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Variations of the chemical components and biological activities of *Thymus capitatus* essential oil from three regions in Palestine

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Thymus capitatus **is a widely utilized medicinal plant in Palestine. The main goal of this study was to assess the phytochemical content of *T. capitatus* essential oils (EOs) from three Palestinian regions using hydro-distillation. Furthermore, the EO extracted from the plant was subjected to biological tests. GC-MS spectrometry was used to identify and quantify the elements in the EOs examined. The DPPH assay and the β-carotene-linoleic acid assay were utilized to determine the levels of antioxidant activity. The plant's anti-lipase activity was carried out using a pancreatic lipase inhibition assay. α-amylase inhibitory activity of the EOs samples was studied compared with the hypoglycemic drug, Acarbose. An antimicrobial assay was conducted against seven common bacteria and fungi types. Additionally, Hep-G2 cells were used to assess the anticancer activity. The EO components were mainly monoterpenes, thymol, and carvacrol. Chemical components of the EOs varied between districts (Ramallah: carvacrol (31.25%), γ-terpinene (30.94%), Jenin: γ-terpinene (67%), cis-b-terpineol (12.91%), Hebron: thymol (40.35%), b-Caryophyllene (13.23%) were the main components of the EOs in the districts. The antioxidant activity of *T. capitatus* EOs was shown to be dose-dependent. The results showed that the three districts had nearly the same IC50, a fourth-fold of gallic acid. The Hebron sample of *T. capitatus* EO showed antibacterial activity with MIC values between 0.1953 and 1.5625 µg/mL. All samples showed anti-lipase activity higher than Orlistat at concentrations equal to or above 200 µg/ml. Furthermore, all three EO samples inhibited α-amylase concentration-dependently. All samples showed promising cytotoxicity results against Hep-G2, with an average percent inhibition of 85% at a concentration of 62.5 µg/mL. The chemical composition of the EO of *T. capitatus* is related to the plant's origin, soil components, genetic variables, and climatic conditions, which in turn reflect the plant's biological activity.**

Keywords *Thymus capitatus*, Anti-lipase, Antioxidant, Antimicrobial, Cytotoxicity, Antiamylase

Medicinal herbs have been seen as a source of therapeutic aids in healthcare systems worldwide[1,2]. Their therapeutic effect is related to their antioxidant, anti-aging, anti-cancer, anti-atherosclerotic, antibacterial, and anti-inflammatory activities. Several studies have been undertaken to find natural compounds extracted from herbs that have the potential to cure a variety of health conditions. Among these herbs are *thyme*, *rosemary*, and *oregano*, mainly used for their antioxidant properties[3–5].

Thymus capitatus (T.capitatus) is known in Palestine as "Zetmane." It's a common type of thyme with various biological effects, including antimicrobial and antioxidant activities[6,7]. *T.capitatus* grows in different regions in Palestine under variable environmental conditions. Since ancient times, people have turned to *T. capitatus* for various medical purposes, including anthelmintic, carminative, antispasmodic, expectorant, sedative, stimulant, tonic, anti-inflammatory, and analgesic. *T. capitatus* EO has been certified for use due to its pharmacological effect, which includes antioxidant and antibacterial properties[8,9]. Moreover, this herb was used in the past to flavor meats, stews, and soups and was also used as raw material without any preliminary preparation[10,11].

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EOs of *Thymus capitatus* are mainly of two chemical groups: terpenoids (sesquiterpenes of low molecular weight and monoterpenes) and less commonly phenylpropanoids[12–14].

T. capitatus belongs to the Lamiaceae family and is native to the eastern Mediterranean region. It is a perennial shrub with fragrant flowers and leaves that grows in many places in the mountains of Palestine.

