play a pivotal role in guiding future therapeutic strategies.

Materials and Methods

Chemicals and Reagents

All standard chemicals used in this study were of analytical grade. Antioxidant reagents included Diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), potassium persulfate, and ascorbic acid, all obtained from Sigma-Aldrich (St. Louis, MO, USA). Anti-inflammatory standards comprised sodium diclofenac and dimethyl sulfoxide (DMSO), also purchased from Sigma-Aldrich. For the antidiabetic assays, pig pancreatic α-amylase enzyme, dinitrosalicylic acid, α-glucosidase, and p-nitrophenyl-α-D-glucopyranoside were obtained from the same supplier. Antibacterial reagents, including Mueller-Hinton broth, Mueller-Hinton agar, and ciprofloxacin, as well as antibiofilm assay reagents such as crystal violet and glacial acetic acid, were procured from HiMedia (Mumbai, India). Additional solvents-chloroform, acetone, and methanol-were also purchased from HiMedia.

Plant Material

Thymus vulgaris leaves were purchased from the local market in Hail, Saudi Arabia, in October 2024. The leaves were thoroughly washed under running tap water to remove dust and debris, followed by rinsing with distilled water. They were then spread on clean paper towels and air-dried at room temperature (25 ± 2 °C) for three days. The dried material was ground into a fine powder using an electric blender operated at high speed for 5 minutes, and the powder was passed through a 40-mesh sieve to obtain a uniform particle size.A portion of the powdered leaves (100 g) was packed into a thimble and extracted with methanol (1 L) using a Soxhlet

apparatus (Model No. 3840, Borosil Glass Works Ltd., Mumbai, India) at 60 °C for 8 hours. The extract was filtered through Whatman No. 1 filter paper, and the solvent was removed under reduced pressure using a rotary vacuum evaporator (Yamato BO410, Scientific Co., Ltd., Tokyo, Japan) at 40 °C, yielding a dark green viscous residue. The dried extract was stored in an airtight container at 4 °C until further analysis. The methanolic extract was subsequently evaluated for its antibacterial, antibiofilm, antioxidant, anti-inflammatory, and antidiabetic activities using various in vitro assays.

Determination of Total Phenolic, Tannin, and Flavonoid Contents

The total phenolic content of the *T. vulgaris* extract was determined according to the method of Siddhuraju and Manian [31]. Briefly, 100 µL of concentrated extract was added to a sterile test tube, followed by 1.0 mL of distilled water. Then, 2.5 mL of sodium carbonate solution (20%) and 0.5 mL of Folin–Ciocalteu reagent (diluted 1:1 with water) were added. The mixture was incubated in the dark for 40 minutes, and absorbance was measured at 725 nm against a reagent blank.Tannin content was assessed using polyvinyl polypyrrolidone (PVPP) as described by Siddhuraju and Becker [32]. A mixture containing 1.0 mL of distilled water, 1.0 mL of *T. vulgaris* extract, and 100 mg of PVPP was vortexed and incubated at 4 °C for 4 hours. The mixture was centrifuged at 3,000 × g for 10 minutes at room temperature, and the supernatant was collected. Tannin concentration was calculated by subtracting non-tannin phenolics from the total phenolics.The flavonoid content was determined using the method of Zhishen et al.[33]. An aliquot of 0.5 mL of extract (1 mg/mL) was mixed with 2.0 mL of distilled water, followed by the addition of 0.15 mL of 5% $NaNO_2$ solution. After 6 minutes, 0.15 mL of 10% $AlCl_3$ solution was added. The mixture was allowed to stand for another 6 minutes before adding 2.0 mL of 4% NaOH solution. Distilled water was added to bring the final volume to 5 mL. The solution was mixed thoroughly and incubated for 15 minutes, after which absorbance was measured at 510 nm against a water blank. Total flavonoid content was expressed as rutin equivalents (RE).All experiments were conducted in triplicate, and results were expressed as gallic acid equivalents (GAE) for phenolics and tannins, and rutin equivalents (RE) for flavonoids.

Characterization of *T. vulgaris* Extract by GC–MS

The methanolic extract of *T. vulgaris* was analyzed by gas chromatography–mass spectrometry (GC–MS) following the method of El-Sherbiny et al. [5]. With minor modifications. The extract was dissolved in spectroscopic-grade methanol and analyzed using a Thermo Scientific Trace GC1310–ISQ mass spectrometer (Austin, TX, USA) equipped with a direct capillary column (30 m length, 0.25 mm internal diameter, 0.25 µm film thickness). A 1 µL aliquot of the sample was injected at 250 °C, with helium used as the carrier gas at a split ratio of 30:1. The oven temperature was initially set at 50 °C for 5 minutes, then gradually increased to 230 °C at a rate of 5 °C/min and held for 2 minutes. The mass spectrometer operated in electron ionization (EI) mode at 70 eV, with an ion source temperature of 200 °C and a scan range of 40–1000 m/z. The obtained mass spectra were compared with reference spectra in the NIST 11 and WILEY 09 libraries (Wiley, New York, NY, USA) for compound identification

Antioxidant Activity of *T. vulgaris* Extract

DPPH Radical Scavenging Assay

The antioxidant capacity of the *T. vulgaris* methanolic extract was assessed using the DPPH radical scavenging method as described by El-Sherbiny et al. [10]. Serial dilutions of the extract were prepared at concentrations of 1000, 500, 250, 125, 62.5, 31.25, 15.62, and 7.81 µg/mL. To each sample, 5 mL of 0.1 mmol/L ethanolic DPPH solution was added and mixed thoroughly. Ascorbic acid was used as a standard control. After incubation for 20 minutes at 27 °C in the dark, absorbance was measured at 517 nm. The IC_{50} values for both ascorbic acid and the extract were calculated, representing the concentration required to reduce the initial DPPH radical concentration by 50%. Antioxidant activity was expressed using the following equation: DPPH scavenging activity = Absorbance of ascorbic acid control - Absorbance of *T. vulgaris* / Absorbance of ascorbic acid control ×100%. All experiments were performed in triplicate, and results are expressed as mean ± standard error (SD).

ABTS Radical Cation Decolorization Assay

The ABTS radical scavenging activity of the extract was determined according to Re et al. [34]. with slight modifications. ABTS cation radicals were generated by mixing 7 mmol/L ABTS solution with 2.4 mmol/L potassium persulfate and allowing the mixture to react in the dark for 12–16 hours at 25 °C. The solution was then diluted with ethanol (1:89, v/v) to obtain an absorbance of 0.70 ± 0.02 at 734 nm. Different concentrations of *T. vulgaris* extract and ascorbic acid (1000–7.81 µg/mL) were tested. After mixing, absorbance was recorded at 734 nm. Results were expressed as mean ± SD of triplicate measurements.

Anti-Inflammatory Activity

The anti-inflammatory effect of *T. vulgaris* extract was evaluated using the human red blood cell (HRBC) membrane stabilization assay [1]. Blood was collected from a healthy volunteer who had not taken NSAIDs for at least one week. Equal volumes of blood and Alsever's solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid, and 0.42% NaCl) were mixed and centrifuged at 3,000 rpm. The cells were washed with saline and resuspended to prepare a 10% HRBC suspension. Different concentrations of *T. vulgaris* extract and sodium diclofenac (positive control) were prepared in DMSO at 4, 8, 16, 32, and 50 µg/mL. Each concentration was mixed with 1 mL of phosphate buffer, 2 mL of hyposaline, and 0.5 mL of HRBC suspension, followed by incubation at 37 °C for 30 minutes and centrifugation at 3,000 rpm for 20 minutes. The hemoglobin content of the supernatant was measured spectrophotometrically at 560 nm. Percentage (%) inhibitions of hemolysis = (control absorbance - absorbance of sample) / (control absorbance) x 100

Antidiabetic Activity

α-Amylase Inhibition Assay

The α-amylase inhibitory activity of the extract was evaluated following the method of Bernfeld. [35], with modifications. Various concentrations of extract (10–100 µg/mL) were pre-incubated with 500 µL of 0.02 mol/L sodium phosphate buffer (pH 6.9 containing 0.006 mol/L NaCl) and 0.5 mg/mL pig pancreatic α-amylase enzyme at 25 °C for 10 minutes. Acarbose served as the positive control. After incubation, 500 µL of 1% soluble starch solution (in the same buffer) was added and incubated for 10 minutes. The reaction was terminated by adding 1.0 mL of dinitrosalicylic acid (DNS) reagent, followed by heating in boiling water for 5 minutes. After cooling, the mixture was diluted with 10 mL of distilled water, and absorbance was measured at 450 nm. % inhibition = [Absorbance control - (Absorbance sample - Absorbance blank) / Absorbance control] x100

α-Glucosidase Inhibition Assay

The α-glucosidase inhibitory activity of *T. vulgaris* extract was assessed using the method described by Kim et al. [36]. Extract concentrations (10–100 µg/mL) were incubated with 100 µL of α-glucosidase (0.5 mg/mL) in 0.1 mol/L phosphate buffer (pH 6.9) at 25 °C for 10 minutes. Acarbose was used as the positive control. The reaction was initiated by adding 50 µL of 5 mmol/L p-nitrophenyl-α-D-glucopyranoside (pNPG) in the same buffer. After incubation for 5 minutes at 25 °C, absorbance was recorded at 405 nm. Inhibition% = [(Absorbance sample – Absorbance sample blank)/ Absorbance control - Absorbance control blank)]x 100.

Antibacterial Activity

Disk Diffusion Assay

The antibacterial activity of *T. vulgaris* extract was tested against *Salmonella typhimurium* ATCC 35987, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 4352, *Escherichia coli* ATCC 25922, and four foodborne bacterial isolates obtained from the Faculty of Science, Al-Azhar University. Bacteria were cultured in Mueller-Hinton broth (MHB) and incubated at 37 °C for 24 hours. A 0.1 mL suspension (adjusted to 0.5 McFarland standard) was spread onto Mueller–Hinton agar (MHA) plates. Sterile filter paper discs impregnated with 100 µL of *T. vulgaris* extract were placed on the inoculated plates. Ciprofloxacin (5 µg/mL) was used as the positive control. Plates were incubated at 37 °C for 24 hours, and inhibition zones were measured in millimeters. All experiments were performed in triplicate [37, 38].

Determination of MIC Values

The minimum inhibitory concentration (MIC) of *T. vulgaris* extract was determined using the microdilution broth method in 96-well microplates according to CLSI guidelines [39]. Briefly, serial two-fold dilutions of the extract (5-70 µg/mL) and ciprofloxacin (0.1-1.3 µg/mL) were prepared in MHB. Wells were inoculated with 100 µL of bacterial suspension (10^6 CFU/mL) and incubated at 37 °C for 24 hours. MIC values were determined as the lowest concentration at which no visible bacterial growth was observed, confirmed by optical density measurement at 630 nm.

Antibiofilm Activity of *T. vulgaris* Extract

The antibiofilm activity of the *T. vulgaris* extract was evaluated using the microplate assay. Bacterial cultures (24 h old) were adjusted to 0.5 McFarland standard and used to prepare the inoculum. A 96-well polystyrene microplate (China) was inoculated in triplicate with 100 µL of the diluted bacterial suspension. To each well, 50 µL of *T. vulgaris* extract at concentrations of 30, 60, and 120 µg/mL was added. Control wells included: extract + growth medium (extract control), growth medium + inoculum (negative control), ciprofloxacin (0.4 µg/mL) + inoculum (positive control), Growth medium only (media control).After incubation, wells were washed repeatedly with phosphate-buffered saline (PBS) to remove non-adherent cells. Biofilms were fixed with methanol for 15 minutes, air-dried, and then stained with 100 µL of 1% crystal violet solution for 30 minutes at

room temperature. Excess stain was removed by washing, and bound dye was solubilized with 100 µL of 33% glacial acetic acid. The wells were washed three times with distilled water and air-dried. Biofilm biomass was quantified by measuring absorbance at 630 nm using an ELISA microplate reader. The percentage of biofilm inhibition was calculated using the formula described by Mohsenipour et al. [40]. % inhibition = (OD negative control- OD medium control) - (OD test-OD extract control) / (OD negative control- OD medium control) X 100. All experiments were performed in triplicate, and results were expressed as mean ± standard error (SD).

Pharmacokinetic Analysis and Drug-Likeness Prediction

Pharmacokinetic and drug-likeness properties of thymol were predicted using the open-source ADMETLab 2.0 platform (Computational Biology & Drug Design Group; **https://admetmesh.scbdd.com/**, accessed February 28, 2024), following the methodology described by Xiong et al. [41]. Parameters assessed included absorption, distribution, metabolism, excretion, and toxicity (ADMET).

Statistical Analysis

All data are presented as mean ± standard deviation (SD). Statistical analyses were conducted using Minitab 18 and Microsoft Excel 365 with additional statistical extensions. A two-way ANOVA with Bonferroni post hoc tests was employed to compare the mean effects of *T. vulgaris* extract and ascorbic acid at each concentration level.

Results and Discussion

Quantitative Determination of Total Phenolic, Tannin, and Flavonoid Content in *T. vulgaris*

The methanolic extract of *T. vulgaris* leaves was found to be rich in phenolic compounds, tannins, and flavonoids. Specifically, the extract contained 225 ± 1.42 mg GAE/g of total phenols, 20 ± 0.57 mg GAE/g of tannins, and 25 ± 0.79 mg RE/g of flavonoids (Table 1). The high concentration of phenolic constituents in the extract can be attributed to the presence of bioactive compounds such as thymol, carvacrol, various phenolic acids, steroids, tannins, and alkaloids [42]. These phytochemicals are widely recognized for their strong antioxidant and free radical scavenging properties, which collectively enhance the therapeutic potential of *T. vulgaris* [43]. It is important to note that the phytochemical composition of *T. vulgaris* extracts can vary considerably depending on environmental factors, geographical origin, and extraction techniques. Previous studies have reported a broad range of phenolic contents in thyme leaf extracts, ranging from 4.75 to 845 µg/10 mL [44,45]. In the present study, the methanolic extract yielded a total phenolic concentration of 225 ± 1.42 mg GAE/g, which falls within this reported range. The flavonoid concentration of the methanolic extract was 25 ± 0.79 mg RE/g, while tannins were quantified at 20 ± 0.57 mg GAE/g. These results are consistent with earlier reports. For example, Koksal et al. [46], reported total phenolic contents of 158 µg GAE/mg in ethanolic extracts and 256 µg GAE/mg in aqueous extracts of dried *T. vulgaris* from Turkey. Similarly, Sarfaraz et al. [47] documented 35.73 mg GAE/g dw of total phenolic content in a methanolic extract of Iranian *T. vulgaris*. In addition, ethanolic extracts of thyme leaves were reported to contain 103.13 µg/10 mL of total flavonoids, while acetone extracts contained 124.60 µg/10 mL.Collectively, these findings highlight the substantial phenolic, tannin, and flavonoid contents of *T. vulgaris* extracts, which underpin their strong antioxidant and therapeutic potential. The contents of tannin and overall phenolics were measured in mg of gallic acid equivalent (GAE) in the semi-dried extracts. The amount of flavonoids in g-dried extracts is stated as mg of rutin equivalent (QE).

GC-MS and FTIR Characterization of *T. vulgaris* Methanolic Extract

As shown in Figure 1A and Table 2, GC-MS analysis of the methanolic extract of *T. vulgaris* revealed that thymol (28.23%) was the predominant compound, followed by n-hexadecanoic acid (14.89%), E,E,Z-1,3,12-nonadecatriene-5,14-diol (12.47%), and phenol, 2-methoxy-4-(2-propenyl)-acetate (7.86%). The identified compounds (Table 2) could be classified into several chemical groups: Phenols (37.55%): including carvacrol, thymol, p-cymene-2,5-diol, eugenol, and trans-isoeugenol. Sesquiterpenes (7.66%): represented by caryophyllene oxide and caryophylla-4(12),8(13)-dien-5α-ol. Fatty acids and esters (34.70%): including hexadecenoic acid methyl ester, n-hexadecanoic acid, 10-octadecenoic acid methyl ester, and 6-octadecenoic acid. Steroids (0.93%): represented by a single compound, stigmast-5-en-3-ol, (3α,24S)-. Other compounds (4.44%): including espintanol, germacycloundecane-6,7-dione, and cinnoline. Approximately 14.72% of compounds could not be classified due to limited spectral information.The predominance of thymol in this study (28.23%) aligns with earlier reports. Saleem et al. [48]. and Bnouham et al. [49] identified thymol (28.88%) as the primary constituent of *T. vulgaris*, alongside eugenol (4.66%), p- cymene (7.77%), and γ-terpinene (3.47%). Similarly, Lemos et al.

[48], reported thymol (47.59%), γ-terpinene (30.90%), and p-cymene (8.41%) as the dominant constituents of thyme essential oil. Such variations in chemical composition have been attributed to environmental conditions, including seasonal changes in temperature and humidity, which may also influence antioxidant and antibacterial activities. The FTIR spectrum of *T. vulgaris* extract (Figure 1B) further confirmed the presence of key functional groups associated with bioactive phytochemicals. A broad absorption band at 3900–3500 cm^{-1} corresponded to O–H stretching vibrations of hydroxyl groups (alcohols, phenols, and carboxylic acids), indicative of strong hydrogen bonding typical of polyphenolic compounds. Peaks near 3000 cm^{-1} and 2780 cm^{-1} were assigned to C-H stretching, with the former attributed to sp^2 hybridized C–H (alkenes or aromatics) and the latter to aliphatic C-H (alkanes or aldehydes). A sharp absorption at ~1740 cm^{-1} indicated C=O stretching of esters, ketones, aldehydes, or carboxylic acids, confirming the presence of carbonyl-containing compounds.Additional characteristic peaks included: 1480 cm^{-1}: C=C stretching of aromatic rings and CH_2/CH_3 bending, 1250 cm^{-1}: C-O stretching vibrations of phenolics, ethers, or esters, 1050 cm^{-1}: C-O-C stretching, indicating ethers, polysaccharides, or secondary alcohols, 630 cm^{-1}: aromatic C-H out-of-plane bending, consistent with substituted aromatic structures. Overall, the FTIR analysis highlighted the presence of hydroxyl (O-H), carbonyl (C=O), aromatic (C=C), and C-O functional groups. These findings support the presence of polyphenols, flavonoids, tannins, and terpenoids, which are consistent with the strong antioxidant, antimicrobial, and therapeutic potential of *T. vulgaris* extract [7,50].

Antioxidant activity *T. vulgaris* extract

The methanolic extract of *Thymus vulgaris* leaves demonstrated high antioxidant activity, as determined by DPPH and ABTS radical scavenging tests. The extract demonstrated a considerably greater DPPH scavenging activity (IC_{50} = 27 µg/ mL) compared to normal ascorbic acid (IC_{50} = 8.2 µg/mL) (Figure 2A). Similarly, the extract has moderate ABTS radical scavenging activity (IC_{50} = 37 µg/mL) compared to ascorbic acid (IC_{50} = 7.5 µg/ mL) (Figure 2B). The antioxidant activity of thyme extract and ascorbic acid (standard antioxidant) was evaluated at various concentrations using the DPPH and ABTS radical scavenging assay. The results were analyzed using a two-way ANOVA with Bonferroni post-hoc tests to compare the means between thyme and ascorbic acid at each concentration level. At the highest concentration of 1000 µg/mL, ascorbic acid showed significantly higher antioxidant activity compared to thyme extract ($p < 0.001$). This trend continued at lower concentrations, with ascorbic acid maintaining significantly higher antioxidant activity than thyme ($p < 0.001$). These results indicate that at higher concentrations, ascorbic acid exhibits significantly greater antioxidant activity than thyme extract although it has moderate antioxidant activity. However, as the concentration decreases, the antioxidant activity of thyme becomes progressively lower compared to ascorbic acid, suggesting that thyme extract may not be as potent as ascorbic acid at lower doses. These findings indicate that the methanolic extract includes a significant number of hydrogen-donating molecules capable of effectively neutralizing free radicals and preventing oxidative damage. *T. vulgaris* leaf extract has considerable antioxidant activity due to its high phenolic component concentration. Thyme's main bioactive ingredients are phenols, notably thymol, and carvacrol, which are recognized for their strong free radical scavenging properties [16,19]. These molecules may prevent the formation of hazardous reactive oxygen species (ROS) such as superoxide, hydrogen peroxide, and hydroxyl radicals, which are formed during a variety of biological activities [57, 58]. By scavenging these radicals, the extract's phenolics protect biological components such as proteins, lipids, and DNA from oxidative damage. *T. vulgaris* extracts have been widely examined for their antioxidant capacity, with DPPH EC_{50} values ranging from 1.8 to 44.7 µg/mL and ABTS EC_{50} values of 0.7-13.8 µg/mL for various solvent extracts [57, 59-62]. The present study's findings fell within this range, indicating the methanolic extract's ability to neutralize free radicals. The extract's somewhat lesser activity compared to ascorbic acid might be attributed to its complex composition, which contains a variety of phenolics, while ascorbic acid is a single pure constituent. The extract's strong DPPH and moderate ABTS radical scavenging properties emphasize its promise as a natural antioxidant with uses in a variety of industries, including food preservation, cosmetics, and medicines. However, additional study is required to identify the individual bioactive components responsible for the antioxidant benefits and assess their effectiveness in vivo.