The antimicrobial activity of *T. capitatus* EO has been tested against a wide range of bacteria and fungi, including gram-negative bacteria as *Escherchia coli* and *Klebsiella pneumoniae*, gram-positive bacteria as *Salmonella analum and Listeria monocytogenes*, fungi as *Mucorramamnianus* and *Aspergillus ochraceus*, and yeast species like *Saccharomyces cerevisiae* and *Candida albicans*. The results showed that *E. coli*, *L. monocytogenes*, *and K. pneumonia* bacteria were suppressed by the tested *T. capitatus* EOs. Furthermore, considerable efficacy against fungi and yeasts was observed[15]. The radical cation 2,20-azinobis(3-ethylbenzothiazoline-6-sulfonate) and the free radical 2,2-diphenyl-1-picrylhydrazyl were examined in antioxidant activity experiments of *T. capitatus* EO, and the findings revealed potent inhibitory concentration values[16,17]. Tepe et al. researched the in vitro antioxidant properties of EOs of two Thymus species extracts and presented their findings. About 71 compounds were identified and characterized by high monoterpene, especially phenolic carvacrol, thymol, *p*-cymene, and γ-terpinene. The oils were tested for their possible antioxidant activity by using (DPPH), and β-carotene/linoleic acid assays, and both of them showed high antioxidant activity in different proportions[16–19].

Obesity, diabetes, and cancer remain significant global health challenges due to their prevalence, complex underlying mechanisms, and limited effectiveness of current treatments. The enzymatic inhibition of lipase has shown promise in managing obesity by reducing the absorption of dietary fats through inhibition of fat digestion in the small intestine[20,21]. Similarly, the inhibition of pancreatic α-amylase has emerged as a strategy for diabetes management, as it delays carbohydrate digestion, reduces the glucose absorption rate, and mitigates postprandial plasma glucose spikes[22]. Medicinal plants, such as Thymus species, have garnered interest for their ability to inhibit α-amylase due to their bioactive chemical constituents, offering potential antidiabetic effects[23–25].

Thymus essential oils (EOs) have been extensively studied for their anticancer activities, attributed to key components such as carvacrol, thymol, terpinene, and p-cymene[26,27].

In this research, *T. capitatus* dried aerial parts from three regions in Palestine were extracted using hydrodistillation to evaluate the efficiency of this extraction method. Gas chromatography-mass spectroscopy was employed to characterize the chemical components of *T. capitatus* EOs. This study explores the potential of *T. capitatus* as a source of bioactive phytochemicals with therapeutic properties, including antioxidant, antimicrobial, anti-obesity, antidiabetic, and anticancer activities. Lastly, the essential oils (EO) from three regions were analyzed to assess how variations in chemical composition influence biological activities. The study also explored the effects of environmental conditions on the chemical composition of T. capitatus post-extraction and its impact on EO activity.

Materials and methods

Materials and chemicals

The present study employed a variety of chemical reagents sourced from reputable manufacturers to evaluate pharmacological activities. Dimethyl sulfoxide (DMSO) from Riedel-de Haën, Germany, and high-purity methanol from Loba Chemie, India, were utilized as solvents. Pharmacological assays involved compounds such as 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), p-nitrophenyl butyrate (PNPB), and orlistat obtained from Sigma-Aldrich, USA, along with 2,2-Diphenyl-1-picrylhydrazyl (DPPH) from Sigma-Aldrich, Denmark, and α-amylase from Sigma-Aldrich, India. Porcine pancreatic lipase, type II, acarbose, and 3,5-Dinitrosalicylic acid (DNSA) were procured from Sigma-Aldrich, USA, facilitating specific enzymatic assays. Additionally, chloroform and Tween 40 from Loba Chemie, India, linoleic acid, and β-carotene from Sigma-Aldrich, USA, and potassium phosphate from Sigma-Aldrich, USA, were utilized for various experimental procedures. Microbiological assessments utilized analytical grade RPMI 1640 culture medium and essential supplements like glutamine, trypsin, amphotericin B, fetal calf serum, Hank's balanced solution, Trypan blue solution, penicillin, and gentamicin. A diverse range of microbial strains, including *Klebsiella pneumoniae* (ATCC 13883), *Proteus vulgaris* (ATCC 8427), *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 9027), *MRSA* (Clinical sample) and *Candida albicans* were employed for evaluating antimicrobial activities.