The anti-inflammatory action of the extract of *T. vulgari*

The methanolic extract of *Thymus vulgaris* exhibited strong anti-inflammatory activity, as demonstrated by its ability to inhibit human erythrocyte hemolysis in a concentration-dependent manner. The extract suppressed hemolysis by 55.60% at 4 µg/mL and up to 74.81% at 50 µg/mL (Table 3). In comparison, the reference drug sodium diclofenac inhibited hemolysis by 64.32%–80.38% at equivalent concentrations. The pronounced anti-inflammatory efficacy of *T. vulgaris* extract is likely attributable to its rich content of

Table 1. The amount of tannin, total phenolics, and flavonoids in the extract of leaves of *T. vulgaris*

Solvent extract	Total phenolics (mg GAE/g extract)	Tannin (mg GAE/g extract)	Flavonoids (mg RE/g extract)
Methanol	225±1.42	20± 0.57	25± 0.79

bioactive phytochemicals, particularly phenolic compounds such as thymol and carvacrol. These molecules are known to modulate inflammatory pathways by targeting pro-inflammatory mediators and signaling cascades [24, 63, 64]. One of the principal mechanisms underlying the anti-inflammatory effect of *T. vulgaris* involves the downregulation of pro-inflammatory cytokines and enzymes, including iNOS, COX-2, and 5-LOX, as well as the inhibition of transcription factors such as NF-κB, which control the expression of numerous inflammatory genes [42, 65-69]. Additionally, the extract reduces nitric oxide (NO) production and other reactive species, further contributing to its protective effects. The therapeutic significance of plant extracts in managing inflammation is well documented. For example, the methanolic extract of *Premna esculenta* roots significantly inhibited carrageenan-induced paw edema in rats at doses of 200 and 400 mg/kg, showing comparable activity to diclofenac sodium [70]. Similarly, extracts of *Piper chaba* demonstrated potent anti-inflammatory effects in vitro through membrane stabilization assays, underscoring the broad potential of plant-derived compounds as natural alternatives to conventional anti-inflammatory drugs [71]. Importantly, the anti-inflammatory activity of *T. vulgaris* is closely linked to its strong antioxidant capacity, which synergistically enhances its effects. Reactive oxygen species (ROS) are key drivers of inflammation, and their overproduction activates transcription factors such as NF-κB and AP-1, leading to elevated expression of cytokines (TNF-α, IL-1β, IL-6) and inflammatory enzymes (COX-2, iNOS). By scavenging ROS, antioxidants inhibit this signaling cascade, thereby suppressing inflammatory mediator production. In addition, antioxidants stabilize cell membranes by preventing lipid peroxidation and reducing the release of damage-associated molecular patterns (DAMPs), which otherwise amplify inflammation. Since oxidative stress and inflammation are interlinked in a self-perpetuating cycle-ROS drive inflammation, and inflammation generates more ROS-antioxidant activity interrupts this cycle, thereby lowering both the severity and duration of the inflammatory response.Together, these findings suggest that *T. vulgaris* extract, with its rich polyphenolic profile and dual antioxidant/anti-inflammatory mechanisms, holds strong potential as a natural therapeutic agent for managing inflammatory disorders [11, 14, 28].

Antidiabetic activity of *T. vulgaris* extract

Thymus vulgaris leaf extract inhibits important enzymes α-amylase and α-glucosidase, making it effective for diabetes treatment. The extract reduced α-amylase activity in a dose-dependent manner, with inhibition ranging from 31% to 65% across tested doses (Figure 3). This contrasts with the typical medication acarbose, which had a larger inhibition range of 36-74% at the same doses. The *T. vulgaris* extract inhibits α-amylase due to its high amount of phenolic components. Plant extracts high in phenols have been shown to inhibit α-amylase more efficiently than other substances [72]. The phenolics and other antioxidants included in the *T. vulgaris* extract are anticipated to play an important role in enzyme inhibition since plant-based natural antioxidants are favored over manufactured medicines owing to their lower side effects [27]. The extract inhibited the α-glucosidase enzyme by 62.0% at doses of 30 to 100 µg/ml. This action was equivalent to the 74.0% inhibition obtained with the conventional medication acarbose (Figure 3). The *T. vulgaris* extract inhibited α-glucosidase with an IC_{50} value of 0.05 g/mL (72%) according to a study by Dessalegn et al., [73]. The *T. vulgaris* extract inhibits α-glucosidase due to its high phenolic and flavonoid content, as well as its antioxidant properties. Phytochemicals block α-glucosidase and scavenge free radicals, making them effective anti-diabetic treatments. Elevated blood sugar levels may have a variety of negative health consequences for diabetics. The basic objective of diabetes treatment is to keep blood glucose levels stable. α-Glucosidase inhibitors, which prevent the conversion of disaccharides to monosaccharides, are often used as oral hypoglycemic medicines to manage blood glucose levels. The *T. vulgaris* extract inhibits α-amylase and α-glucosidase enzymes, making it a potential natural treatment for diabetes and related problems. The extract's antioxidant capabilities and high concentration of bioactive phenolic components are likely contributing to its antidiabetic potential [74, 75].

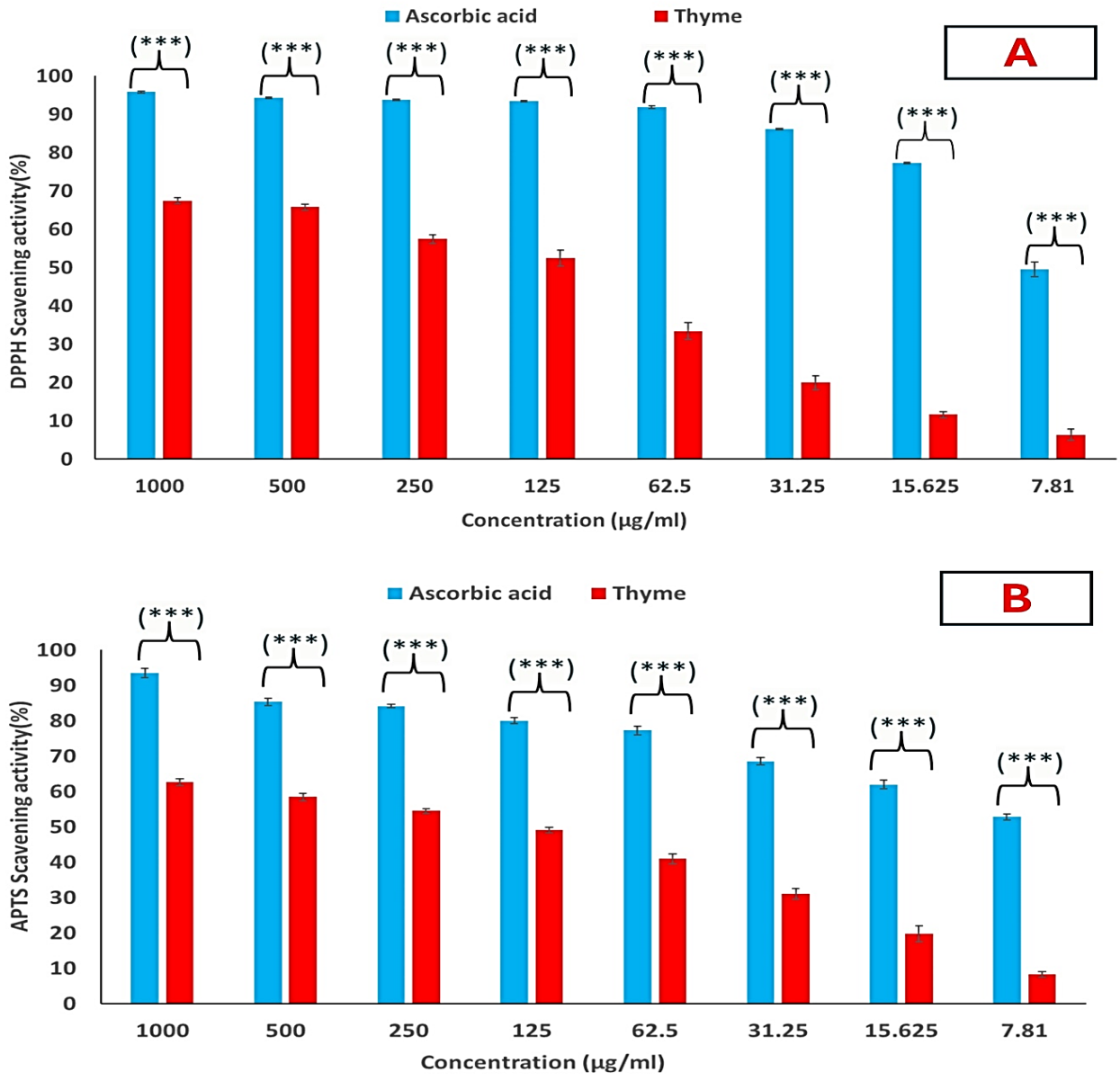

Figure 2. The antioxidant activity of *T. vulgaris* extract (A) using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and (B) 2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS) techniques

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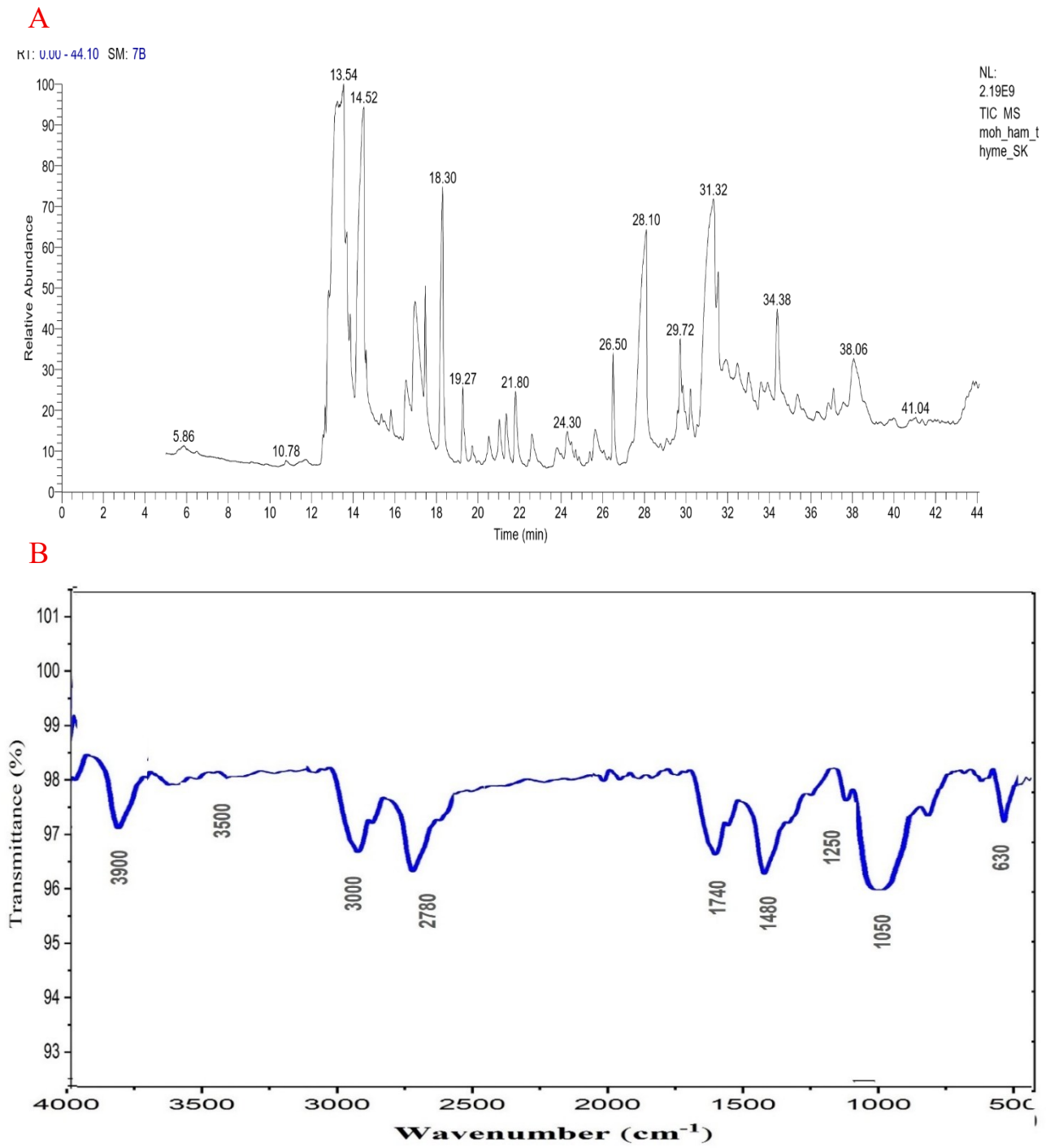

Figure 1. Spectroscopic analysis of *T. vulgaris* methanolic extract, (A) GC-MS and (B) FTIR

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Table 2. Chemical profiling of the methanolic extract of *T. vulagris* by GC-MS spectrometr

Peak	Rotation Time	Contents %	Compound	Molecular Formula	Molecular Weight	Activity	Ref.
1	5.86	1.07	Phenol, 2-methyl-5-(1-methylethyl) carvacrol	C 10 H14 O	148	Antimicrobial antioxidant	[5]
2	10.78	0.42	Eugenol	$C_{10}H_{12}O_2$	162	Anti-inflammatory, antibacterial, antiulcer, anticancer,	[1]
3	13.54	28.32	Thymol	$C_{10}H_{14}O$	150	Antimicrobial antioxidant	
4	14.52	3.79	Eugenol	$C_{10}H_{12}O_2$	164	anti-inflammatory, antibacterial, antiulcer, anticancer	[1]
5	14.63	0.22	Phenol, 2,3,5,6-tetramethyl-	$C_{10}H_{14}O$	150	anti-inflammatory, antibacterial	[1]
6	15.82	0.52	7-(1,3-Dimethylbuta-1,3-dienyl)-1, 6,6-trimethyl-3,8-dioxatricyclo [	$C_{15}H_{22}O_2$	234	Not Found	----
7	16.53	1.75	trans-Isoeugenol	$C_{10}H_{12}O_2$	164	Antimicrobial antioxidant	[1]
8	16.94	3.69	p-Cymene-2,5-diol	$C_{10}H_{14}O_2$	166	Anti-inflammatory, antibacterial, antiulcer, anti-cancer,	[51]
9	17.47	2.15	3,4-pyridinic carboxylic acid	$C_{14}H_{20}N_2O_5$	296	Not Found	----
10	18.30	7.86	Phenol, 2-methoxy-4-(2-propenyl)-, acetate	$C_{12}H_{14}O3$	206	Antimicrobial antioxidant	[52]
11	19.27	1.78	Caryophyllene oxide	$C_{15}H_{24}O$	220	Antimicrobial antioxidant	[52]
12	20.52	0.70	Caryophylla-4(12),8(13)-dien-5à-ol	$C_{15}H_{24}O$	220	Antimicrobial antioxidant	[52]
13	21.03	0.89	Caryophyllene oxide	$C_{15}H_{24}O$	220	Antimicrobial antioxidant	[52]
14	21.36	1.31	Caryophyllene oxide	$C_{15}H_{24}O$	220	Antimicrobial antioxidant	[52]
15	21.80	1.83	2',3',4' Trimethoxyacetophenone	$C_{11}H_{14}O_4$	210	Not Found	
16	22.59	0.68	Espintanol	$C_{12}H_{18}O_3$	210	Anti-inflammatory, antioxidants, antiparasitic	[83]
17	25.63	1.07	4,4,8-Trimethyltricyclo[6.3.1.0(1,5)]dodecane-2,9-diol	$C_{15}H_{26}O_2$	238	Not Found	----
18	26.50	2.34	Hexadecenoic acid methyl ester	$C_{17}H_{34}O_2$	270	Anti-inflammatory and antioxidant	[84]
19	28.09	14.89	n-Hexadecanoic acid	$C_{16}H_{32}O$	256	Anti-inflammatory and antioxidant	[84]
20	29.72	3.43	10-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	Antibacterial and antifungal	[84]
21	31.17	12.47	E, E, Z-1,3,12-Nonadecatriene-5,14-diol	$C_{19}H_{34}O_2$	294	Not found	[84]
22	31.32	4.61	6-Octadecenoic acid	$C_{18}H_{34}O_2$	282	Antimicrobial	[84]
23	33.01	0.93	Stigmast-5-en-3-ol, (3á,24S)-	$C_{29}H_{50}O$	414	Antihyperlipidemic and Anti-tumor	[85]
24	34.38	1.50	3-t-Butyl-6,6,9-trimethyl-6a,7,10,10a-tetrahydro-6H-benzo[c]chromen -1-ol	$C_{20}H_{28}O_2$	300	Not Found	----
25	37.09	0.63	Cyclopropa[5,6]-A-nor-5à-androstane-	$C_{23}H_{32}O_4$	392	Not Found	----
26	38.06	2.81	Germacycloundecane-6,7-dione	$C_{14}H_{26}GeO_2$	300	Not Found	----

Table 3. Estimation of the anti-inflammatory action of the *T. vulgaris* extract

Treatment	Concentration (µg/mL)	% Mean of Inhibition of hemolysis (mean ± SD)
Control	0.0	0.0
T. vulgaris extract	4	55.6±0.139
	8	59.3±0.261
	16	62.6±0.28
	32	65.2±0.247
	50	74.8±0.235
Sodium diclofenac	4	64.3±0.166
	8	67.8±0.379
	16	69.8±0.248
	32	72.6±0.515
	50	80.5±0.329

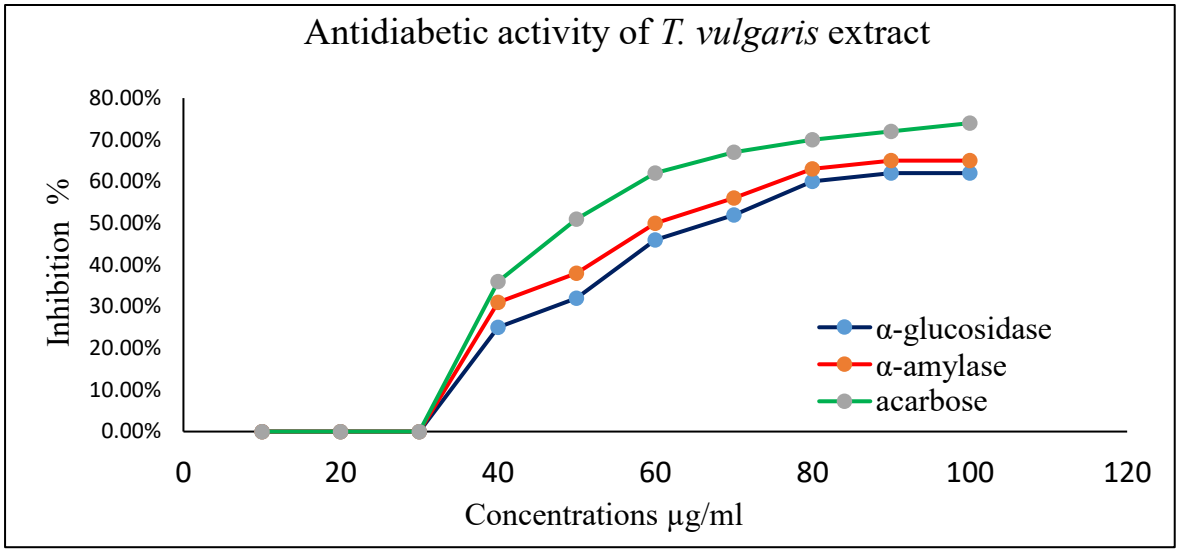

Figure 3. Antidiabetic activity of *T. vulgaris* extract

Antibacterial activity of *T. vulgaris* extracts and MIC

The methanolic extract of *T. vulgaris* leaves demonstrated considerable antibacterial activity against a wide range of standard bacterial strains as well as clinically identified foodborne pathogenic species. In the disc diffusion assay, the extract produced inhibition zones ranging from 11 ± 0.45 mm to 14 ± 0.82 mm, which were comparable to those of the standard antibiotic ciprofloxacin (12 ± 0.49 mm to 16 ± 0.67 mm) (Table 4). The significant antibacterial effect of *T. vulgaris* extract is likely attributable to its rich phytochemical composition, particularly terpenoids, tannins, and alkaloids. These compounds are known to exert antimicrobial effects by increasing membrane permeability, inhibiting bacterial enzymes, and interfering with the synthesis of cellular structures and genetic material, particularly in Gram-positive bacteria. Phytochemical-based antibacterial activity has been widely documented, with numerous studies confirming the broad-spectrum efficacy of plant extracts against microbial pathogens. In our study, GC–MS analysis identified thymol as the most abundant active constituent. Thymol is a well-characterized membrane-active antimicrobial compound; its hydrophobic nature enables it to integrate into bacterial lipid bilayers, disrupting their structural integrity. This disruption increases membrane permeability, leading to the leakage of ions, ATP, and other cellular contents, ultimately causing cell lysis. Because this mechanism is non-specific, thymol exhibits activity against both Gram-positive and Gram-negative bacteria [1, 9, 21]. Comparable findings have been reported in other plants with similar phytochemical profiles. For example, the leaf extract of *Azadirachta indica* (neem) exhibits antimicrobial activity against diverse bacterial and fungal strains, attributed to its alkaloids, flavonoids, tannins, saponins, and terpenoids. These compounds disrupt microbial growth by mechanisms such as membrane destabilization and enzyme inhibition [76]. Such similarities emphasize the therapeutic potential of *T. vulgaris* as a natural antimicrobial agent. Several studies have reinforced the antibacterial effects of *T. vulgaris*. An ethanolic–water extract showed strong inhibition against *S. aureus, Streptococcus sp., M. luteus, Vibrio tubiashii, Cellulosimicrobium cellulans, Legionella pneumophila,* and *B. cereus*. Similarly, a methanolic extract demonstrated inhibitory activity against *S. aureus, B. cereus, E. coli,* and *S. typhimurium*, with inhibition zones ranging from 17.11 to 24.87 mm [17,77,78]. These data indicate that *T. vulgaris* extracts can effectively suppress the growth of both Gram-positive and Gram-negative bacteria. In our study, the methanolic extract exhibited minimum inhibitory concentrations (MICs) ranging from 95 to 450 µg/mL (Table 5). Previous reports have shown MIC values for ethanolic and methanolic extracts between 78–312 µg/mL and 0.156–2.5 mg/mL, respectively [37, 78]. The relatively low MIC values observed suggest strong antibacterial potency, likely due to the synergistic effects of multiple bioactive constituents. The ability of *T. vulgaris* extract to inhibit both reference strains and clinically isolated pathogenic bacteria, including drug-resistant strains, underscores its therapeutic promise. This is particularly important given the growing threat of antibiotic resistance, highlighting thyme extract as a potential natural alternative for infection management. Indeed, thyme's antibacterial activity is often attributed to carvacrol and thymol, which increase membrane permeability, inhibit bacterial enzymes, and disrupt the synthesis of cellular components, especially in Gram-positive species [79]. Further supporting evidence

comes from Patil et al. [27], who demonstrated that essential oil from *T. vulgaris* produced 35–40 mm inhibition zones against drug-resistant *S. aureus*. Likewise, Yassin et al. [80] reported that the hexane extract of *T. vulgaris* showed maximum inhibition zone diameters of 33.24 mm, 29.86 mm, and 24.94 mm against *E. coli, S. typhi, S. aureus,* and MRSA, with MIC values ranging from 1250 µg/mL to <78 µg/mL. Similarly, Mohsenipour and Hassanshahian [40] found that methanol and ethanol extracts of *T. vulgaris* inhibited *S. pneumoniae, S. aureus, B. cereus, K. pneumoniae, P. aeruginosa,* and *E. coli*, with MICs of 0.156–1.25 mg/mL and 0.625–2.5 mg/mL, respectively. Furthermore, the n-hexane extract exhibited MICs of 0.25 mg/disk against *E. coli, E. faecalis,* and *S. typhi*, and 0.50 mg/disk against *S. aureus* and MRSA. Taken together, these findings demonstrate that *T. vulgaris* extract exhibits strong and broad-spectrum antibacterial activity, attributable to both major compounds like thymol and synergistic effects of other phytochemicals. Its efficacy against conventional and drug-resistant pathogens highlights its potential as a natural antimicrobial candidate in the era of rising antibiotic resistance.