Instrumentation

The following instruments were utilized in this research: An oven (Ari Levy, Inc, Israel) for controlled heating processes, a precision balance (AS 220/C/2, Radwag, Poland) for accurate mass measurements, a micropipette (MRC, Ltd., Israel) for precise liquid handling, a grinder (Uno Moulinex, China) for sample preparation, micropipettes (Macherey-Nagel, USA) for varied volumes, a vortex mixer (Heidolph, Germany) for efficient mixing, a microplate reader (Unilab, USA) for measurements, a water bath (BPXOP1001040, Lab Tech, South Korea) for controlled temperature applications, and a gas chromatography-mass spectrometry (GC-MS) system (Perkin Elmer, UK) for chemical analysis.

Collection of plant materials

T. capitatus aerial parts were collected in March (2021) from three cities that resembled three regions of Palestine: Jenin (north), Ramallah (middle), and Hebron (south). All necessary permissions and licenses for collecting *T. capitatus* have been obtained from the relevant authorities, including An-Najah National. Our collection follows all legal and ethical guidelines. Professor Nidal Jaradat, a pharmacognosist, recognized the plant samples in the Natural Products Laboratory, Faculty of Pharmacy at An-Najah National University. A voucher specimen was deposited in the abovementioned laboratory under the code (Pharm-PCT-2813).

Areal parts of the plant were carefully separated, washed twice with distilled water, dried for 15 days in the shade, grounded well, and stored in cloth bags until the extraction process began.

Extraction process from *T. capitatus* aerial parts

Hydro-distillation methods

Three T. capitatus aerial parts samples were dried and powdered. The extraction method for the EOs of this plant was utilized by hydro-distillation, which was used by Jaradat and colleagues[28]. The essential oil (EO) was extracted using a Clevenger apparatus at atmospheric pressure for 180 min, maintaining a temperature of 100 °C and a hydro-distillation rate of 0.54 mL/min. This process involved suspending 100 g of dry powder in 1 L of distilled water. After extraction, the EO was chemically dried with calcium carbonate and stored in the refrigerator at 4 °C until it was needed. The following formula was used to compute the yield of each sample.

$$\text{Yield percent } = (\text{extract weight}/\text{dry weight of } T.\ capitatus) * 100$$

Chemical composition identification by gas chromatography/mass spectrometry

The chemical composition of the three EO samples examined was determined using the GC-MS method. Shimadzu QP-5000 GC-MS with Rtx-5ms column (30 m long, 0.25 m thickness, 0.250 mm inner diameter) was used to record GC-MS chromatograms. Helium was used as the carrier gas at a 1 mL/min flow rate. 220 °C was the injector temperature. The oven temperature was programmed to rise from 50 °C (1 min hold) to 130 °C at 5 °C/min, then to 250 °C at 10 °C/min, and kept isothermally for 15 min. The temperature on the transfer line was 290 °C. An electron ionization system with detector voltages of 1.7 KV was employed for GC-MS detection. The mass range was 38-450 M/Z, with a scan rate of 0.5 s and a scan speed of 1000 amu/Sect[29].

NIST mass spectrometry data center and literature references were used to compare MS retention times and Kovats indices. This allowed us to determine which chemical constituents were present in the EOs. electronically produced quantitative data from integrated peaks[30].

Antioxidant assay for samples of EOs from *T. capitatus*

DPPH assay

The methodology for antioxidant analysis followed the same approach as in our previous studies[31,32]; Methanol, a 100 µg/mL stock solution for the three *T. capitatus* EO samples, was produced. In addition, a Trolox and gallic acid solution of 100 µg/mL was produced (the reference standard). Each sample's serial dilutions were made from the stock solutions, yielding (5,10,20,30,40,50,80, and 100 µg/mL). One milliliter of each sample dilution was combined with one milliliter of 0.002 g/mL DPPH in methanol. To make a final working volume of 3 mL, 1 mL of methanol was added. The DPPH solutions were made fresh because they are light-sensitive. A blank controlled with DPPH in methanol at a 1:2 ratio was also made without adding an extract. For around 30 min, all working solutions were incubated at room temperature (25^0 C) in the dark. A spectrophotometer was used to detect optical densities at a wavelength of 517 nm. The following equation was used to compute the percent DPPH inhibition for three samples of EO from *T. capitatus.*

with trolox or gallic acid as the standard compound:

$$\text{DPPH inhibition } \% = (\text{ABl - Ats})/\text{ABl} \times 100$$

ABl: the absorbance measured for the blank solution, Ats: The absorbance was measured for the tested sample of *T. capitatus* solution.