Table 4. Antibacterial action of *T. vulgaris* extracts

Bacterial strains	Mean of inhibition zone diameter mm (mean ± SD)	
	T. *vulgaris* extract	Ciprofloxacin (5µg/mL)
Klebsiella pneumoniae ATCC 4352	12±1.52	15±0,58
Pseudomonas aeruginosa ATCC 27853	11±0.45	12±0.49
Escherichia coli ATCC 25922	13±0,15	15±0.37
Bacillus subtilis ATCC 6633	14± 0.82	15±0.80
Staphylococcus aureus ATCC 29213	14± 1.0	16± 0.67
Salmonella typhimurium ATCC 35987	13±0.54	14± 1.0
Listeria monocytogenes	12± 0.27	14± 0.57
Enterococcus faecalis	10± 0.73	14± 0.56
Staphylococcus aureus	13±0.44	12±0.89
Escherichia coli	14± 0.36	14± 0.57

Table 5. MIC of *T. vulgaris*

Bacterial strains	*T. vulgaris* methanol extract (µg/mL)	Ciprofloxacin (µg/mL)
Klebsiella pneumoniae ATCC 4352	150	0.5
Pseudomonas aeruginosa ATCC 27853	175	0. 5
Escherichia coli ATCC 25922	95	0. 5
Bacillus subtilis ATCC 6633	132	0. 5
Staphylococcus aureus ATCC 29213	150	0. 5
Salmonella typhimurium ATCC 35987	105	0. 5
Listeria monocytogenes	180	0. 6
Enterococcus faecalis	450	0. 7
Staphylococcus aureus	350	0. 5
Escherichia coli	175	0. 5

Antibiofilm activity of *T. vulgaris* extract

The present study investigated the antibiofilm potential of the *T. vulgaris* leaf extract against four foodbrone bacterial isolates.The findings revealed that the bacterial isolates exhibited varying degrees of biofilm formation. Interestingly, *T. vulgaris* extract demonstrated potent inhibitory effects on the biofilm formation of these bacterial strains. At concentrations of 30 and 60 µg/mL, the extract significantly reduced biofilm formation, and at 120 µg/mL it achieved complete suppression (100%) (Figure 4). In comparison, the standard antibiotic ciprofloxacin inhibited biofilm formation by approximately 68% at a much lower concentration (0.4 µg/mL). While this indicates that ciprofloxacin is more potent on a concentration basis, the extract displayed a greater maximum inhibitory effect, completely eliminating biofilm formation at higher concentrations. These results suggest that although *T. vulgaris* extract requires higher doses, it possesses strong antibiofilm potential that may complement or enhance conventional antibiotic therapy.The antibiofilm activity of the *T. vulgaris* extract can be attributed to its major bioactive compound, thymol. Previous studies have reported that thymol effectively suppresses biofilm formation by inhibiting the production of intracellular polysaccharide adhesins, the release of extracellular DNA (eDNA), and the expression of biofilm-regulated genes in various bacterial species, including MRSA [81-83]. These components are essential for the aggregation, adhesion, and maturation of bacterial biofilms. Biofilm formation is an important virulence factor in many bacterial species, as it allows them to adhere to host tissues and cells and confers increased resistance to antibiotics. The biofilm matrix is controlled by the Quorum Sensing (QS) system, which regulates the expression of genes involved in biofilm development. Four stages comprise the biofilm cycle: bacterial adhesion, microcolonization, biofilm maturation, and cell dispersal. Intracellular adhesin, eDNA, and polysaccharides are essential components of MRSA biofilms. The aggregation and adhesion processes are significantly influenced by intracellular adhesin, a polysaccharide [82,83]. Previous studies have reported the effective suppression of biofilm formation by *T. vulgaris* extracts against a range of bacterial species, including *Streptococcus pneumoniae, Staphylococcus aureus, Bacillus cereus, Klebsiella pneumoniae, Pseudomonas aeruginosa*, and *Escherichia coli* [1, 40]. Also, Carezzano et al. [84]. reported the antibiofilm activity of thymol oil extracted from *T. vulgaris a*gainst *Pseudomonas. syringae.*

Pharmacokinetic characteristics of thymol by (ADMET)

The pharmacokinetic characteristics of thymol, the major active compound in the methanolic extract of *T. vulgaris*, were assessed using the ADMETlab 2.0 platform. The results are summarized in Table 6. and illustrated in a radar diagram depicting 13 distinct physicochemical properties (Figure 5). Overall, the data indicate that thymol possesses favorable physicochemical attributes consistent with drug-like behavior. Specifically, thymol successfully passed the Lipinski and GSK drug-likeness filters but did not meet the Pfizer and Golden Triangle criteria, primarily due to low QED values associated with certain drug-likeness parameters. Compounds meeting the Golden Triangle criteria typically exhibit a more favorable ADMET profile, according to ADMETlab 2.0. Regarding absorption and transport, thymol was identified as both a substrate and inhibitor of P-glycoprotein (P-gp), an efflux pump that reduces intracellular drug accumulation by actively transporting xenobiotics out of cells. P-gp plays an essential role in central nervous system protection and xenobiotic clearance, as described by Obakiro et al. [85]. Thymol also demonstrated excellent human intestinal absorption (HIA), an important indicator of oral bioavailability. Moreover, it exhibited low plasma protein binding (PPB), suggesting a reduced tendency to bind plasma proteins, which may lower toxicity risks and increase the therapeutic index. The extract also showed a high fraction unbound (Fu) and a large volume of distribution (Vd), both of which suggest efficient tissue penetration and favorable systemic distribution. However, thymol displayed poor blood–brain barrier (BBB) permeability. Only certain lipid- and water-soluble molecules, often transported via carriers such as P-gp or glucose transporters, can effectively cross the BBB [86]. The hydrophilic or lipophilic character of a compound significantly influences its HIA, PPB, Vd, Fu, and BBB penetration profiles [87]. In terms of metabolism, thymol demonstrated inhibitory effects on multiple cytochrome P450 (CYP450) isoenzymes, which are responsible for metabolizing approximately 60% of drugs, as well as xenobiotics, steroids, and eicosanoids. While inhibition of CYP450 enzymes can lead to clinically significant drug–drug interactions, compounds serving as CYP450 substrates are often rapidly metabolized, potentially resulting in either deactivation or generation of active/toxic metabolites [88]. Thymol exhibited a moderate clearance rate, suggesting a reduced risk of accumulation within the body. Clearance influences drug half-life and dosing regimens; while lower clearance rates may reduce dosing frequency, they can also raise toxicity concerns if the compound is concentration-dependent [85]. Importantly, thymol did not inhibit the hERG potassium channel, which is critical

for cardiac repolarization. Inhibition of this channel can cause arrhythmias and sudden cardiac death [89, 90]. Despite these favorable pharmacokinetic features, thymol demonstrated concerning toxicity predictions in silico. According to ADMETlab 2.0, thymol tested positive in the Ames mutagenicity assay, showed acute toxicity in rats, and was associated with moderate carcinogenicity and hepatotoxicity in humans. Acute toxicity studies in mammalian models remain essential for further safety evaluations [41]. Taken together, the ADMET predictions suggest that while thymol displays promising pharmacokinetic and drug-likeness properties, its potential toxicities warrant caution and necessitate further toxicological validation. Nevertheless, the growing interest in thymol as a natural therapeutic candidate is supported by extensive evidence of its antioxidant, anti-inflammatory, antimicrobial, and antitumor properties [19, 21]. Thymol-rich plants have long been used in traditional medicine for managing cancers, cardiovascular diseases, diabetes, and neurodegenerative disorders. Although numerous *in vitro* studies highlight its therapeutic potential, discrepancies between effective laboratory concentrations and achievable *in vivo* doses-especially in cancer models-remain a challenge [89]. Importantly, thymol is abundant, cost-effective, and accessible through dietary sources, enhancing its appeal as a functional food component and therapeutic candidate. Despite limitations, the cumulative data strongly support thymol as a promising phytochemical with polypharmacological actions. With its broad-spectrum activity and favorable pharmacokinetic features, thymol remains a strong contender in natural product–based drug discovery[91].

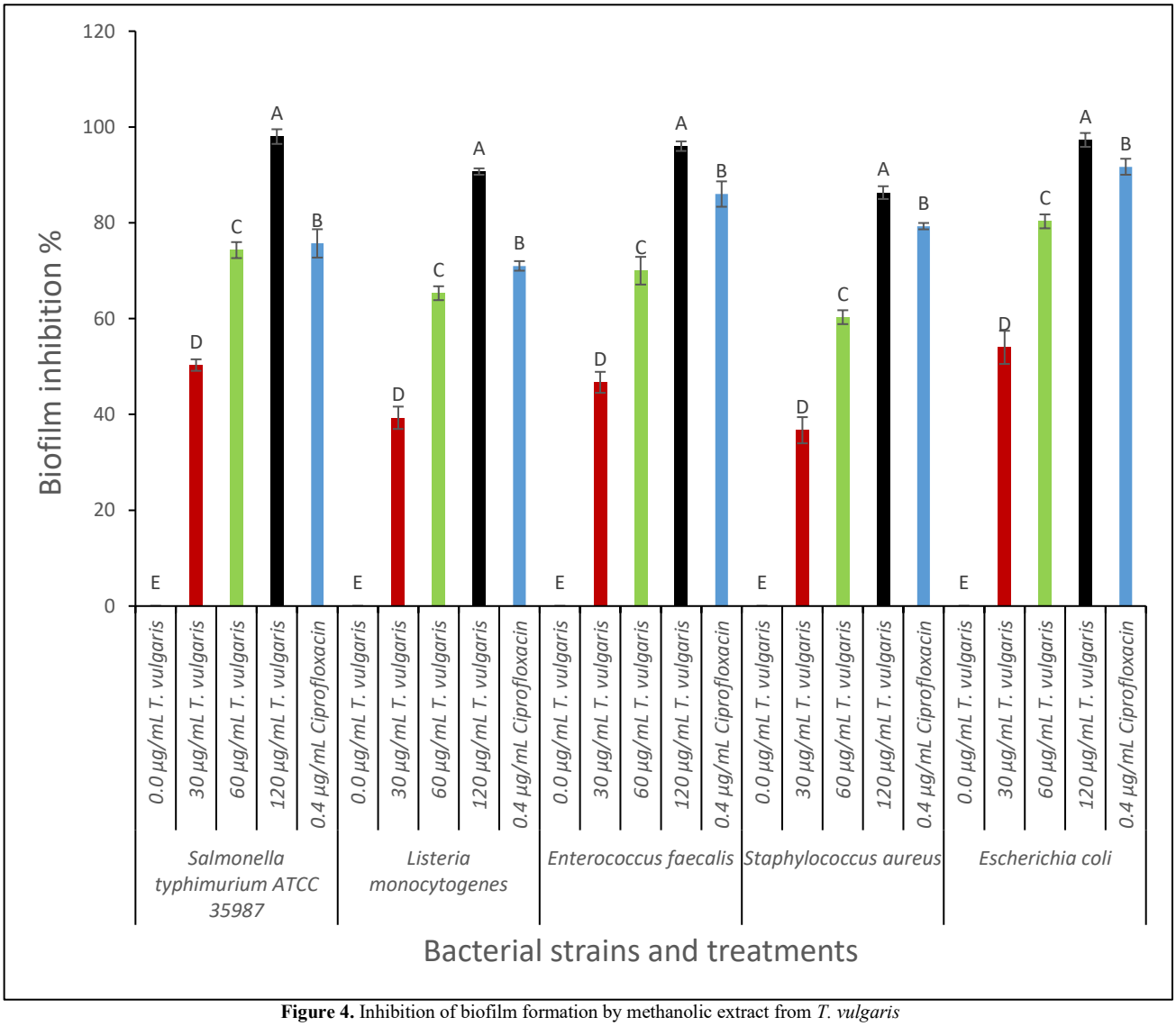

Figure 4. Inhibition of biofilm formation by methanolic extract from *T. vulgaris*