Assay of β carotene-linoleic acid

The antioxidant activity is measured by the β-carotene-linoleic acid system model for the three EO samples from *T. capitatus* by Miller[33]. Based on the oxidative breakdown products of the linoleic acid, this assay measures the oxidation of β-carotene-linoleic acid. 1 mg of carotene was dissolved in 2mL chloroform with 20 mg of linoleic acid and 200 mg of Tween 40. Then, the chloroform was evaporated entirely with a rotary evaporator at a low temperature and lower pressure. Then, we added 200mL of distilled water saturated with oxygen, shaking vigorously for 30 min. (0.1 mL) of the EO samples from *T. capitatus* aerial parts and the positive control (α-tocopherol) were combined with aliquots (5mL) of these prepared solutions. A sample without antioxidants was also made as a control sample.

After an initial absorbance reading at 470 nm, the mixture was maintained in a thermostatic bath at 50 °C, and absorbance was recorded at 15- minutes to 120 min.

Pancreatic lipase Inhibition assay for *T. capitatus* EOs

The steps of the protocol for the porcine pancreatic lipase inhibitory assay were mainly the same as those described by Bustanji et al. (2010), with a few changes[34,35].

The three samples of EOs from *T. capitatus* were used to make stock solutions of 500 µg/mL in 10% DMSO. From the stock solution, serial dilutions of five concentrations (50, 100, 200, 300, and 400 µg/mL) were made. Just before usage, a 1 mg/mL stock of porcine pancreatic lipase in Tris-HCl buffer was produced fresh. 20.9 mg of p-nitrophenyl butyrate (PNPB) was dissolved in 2 mL of acetonitrile to make the substrate.

Each working solution contained 0.1mL of 1 mg/mL porcine pancreatic lipase and 0.2mL of each dilution series member of the EO. Tris-HCL was added to produce the working solutions' final volume of 1 mL, and they were incubated for 15 min at 37 °C. After incubation, each test tube received a 0.1 mL p-nitrophenyl butyrate solution. The mixture was then incubated at 37 °C for another 30 min. A UV spectrophotometer was used to measure the hydrolysis of PNPB into p-nitrophenolate at 410 nm, which was used to estimate pancreatic lipase

activity. Using Orlistat as a standard reference chemical, the same technique was used. The following equation was used to compute the percentage of lipase inhibition by EOs in the three regions:

$$\text{Lipase inhibition } \% = (\text{ABl} - \text{Ats})/\text{ABl} \times 100\%$$

A_{Bl} is the obtained absorbance of the blank solution, and A_{ts} is the obtained absorbance of the tested sample solution.

In-vitro evaluation of α-amylase Inhibition

α-Amylase inhibitory activity of the three samples was assessed by the standard method of Wickramaratne, M.N., et al. (2015) with minor modifications[36–38].

Each *T. capitatus* EO sample was diluted in a few milliliters of 10% DMSO, then further dissolved in (Na_2HPO_4 /NaH $_2PO_4$(1:1) (0.02 M), NaCl (0.006 M) at pH 6.9) to yield stock solutions with 1000 µg/mL concentrations. The following dilutions were made from these: 50, 100, 200, 300, 400, and 500 µg/mL, using 10% DMSO as the diluent. A 0.2 mL amount of 2 units/mL porcine pancreatic amylase was mixed with 0.2 mL of EO-prepared solutions and incubated for 10 min at 30 °C. Following incubation, the tubes were given 0.2 mL of a freshly prepared 1% starch solution in water and incubated for at least three minutes more. The reaction was then paused by adding 0.2mL (3,5-dinitro salicylic acid (DNSA) reagent, diluted with 5 mL distilled water before being heated in a water bath at 90 °C for 10 min. The combination was then allowed to cool to the ambient temperature before being measured at 540 nm. The blank control was made using the identical ingredients as the above but with 0.2mL buffer instead of EOs. Following the process outlined above, acarbose was utilized as a standard reference. The -amylase inhibitory activity was estimated using the following equation for the three EO samples from *T. capitatus*:

$$\%\ \text{Of } \alpha\text{-amylase inhibition} = (\text{ABl} - \text{ATs})/\text{ABl} \times 100\%$$

ABl: the absorbance of the blank sample.

A_{Ts}: the absorbance of the test sample.

Antimicrobial activity of *T. capitatus* EOs

Microorganisms and conditions for cultivation

EOs samples of *T.capitate* were tested against the following bacteria strains: *Klebsiella pneumoniae* (ATCC 13883), *Pseudomonas aeruginosa* (ATCC 9027), MRSA (Clinical sample), *Proteus vulgaris* (ATCC 8427), *Staphylococcus aureus* (ATCC 6538), and *Escherichia coli* (ATCC 25922). The Antifungal activity of the EOs was examined against the growth of a diagnostically confirmed *Candida albicans* clinical isolate.

Antimicrobial assays for T. capitatus EOs for the three samples

The antimicrobial activity of the EO samples from three regions of Palestine was assessed by using the broth micro-dilution method according to the previous protocols by Balouiri, M. et al. (2016) and Bariş, Ö. et al. (2006) with some modifications[39,40].

Bacteria stains developed in cultured broth for 18 h. 10% DMSO was used to dissolve each isolated *T. capitatus* EO. After filter sterilization, the *T. capitatus* EOs solutions were serially micro-diluted ten times in sterile nutritional broth before use. The dilution procedures were carried out in an aseptic environment in 96-well plates.

Antibacterial and anti-fungal assay for T. capitatus EOs for three samples

For the antibacterial assay, each 200 µL of the isolated EOs samples was dissolved in 150 µL of 10% DMSO, diluted with 150 µL distilled water, and left on UV for 15 min.

Bacterial suspensions were made; a swab was collected from the different types of bacteria and then placed in normal saline. Turbidity was measured using the UV at λ = 620. It should be between (0.08 and 0.12). If it was less than that, bacteria were introduced, and if it was more significant than this value, normal saline was added. Then, 50µL of the bacterial suspension was combined with 5mL of the media to obtain the final bacterial suspension. The prepared *T. capitatus* EOs solutions were filtered, sterilized, and then micro-diluted serially 10 times, starting with 50 µL of the EOs solutions added to a sterile nutrient broth containing 50 L of the media. The initial obtained concentration was 20% v/v of the extracted EOs at the first line of the 96 well plates. This process was repeated until plate 10, at which 50 µL taken from it and removed. Then, 50µL of the prepared bacterial or fungal suspensions were added (one type of the mentioned bacterial strains for each line) to each plate, except the 12th vertical line of the 96 well plates, so that the final concentration obtained at the first vertical line of the EO became 10% v/v, equal to 100 µg of the EO per ml. The EOs-free nutrient broth on vertical line 11 was used as a positive control for bacterial growth. Vertical line number 12, on the other hand, contained EOs-free nutrient broth that had not been inoculated with any of the tested bacterial cells and served as a negative control for the media. We also had a compound control (compound + media) to ensure that there was no contamination or turbidity and that the change at the last horizontal line of the 96 well plates was not due to the compound itself.

The *T. capitatus* EO samples were tested in triplicate on each bacterial cell included in this investigation. At 35 °C, all of the inoculation plates were incubated. The incubation period was around 18 h long. The minimum inhibitory concentration of *T. capitatus* EOs was defined as the lowest concentration at which no observable bacterial growth in that micro-well was observed.

Anticancer activity

Numerous forms of cancer are classified according to the site of infection or the underlying biological process[41]. In our study, we used hepatic G2 cancer cells (ATCC, Rockville, MD, USA) to study the anticancer activity of the *T. capitatus* EOs.