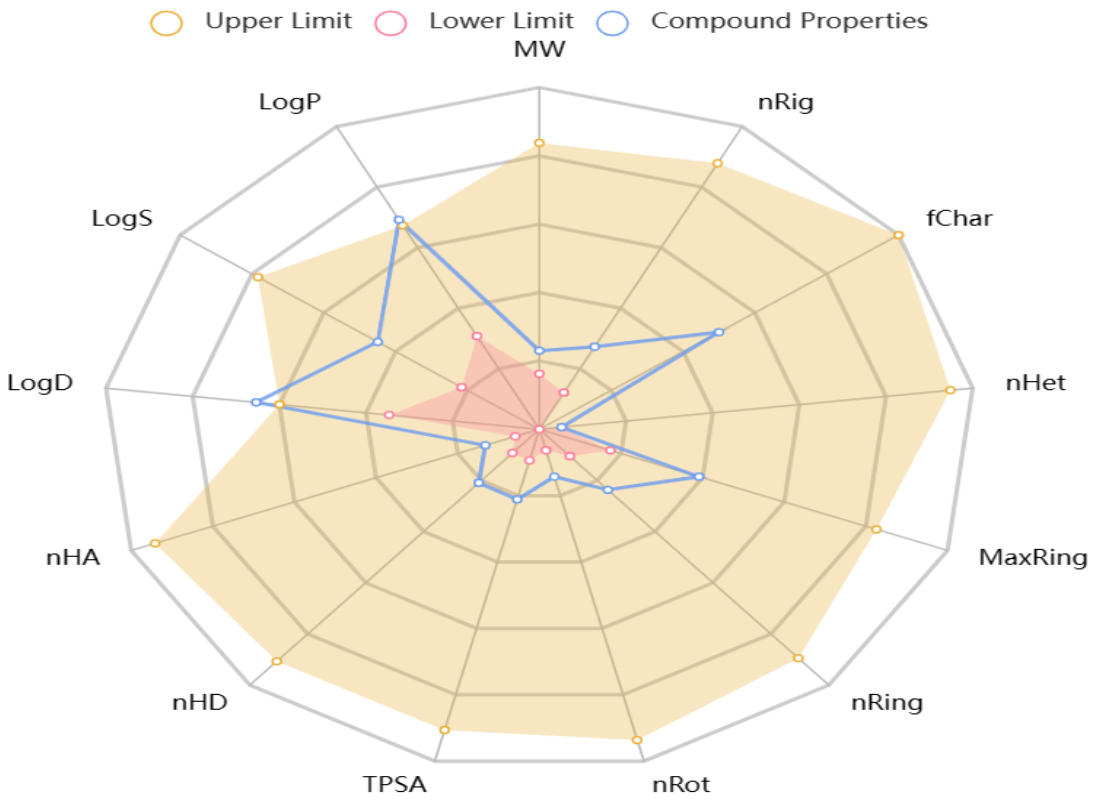

Figure 5. Radar graphic for the physiochemical characteristics of thymol predicated by ADMET

Conclusions

Phytochemical profiling of *Thymus vulgaris* leaf extract revealed a high abundance of bioactive secondary metabolites, particularly tannins, flavonoids, and phenolic compounds, with thymol identified as the major constituent through GC–MS analysis. Comprehensive *in vitro* evaluations demonstrated its broad therapeutic potential, including pronounced antioxidant, antidiabetic, anti-inflammatory, antibacterial, and antibiofilm activities, underscoring its versatility as a natural therapeutic candidate. Its strong free radical scavenging capacity suggests a protective role against oxidative stress–mediated cellular damage, which is central to aging and many chronic diseases. The inhibition of α-amylase and α-glucosidase highlights its potential as a natural antidiabetic agent for managing postprandial hyperglycemia, while its anti-inflammatory efficacy—evidenced by suppression of erythrocyte hemolysis—supports applications in inflammatory disorders. Importantly, the extract also showed potent antibacterial and antibiofilm effects, including activity against drug-resistant strains, suggesting value as a complementary or alternative therapy to conventional antibiotics. ADMET predictions further confirmed thymol's favorable pharmacokinetic and safety profiles, validating its promise as a lead compound for drug development. The anti-inflammatory effects of *T. vulgaris* are closely linked to its antioxidant capacity, which disrupts ROS-driven cascades, prevents lipid peroxidation, and interrupts the self-perpetuating cycle between oxidative stress and inflammation. Collectively, these findings position *T. vulgaris* as a multifunctional medicinal herb with broad-spectrum biomedical applications, offering promising opportunities for the development of safe, natural, and effective therapeutic agents.

Future Recommendations

Further in vivo and clinical studies are needed to validate the therapeutic efficacy and safety of *T. vulgaris* extract in humans. Detailed mechanistic studies should be carried out to better understand its molecular targets and pathways. Additionally, formulation development, dosage optimization, and toxicity profiling will be essential for its translation into clinical practice. Exploring synergistic effects with conventional drugs may also enhance its therapeutic potential against multidrug-resistant pathogens and chronic diseases.

Table 6. Computed ADMIT characteristics of thymol using ADMETlab 2.0

Properties			
Physicochemical		Medicinal Chemistry	
MW (molecular weight; optimal:100–600)	150.10	QED (> 0.67: excellent; ≤ 0.67: poor)	Excellent
nHA (H-bond acceptors; optimal:0–12)	1	Lipinski (MW≤ 500; log P ≤ 5; nHA ≤ 10; nHD ≤ 5)	Accepted
nHD (H-bond donors; optimal:0–7)	1	Pfizer (log P < 3; TPSA > 75)	Rejected
nRot (number of rotatable bonds; optimal:0–11)	1	GSK (MW≤400; logP≤4)	Accepted
nRing (number of rings; optimal:0–6)	1	Golden Triangle (200≤MW≤50; −2≤logD≤5)	Rejected
MaxRing (atoms number in the biggest ring:0–18)	6	Absorption	
nHet (number of heteroatoms; optimal:1–15)	1	Caco-2 permeability (>-5.15: excellent; otherwise: poor)	Excellent
fChar (formal charge; optimal: −4 to 4)	0	Pgp-inhibitor (0-0.3: excellent; 0.7-1.0(++): poor)	Excellent
nRig (number of rigid bonds; optimal:0–30)	6	Pgp-substrate (0-0.3: excellent; 0.7-1.0(++): poor)	Excellent
TPSA (topological polar surface area; optimal:0–140)	20.239	HIA (0-0.3: excellent; 0.7-1.0(++): poor)	Excellent
LogS (solubility; optimal; −4 to 0.5 log mol/L)	-2.147	Distribution	
LogP (distribution coefficient P; optimal: 0–3)	3.153	PPB (≤ 90%: excellent; otherwise: poor)	Poor
LogD7.4 (logP at physiological pH 7.4; optimal: 1~4)	3.427	VD (0.04-20: excellent; otherwise: poor)	Excellent
Metabolism		BBB Penetration (0-0.3: excellent; 0.7-1.0(++): poor)	Poor
CYP1A2 (Inhibitor)	Positive	0%: High Fu; 5-20%: medium Fu; <5% low Fu)	Moderate
CYP2C19 (Inhibitor)	Positive	Excretion	
CYP2C9 (Inhibitor)	Positive	CL (clearance)High ≥ 15; moderate 5-15; low <5 ml/min/kg	Moderate
CYP2D6 (Inhibitor)	Positive	Toxicity	
CYP3A4 (Inhibitor)	Positive	hERG Blockers	Inactive
Toxicophoric Rules		H-HT	Excellent
Acute Toxicity Rule	0 alerts	AMES Toxicity	Excellent
Genotoxic Carcinogenicity Rule	0 alerts	Rat oral acute toxicity	Moderate
Aquatic Toxicity Rule	0 alerts -	Carcinogenicity	Moderate

Authors' Contributions

M.A.F. G.M.E-S. & **M. H.K.** conceived the presented idea, co-wrote the paper, and supervised the research; **N.M.E.** & **N.E.E.** conceptualization, examination, and original draft writing; **M.S.O.** & **A.A.R.** conceptualization, investigation, original draft writing, supervision; **F.A.H.,** & **D.M.E.** conceptualization, examination, writing original draft preparation, and investigation; **M.A.F. G.M.E-S.** & **M. H.K.** conceptualization, examination, writing original draft preparation, and investigation. All authors have read and approved the version of the manuscript that has been published.

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Competing interests / COI statement

The author(s) did not report any potential conflict of interest.

References

[1] Elbestawy, M.K.M., El-Sherbiny, G.M. and Moghannem, S.A. 2023. Antibacterial, Antibiofilm and Anti-Inflammatory Activities of Eugenol Clove Essential Oil against Resistant Helicobacter pylori. *Molecules*, 28, 2448.

[2] Watson, K.B., Wiltz, J.L., Nhim, K., Kaufmann, R.B., Thomas, C.W. and Greenlund, K.J. 2025. Trends in Multiple Chronic Conditions Among US Adults, By Life Stage, Behavioral Risk Factor Surveillance System, 2013–2023. *Prev Chronic Dis*, 22, 240539.

[3] Wang, N., Hu, B., Zhao, Y., Kuang, G., Zhao, Y., Liu, Q., et al. 2021. Applications of System Dynamics Models in Chronic Disease Prevention: A Systematic Review. *Prev Chronic Dis*, 18, 210175.

[4] Shawky, M., Kalaba, M.H. and El-Sherbiny, M. 2025. Tackling carbapenem-resistant Acinetobacter baumannii (CRAB) and their virulence inside factors using biosynthesized silver nanoparticles combined with imipenem. *Biotechnol Notes*, 6, 183–195.

[5] Kalaba, M.H., El-Sherbiny, G.M., Ewais, G.M., Darwesh, O.M. and Moghannem, S.A. 2024. Green synthesis of zinc oxide nanoparticles (ZnO-NPs) by Streptomyces baarnensis and its active metabolite (Ka): a promising combination against multidrug-resistant ESKAPE pathogens and cytotoxicity. *BMC Microbiol*, 24, 254.

[6] El-Sherbiny, G.M., Farghal, E.E., Lila, name, Shetaia, Y.M., Mohamed, S.S. and Elswify, M.M. 2024. Antibiotic susceptibility and virulence factors of bacterial species among cancer patients. *Biotechnol Notes*, 5, 27–32.

[7] Bulbul, I.J., Hossain, M., Haque, M.R., et al. 2024. Two rare flavonoid glycosides from Litsea glutinosa (Lour.) C. B. Rob.: experimental and computational approaches endorse antidiabetic potentiality. *BMC Complement Med Ther*, 24, 69.