Cytotoxicity assay: RPMI 1640 media supplemented with 10% heated fetal bovine serum, 1% of 2 mM l-glutamine, 50 IU/mL penicillin, and 50 µg/mL amphotericin B was used to culture hepatic G2 carcinoma cells. Once mycoplasma and bacteria were ruled out, cells were cultured in RPMI 1640 media with 10% calf serum as a monolayer confluent at 35 °C. As a precaution, antibiotics were not administered to avoid sensitizing the cell membranes. Phosphate buffer saline (PBS) was used to wash cells three times for the experiment. PBS was decanted, and cells detached with 0.025% Trypsin–EDTA and RPMI 1640 medium were added to make up a volume of 10 mL. To make a single-cell suspension, the cell suspension was centrifuged at 1000 xg for 10 min, and the pellet was resuspended in 10 mL of media. Trypan blue exclusion was used to measure cell viability more significantly than 96% in a hemocytometer. After inoculation, stock cultures were duplicated weekly. The cell line was grown in 6-well tissue culture plates (9.8 cm^2) at 35 degrees Celsius in a humidified atmosphere containing 5% CO_2. The cells were treated with the isolated EOs after 24 h. 0.1 mL of each EO extract from the three districts was serially diluted to 1000, 500, 250, 125, and 62.5 µg/mL.

Statistical analysis and IC50 calculations

The tests were carried out in triplicate. The results were reported as means ± standard deviation (SD). The means of the different districts were compared using an unpaired one-way ANOVA test. The statistical significance was established based on a p-value of less than 0.05. The IC_{50} value of the biological tests was calculated based on the inhibition percentage and the test material concentration. These values were plotted against the corresponding concentrations to create a dose-response curve. The IC_{50} value is obtained through nonlinear regression analysis or interpolating from the curve.

Results and discussion

Extraction yield of the collected EOs from *T. capitatus*

The dried aerial parts of *T. capitatus* from three districts in Palestine were grinded and subjected to extraction by hydrodistillation. Ramallah district, which has an average elevation of 880 m above sea level and an average humidity and average temperature of (47%), (40 °F to 84 °F) respectively, gave the highest yield in both methods (2.8), followed by Hebron, which has an average elevation of 930 m above sea level and an average humidity (62%) and an average temperature of (27 °F to 78 °F), gave a yield of (1.26%). Jenin, located 250 m above sea level with an average temperature of (52 °F to 89 °F) and an average humidity of 69%, gave a yield of 1.15%. In addition to the varied geographical locations and environmental conditions, the three districts have different soil types, components, and pH. All these factors worked together, not separately, to cause the quantity changes seen between the three samples.

Our results are consistent with other studies on different plant types, emphasizing the effect of environmental factors on the chemical composition and amount of EOs[42,43].

Components of the essential oils

GC-MS was used to identify all the EO components of *T. capitatus*, their concentrations, and output orders of the EOs. An EO chromatogram and the reference substances in a spectrum library with a computerized data bank were compared. The GC-MS method was used to get the different mass spectra and retention indices of compounds in this extract[40,41]. *T. capitatus* EO has been meticulously investigated, and the compositional diversity of plants growing in different nations and even in other regions of the same country has produced numerous chemotypes[44].

Table 1 shows the GC-MS analysis of EO components from three districts in Palestine (Ramallah, Jenin, Hebron). It showed more than 21 compounds were separated and identified; the EOs percentages yield of *T.capitatus* were (99.73%, 99.46%, and 94.41%) for Ramallah, Jenin, and Hebron, respectively. Components identified included sesquiterpenes, monoterpenes, and other compounds like alcohols, phenols, and organic acids.

Differences in the chemical composition of essential oils arise from their geographical origins. This variation, known as chemotypic variation or chemical polymorphism, is influenced by environmental factors such as climate, soil type, and altitude. The detailed results are described in Table 1. In the Ramallah district, carvacrol (31.25%), γ-terpinene (30.94%), o-cymene (16.84%), and linalool (6.19%) were the main components (Supplementary Figure S1). While, γ -terpinene (67%), cis-b-terpineol (12.91%), carvacrol (6.44%), and thymol (5.51%) were the main components in Jenin (Supplementary Figure S2). On the other hand, thymol (40.35%), b-caryophyllene (13.23%), (carvacrol, methyl ether) (10.7%), p-cymene (8.41%), and camphene (5.56%) were the main components in Hebron sample (Supplementary Figure S3). The comprehensive results and mass spectrometry graphs are presented in the supplementary documents. The results indicate the presence of three key components in the essential oil extracted from the three districts. However, significant variations in the levels of γ-terpinene, carvacrol, and thymol were observed across the regions. These differences are likely influenced by geographical factors, which play a crucial role in determining their relative proportions.The variation of chemical components of the EO samples for the chosen districts can be explained by the plant's origin, harvest period, soil components, genetic variables, and climatic conditions on the chemical structure of EOs[45,46]. In their research, Vaičiulytė V et al.[44] studied the effects of soil PH and 14 chemical components of the soil on the chemical composition of *Thymus pulegioides.* Results showed that the amount of aluminum, copper, iron, potassium, and manganese in soil increased, leading to decreased EOs in the raw material of *T. pulegioides.* Also, an abundance of higher amounts of phosphorus in the soil led to increased biosynthesis of α-terpinyl acetate. This concludes that