[8] El-Sherbiny, M.S. and Elbestawy, M.K.M. 2022. A review – plant essential oils active against Helicobacter pylori. *Journal of Herb Med*, 34, 203–215.

[9] M.E., S., El-Sherbiny, M., Sharaf, M.H., et al. 2024. Phytochemical analysis, bold antimicrobial, antioxidant, and cytotoxicity activities of Schinus molle (L.) extracts. *Biomass Conv. Bioref.*

[10] El-Sherbiny, G.M., Gazelly, A.M., Sharaf, in M.H., Moghannemm, S.A., Shehata, M.E., Ismail, M.K.A. and El-Hawary, S.S. 2023. Exploitation of the antibacterial, antibiofilm and antioxidant activities of Salvadora persica (A) (Miswak) extract. *Journal of Bioresources and Bioproducts*.

[11] Akbar, S. 2020. Handbook of 200 Medicinal Plants. *Springer Nature Switzerland AG*, 1795–1810.

[12] Pandiyan, I., Sri, S.D., Indiran, M.A., Rathinavelu, P.K., Prabakar, J. and Rajesh, S. 2022. Antioxidant, anti-inflammatory activity of Thymus vulgaris-mediated selenium nanoparticles: An in vitro study. *J Conserv Dent*, 25, 241–245.

[13] Hammoudi Halat, D., Krayem, M., Khaled, S. and Younes, S. 2022. A Focused Insight into Thyme: Biological, Chemical, and Therapeutic Properties of an Indigenous Mediterranean Herb. *Nutrients*, 14, 2104.

[14] Kuete, V. 2017. Thymus vulgaris, Therapeutic Potential Against Metabolic, Inflammatory, Infectious and Systemic in Diseases. *Medicinal Spices and Vegetables from Africa*, 599–609.

[15] Nadi, A., Shiravi, A.A., Mohammadi, Z., Aslani, A. and Zeinalian, M. 2023. Thymus vulgaris, a natural pharmacy against COVID-19: A molecular review. *J Herb Med*, 38, 100635.

[16] Mokhtari, R., Fard, M.K., Rezaei, M., Moftakharzadeh, S.A. and Mohseni, A. 2023. Placeholders Antioxidant, Antimicrobial Activities, and Characterisation of Phenolic Compounds of Thyme (Thymus vulgaris L.), Sage (Salvia officinalis L.), and Thyme–Sage Mixture Extracts. *J. Food Qual.*, 9, 2602454.

[17] Pluhár, Z., Szabó, D. and Sárosi, S. 2016. Effects of different factors influencing the essential oil properties of Thymus vulgaris L. *PST*, 3, 312.

[18] Ács, K., Balázs, V.L., Kocsis, B., Bencsik, T., Böszörményi, A. and Horváth, G. 2018. Antibacterial activity evaluation of selected essential oils in liquid and vapor phase on respiratory tract pathogens. *BMC Complement Med Ther*, 18, 227.

[19] Bakó, C., Balázs, V.L., Kerekes, E., et al. 2023. Flowering phenophases influence the antibacterial and anti-biofilm effects of Thymus vulgaris L. essential oil. BMC Complement Med Ther, 23, 168.

[20] Pruteanu, A., Popescu, C., Vladut, V. and Gageanu, G. 2018. Biochemical analysis of some vegetal extracts obtained from indigenous spontaneous species of Thymus serpyllum L. *Romanian Biotechnological Letters*, 23, 14013.

[21] Pedonese, F., Fratini, F., Pistelli, L., Porta, F.M., DiCiccio, P., Fischetti, R., Turch, B. and Nuvoloni, R. 2017. Antimicrobial activity of four essential oils against pigmenting Pseudomonas fluorescens and biofilm producing Staphylococcus aureus of dairy origin. *Ital. J. Food Saf.*, 6, 6939.

[22] Bnouham, M., et al. 2006. Medicinal plants with potential antidiabetic activity-A review of ten years of herbal medicine research (1990-2000). *Int J Diabetes Metabol*, 14, 1–7.

[23] Koski, R.R. 2006. Practical review of oral antihyperglycemic agents for type 2 diabetes mellitus. *J. Food Qual.*, 32, 869–877.

[24] Kifle, Z.D., Abdiwahab, M., Melak, A.M., Genet, G.M., Meseret, T. and Adugna, M. 2022. Pharmacological evaluation of medicinal plants with antidiabetic activities in Ethiopia: A review. *Metabolism. Open*, 13, 100174.

[25] Dauqan, E.M. and Abdullah, A. 2017. Medicinal and functional values of thyme (Thymus vulgaris L.) herb. *Journal of Applied Biology & Biotechnology*, 5, 17–22.

[26] Hammoudi Halat, D., Krayem, M., Khaled, S. and Younes, S. 2022. A focused insight into thyme: Biological, chemical, and therapeutic properties of an indigenous Mediterranean herb. *Nutrients*, 14, 210.

[27] Patil, S.M., Ramu, R., Shirahatti, P.S., Shivamallu, C. and Amachawadi, R.G. 2021. A systematic review on ethnopharmacology, phytochemistry and pharmacological aspects of Thymus vulgaris Linn. *Heliyon*, 7, e07054.

[28] Rahaman, M.M., Rakib, A., Mitra, S., Tareq, A.M., Emran, T.B., Shahid-Ud-Daula, A.F.M., et al. 2020. The genus curcuma and inflammation: overview of the pharmacological perspectives. *Plants*, 10, 63.

[29] Shamsuddin, T., Hosen, M.A., Alam, M.S., Emran, T.B. and Kawsar, S.M.A. 2021. Uridine derivatives: Antifungal, PASS outcomes, ADME/T, drug-likeliness, molecular docking and binding energy calculations. *Med. Sci. Int. Med. J*, 10, 1373–1386.

[30] Shovo, M.A.R.B., Tona, M.R., Mouah, J., Islam, F., Chowdhury, M.H.U., Das, T., et al. 2021. Computational and pharmacological studies on the antioxidant, thrombolytic, anti-inflammatory, and analgesic activity of molineria capitulata. *Current Issues in Molecular Biology*, 43, 434–456.

[31] Siddhuraju, P. and Manian. 2007. The antioxidant activity and free radical scavenging capacity of dietary phenolic extracts from horse gram (Macrotyloma uniflorum (Lam.) Verdc.) seeds. *Food Chem.*, 105, 950–958.

[32] Siddhuraju, P. and Becker, K. 2003. Studies on antioxidant activities of mucuna seed (Mucuna pruriens var. utilis) extracts and certain non-protein amino/imino acids through in vitro models. *J. Sci. Food Agric*., 83, 1517–1524.

[33] Zhishen, J., Mengecheng, T. and Jianming, W. 1999. The determination of flavonoid contents on mulberry and their scavenging effects on superoxide radical. *Food Chem*., 64, 555–559.

[34] Re, R., Pellegrini, N., Proteggente, A., et al. 2000. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.*, 26, 1231–1237.

[35] Saravanan, S. and Parimelazhagan, T. 2014. In vitro antioxidant, antimicrobial and anti-diabetic properties of polyphenols of Passiflora ligularis Juss. fruit pulp. *Food Science and Human Wellness*, 3, 56–64.

[36] Apostolidis, E. and Lee, C. 2010. In vitro potential of Ascophyllum nodosum phenolic antioxidant-mediated α-glucosidase and α-amylase inhibition. *J. Food Sci.*, 75, H97–H102.

[37] Foda, A.M., Kalaba, M.H., El-Sherbiny, G.M., Saad, A.M. and El-Fakharany, E.M. 2022. Antibacterial activity of essential oils for combating colistin-resistant bacteria. *Expert Review of Anti-infective Therapy*.

[38] Sharaf, M.H., El-Sherbiny, G.M., Moghannem, S.A., et al. 2021. New combination approaches to combat methicillin-resistant Staphylococcus aureus (MRSA). *Sci Rep.*, 11, 1–16.

[39] CLSI. 2021. Performance Standards for Antimicrobial Susceptibility Testing. 31st ed. CLSI supplement M100. *Clinical and Laboratory Standards Institute*.

[40] Mohsenipour, Z. and Hassanshahian, M. 2015. The inhibitory effect of Thymus vulgaris extracts on the planktonic form and biofilm structures of six human pathogenic bacteria. *Avicenna J Phytomed.*, 5, 309–318.

[41] Xiong, G., Wu, Z., Yi, J., Fu, L., Yang, Z., Hsieh, C., Yin, M., Zeng, X., Wu, C., Lu, A., Chen, X., Hou, T. and Cao, D. 2021. ADMETlab 2.0: an integrated online platform for accurate and comprehensive predictions of ADMET properties. *Nucleic Acids Res.*, 49, W5–W14.

[42] Afonso, A.F., Pereira, O.R. and Cardoso, S.M. 2020. Health-Promoting Effects of Thymus Phenolic-Rich Extracts: Antioxidant, Anti-inflammatory and Antitumoral Properties. *Antioxidants*, 9, 814.

[43] Hossain, M.A., AL-Raqmi, K.A.S., AL-Mijizy, Z.H., Weli, A.M. and Qasim Al-Riyami, Q. 2013. Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown Thymus vulgaris. *Asian Pac J Trop Biomed*, 3, 705–710.

[44] Nieto, G., Bannon, S., Garrido, M.D., et al. 2011. Effect of supplementing ewes' diet with thyme (Thymus zygis ssp. gracilis) leaves on the lipid oxidation of cooked lamb meat. *Food Chem*., 125, 1147–1152.

[45] Roby, M.H.H., Sarhan, M.A., Selim, K.A.H. and Khalel, K.I. 2013. Evaluation of antioxidant activity, total phenols and phenolic compounds in thyme (Thymus vulgaris L.), sage (Salvia officinalis L.), and marjoram (Origanum majorana L.) extracts. *Ind. Crop. Prod*., 43, 827–831.

[46] Köksal, E., Bursal, E., Gülçin, İ., Korkmaz, M., Çağlayan, C., Gören, A.C. and Alwasel, S.H. 2016. Antioxidant activity and polyphenol content of Turkish thyme (Thymus vulgaris) monitored by liquid chromatography and tandem mass spectrometry. *Int. J. Food Prop*., 20, 514–525.

[47] Sarfaraz, D., Rahimmalek, M. and Saeidi, G. 2021. Polyphenolic and molecular variation in Thymus species using HPLC and SRAP analyses. *Sci. Rep*., 11, 5019.

[48] Saleem, A., Afzal, M., Naveed, M., Makhdoom, S.I., Mazhar, M., Aziz, T., Khan, A.A., Kamal, Z., Shahzad, M., Alharbi, M., et al. 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, 8512.

[49] Bnouham, M., et al. 2006. Medicinal plants with potential antidiabetic activity-A review of ten years of herbal medicine research (1990-2000). *Int J Diabetes Metabol*, 14, 1–7.

[50] Lemos, M.F., Lemos, M.F., Pacheco, H.P., Guimarães, A.C., Fronza, M., Endringer, D.C. and Scherer, R. 2017. Seasonal variation affects the composition and antibacterial and antioxidant activities of Thymus vulgaris. *Industrial Crops and Products*, 95, 543–548.

[51] Borugă, O., Jianu, C., Mişcă, C., Goleţ, I., Gruia, A.T. and Horhat, F.G. 2014. T. vulgaris essential oil: chemical composition and antimicrobial activity. *J Med Life*, 7, 56–60.

[52] Prinsloo, G. and Nogemane, N. 2018. The effects of season and water availability on chemical composition, secondary metabolites, and biological activity in plants. *Phytochem. Rev*., 17, 889–902.

[53] Brahmkshatriya, P.P. and Brahmkshatriya, P.S. 2013. Terpenes: Chemistry, Biological Role, and Therapeutic Applications. In: Ramawat, K., Mérillon, J.M. (eds) Natural Products. *Springer*, Berlin, Heidelberg.

[54] Shehata, M.E., El-Sherbiny, G.M., Sharaf, M.H., et al. 2024. Phytochemical analysis, antimicrobial, antioxidant, and cytotoxicity activities of Schinus molle (L.) extracts. *Biomass Conv. Bioref.*

[55] Iyer, D. and Patil, U.K. 2012. Efficacy of Stigmast–5–en–3β–ol Isolated from Salvadora persica L. as Antihyperlipidemic and Antitumor agent: Evidence from animal studies. *Asian Pacific Journal of Tropical Disease,* 2, S849–S855.

[56] Taek Han, Y., Jung, J.W. and Kim, N.J. 2017. Recent advances in the synthesis of biologically active cinnoline, phthalazine and quinoxaline derivatives. *Curr Org Chem*, 21, 1265–1291.

[57] Afonso, A.F., Pereira, O.R. and Cardoso, S.M. 2020. Health-Promoting Effects of Thymus Phenolic-Rich Extracts:

Antioxidant, Anti-inflammatory and Antitumoral Properties. *Antioxidants*, 9, 814.

[58] Pandiyan, I., Sri, S.D., Indiran, M.A., Rathinavelu, P.K., Prabakar, J. and Rajeshkumar, S. 2022. Antioxidant, anti-inflammatory activity of Thymus vulgaris-mediated selenium nanoparticles: An in vitro study. *J Conserv Dent*, 25, 241–245.

[59] Kannat, S.R., Chander, R. and Sharma, A. 2007. Antioxidant potential of mint (Mentha spicata L.) in radiation-processed lamb meat. *Food Chem*., 600, 451–458.

[60] Rao, K.S., Chaudhury, P.K. and Pradhan, A. 2010. Evaluation of antioxidant activities and total phenolic content of Chromolaena odorata. *Food. Chem. Toxicol*., 48, 729–732.

[61] Brahmi, N., Scognamiglio, M., Pacifico, S., Mekhoukhe, A., Madani, K., Fiorentino, A. and Monaco, P. 2015. 1H NMR based metabolic profiling of eleven Algerian aromatic plants and evaluation of their antioxidant and cytotoxic properties. *Food Res. Int*., 76, 334–341.

[62] Kozics, K., Klusová, V., Srančíková, A., Mučaji, P., Slameňová, D., Hunáková, L', Kusznierewicz, B. and Horváthová, E. 2013. Effects of Salvia officinalis and Thymus vulgaris on oxidant-induced DNA damage and antioxidant status in HepG2 cells. *Food Chem*., 141, 2198–2206.

[63] Patil, S.M., Ramu, R., Shirahatti, P.S., Shivamallu, C. and Amachawadi, R.G. 2021. A systematic review on ethnopharmacology, phytochemistry and pharmacological aspects of Thymus vulgaris Linn. *Heliyon*, 7, e07054.

[64] Elbestawy, M.K.M., El-Sherbiny, G.M., Moghannem, S.A. and Farghal, E.E. 2023. Antibacterial, Antibiofilm, and Anti-Inflammatory Activities of Ginger Extract against Helicobacter pylori. *Microbiology Research*, 14, 1124–1138.

[65] Lopez-Lazaro, M. 2009. Distribution and Biological Activities of the Flavonoid Luteolin. *Mini-Reviews Med. Chem*., 9, 31–59.

[66] Leyva-Lopez, N., Gutierrez-Grijalva, E.P., Ambriz-Perez, D.L. and Basilio Heredia, J. 2016. Flavonoids as cytokine modulators: A possible therapy for inflammation-related diseases. *Int. J. Mol. Sci*., 17, 921.

[67] Debnath, T., Kim, D.H. and Lim, B.O. 2013. Natural products as a source of anti-inflammatory agents associated with inflammatory bowel disease. *Molecules*, 18, 7253–7270.

[68] Hmidani, A., Dine, E., Bouhlali, T., Khouya, T. and Ramchoun, M. 2019. Antioxidant, anti-inflammatory and anticoagulant activities of three Thymus species grown in southeastern Morocco. *Futur. J. Pharm. Sci*., 5.

[69] Rahman, J., Tareq, A.M., Hossain, M.M., Sakib, S.A., Islam, M.N., Ali, M.H., et al. 2020. Biological evaluation, DFT calculations and molecular docking studies on the antidepressant and cytotoxicity activities of Cycas pectinata Buch.-Ham. compounds. *Pharmaceuticals*, 13, 232.

[70] Al Mahmud, Z., Emran, T.B., Qais, N., Bachar, S.C., Sarker, M. and Nasir Uddin, M.M. 2016. Evaluation of analgesic, anti-inflammatory, thrombolytic and hepatoprotective activities of roots of Premna esculenta (Roxb). *Journal of basic and clinical physiology and pharmacology*, 27, 63–70.

[71] Yesmin, S., Paul, A., Naz, T., Rahman, A.A., Akhter, S.F., Wahed, M.I.I., et al. 2020. Membrane stabilization as a mechanism of the anti-inflammatory activity of ethanolic root extract of Choi (Piper chaba). Clinical Phytoscience, 6, 1–10.

[72] Tarling, C.A., Woods, K., Zhang, R., et al. 2008. The search for novel human pancreatic α-amylase inhibitors: high-throughput screening of terrestrial and marine natural product extracts. *Chem. Biol. Chem*., 9, 433–438.

[73] Dessalegn, F., Bultosa, G., Haki, G.D. and Vasantha Rupasinghe, H.P. 2019. Evaluation of In vitro Antidiabetic Potential of Thymus schimperi R. and Thymus vulgaris L. Journal of Health, Medicine and Nursing, 69, 9–16.

[74] Shobana, S., Sreerama, Y.N. and Malleshi, N. 2009. Composition and enzyme inhibitory properties of finger millet (Eleusine coracana L.) seed coat phenolics: Mode of inhibition of α-glucosidase and pancreatic amylase. *Food Chem*., 115, 1268–1273.

[75] Chen, L. and Kang, Y.H. 2014. In vitro inhibitory potential against key enzymes for hyperglycemia and hypertension of red pepper (Capsicum annuum L.) including pericarp placenta and stalk. Journal of Food. *Biochemistry*, 38, 300–306.

[76] Emran, T.B., Nasir Uddin, M.M., Rahman, A., Uddin, Z. and Islam, M. 2015. Phytochemical, antimicrobial, cytotoxic, analgesic and anti-inflammatory properties of Azadirachta indica: A therapeutic study. *J Bioanal Biomed S*, 12, 2.

[77] Al-Juraifani, A.A. 2011. Antimicrobial activity of some medicinal plants used in Saudi Arabia. Can. J. Pure App. Sci., 5, 1509–1512.

[78] Alkufeidy, R.M., Al Farraj, D.A., Aljowai, R.M., Ali, M.A. and Elshikh, M.S. 2022. Chemical composition of Thymus vulgaris extracts and antibacterial activity against pathogenic multidrug resistance bacteria. *Physiological and Molecular Plant Pathology*, 117, 10174.

[79] Parzhanova, A., Yanakieva, V., Vasileva, I., Momchilova, M., Dimitrov, D., Ivanova, P. and Tumbarski, Y. 2023. Physicochemical, Antioxidant, and Antimicrobial Properties of Three Medicinal Plants from the Western Part of the Rhodope Mountains, Bulgaria. *Life*, 13, 2237.

[80] Yassin, M.T., Mostafa, A.A.F., Al-Askar, A.A. and Sayed, S.R.M. 2022. In vitro antimicrobial activity of Thymus vulgaris extracts against some nosocomial and food poisoning bacterial strains. *Process Biochemistry*, 115, 152–159.

[81] Yuan, Z., Dai, Y., Ouyang, P., Rehman, T., Hussain, S., Zhang, T., Yin, Z., Fu, H., Lin, J., He, C., et al. 2020. Thymol Inhibits Biofilm Formation, Eliminates Pre-Existing Biofilms, and Enhances Clearance of Methicillin-Resistant Staphylococcus aureus (MRSA) in a Mouse Peritoneal Implant Infection Model. *Microorganisms*, 8, 99.

[82] Joo, H.S. and Otto, M. 2012. Molecular basis of in vivo biofilm formation by bacterial pathogens. *Chem. Biol*., 19, 1503–1513.

[83] Figueiredo, A.M.S., Ferreira, F.A., Beltrame, C.O. and Côrtes, M.F. 2017. The role of biofilms in persistent infections and factors involved in ica-independent biofilm development and gene regulation in Staphylococcus aureus. Crit. Rev. *Microbiol*., 43, 602–620.

[84] Carezzano, M.E., Paletti Rovey, M.F., Sotelo, J.P., Giordano, M., Bogino, P., Oliva, MdlM. and Giordano, W. 2023. Inhibitory Potential of Thymus vulgaris Essential Oil against Growth, Biofilm Formation, Swarming, and Swimming in Pseudomonas syringae Isolates. *Processes*, 11, 933.

[85] Obakiro, S.B., Kiprop, A., K'owino, I., Andima, M., Owor, R.O., Chacha, R. and Kigondu, E. 2022. Phytochemical, Cytotoxicity, and Antimycobacterial Activity Evaluation of Extracts and Compounds from the Stem Bark of Albizia coriaria Welw ex. Oliver. *Evid Based Complement Alternat Med*., 2022, 7148511.

[86] Krüger, A., Gonçalves Maltarollo, V., Wrenger, C. and Kronenberger, T. 2020. ADME Profiling in Drug Discovery and a New Path Paved on Silica. *IntechOpen*.

[87] Md Idris, M.H., Mohd Amin, S.N., Mohd Amin, S.N., Wibowo, A., Zakaria, Z.A., Shaameri, Z., Hamzah, A.S., Selvaraj, M., Teh, L.K. and Salleh, M.Z. 2022. Discovery of polymethoxy flavones as potential cyclooxygenase-2 (COX-2), 5-lipoxygenase (5-LOX) and phosphodiesterase 4B (PDE4B) inhibitors. *J Recept Signal Transduct Res.*, 42, 325–337.

[88] Durán-Iturbide, N.A., Díaz-Eufracio, B.I. and Medina-Franco, J.L. 2020. In Silico ADME/Tox Profiling of Natural Products: A Focus on BIOFACQUIM. *ACS Omega*, 5, 16076–16084.

[89] El-Sherbiny, G.M., Alluqmani, A.J., Elsehemy, I.A., et al. 2024. Antibacterial, antioxidant, cytotoxicity, and phytochemical screening of Moringa oleifera leaves. *Sci Rep*, 14, 30485.

[90] Adem, Ş., Yırtıcı, Ü., Aydın, M., Rawat, R. and Eyüpoğlu, V. 2024. Natural flavonoids as promising 6-phosphogluconate dehydrogenase inhibitor candidates: In silico and in vitro assessments. *Arch Pharm (Weinheim),* 357, e2300326.

[91] Nagoor Meeran, M.F., Javed, H., Al Taee, H., Azimullah, S. and Ojha, S.K. 2017. Pharmacological Properties and Molecular Mechanisms of Thymol: Prospects for Its Therapeutic Potential and Pharmaceutical Development. *Front Pharmacol*., 8, 380.