Ramallah	%	Jenin	%	Hebron	%
α-Thujene	0.65	α-Thujene	0.31	a-Pinine	2.52
α-Pinine	0.70	α-Pinine	0.49	Camphene	5.56
Camphene	0.15	Camphene	0.90	β-pinene	0.23
Sabinene	0.17	b-pinine	0.49	Myrcene	0.17
Myrcene	0.73	Myrcene	0.33	α-Terpinene	0.35
α-phellandrene	0.08	α-Terpinene	1.41	p-Cymene	8.41
α-Terpinene	2.25	p-Cymene	1.41	Limonene	0.47
o-Cymene	16.84	γ-Terpinene	67.00	γ-Terpinene	3.61
Sylvestrene	0.40	cis-4-Thujanol	0.19	cis-4-Thujanol	2.98
γ-Terpinene	30.94	cis-b-Terpineol	12.91	Camphor	0.004
Linalool	6.19	Carvacrol methyl ether	0.07	2-methyl isoborneol	2.24
cis-b-Terpineol	0.02	2-Isopropyl-4-methyl phenol	0.01	Terpinene-4-ol	0.02
Thymol methyl ether	0.24	o-Cymenol	0.14	α-Terpineol	0.23
Thymol	3.00	Thymol	5.51	Thymol methyl ether	0.58
Carvacrol	31.25	Carvacrol	6.44	Carvacrol, methyl ether	10.70
β-Caryophellene	4.61	β-Caryophellene	1.65	Isobornyl acetate	0.38
trans-bergamotene	0.28	trans-a-Bergamotene	0.10	Thymol	40.35
α-Caryophellene	0.19	α-Caryophellene	0.02	carvacrol	0.58
Viridiflorene	0.27	Caryophyllene oxide	0.11	β-Caryophyllene	13.23
Caryophyllene oxide	0.77			Caryophyllene oxide	1.80
Sum	99.73		99.46		94.41

Table 1. GC-MS analysis results.

	% inhibition				
Conc. (mg/ml)	Ramallah	Jenin	Khalil	Gallic	Trolox
0	0	0	0	0	0
5	26.0 ± 1.150	14.5 ± 0.451	27.2 ± 0.404	12.8 ± 0.252	84.2 ± 0.321
10	32.1 ± 0.030	20.2 ± 0.404	28.1 ± 0.208	26.5 ± 0.458	90.7 ± 0.702
20	34.4 ± 0.750	22.3 ± 0.513	28.8 ± 0.404	40.9 ± 0.854	91.7 ± 0.751
30	35.5 ± 1.501	23.4 ± 0.322	38.1 ± 0.252	55.1 ± 0.416	96.1 ± 0.100
40	36.8 ± 0.251	23.8 ± 0.300	38.6 ± 0265	64.0 ± 0.85	96.6 ± 0.551
50	36.7 ± 0.643	27.2 ± 0.529	42.3 ± 0.436	74.8 ± 0.700	97.4 ± 0.115
80	42.5 ± 0.458	28.2 ± 0.400	43.8 ± 0.208	80.2 ± 0.874	97.3 ± 0.058
100	43.3 ± 0.643	31.7 ± 0.436	51.8 ± 0.208	81.4 ± 0.709	97.4 ± 0.057
IC50	> 100	> 100	98	28	6

Table 2. Percent inhibition and IC50 of EO's of the districts.

soil chemical compositions of the EO of the plants have a significant effect. Environmental factors, including soil composition and climate, significantly influence the chemical profile of Thymus capitatus essential oil. Variations in soil mineral content, such as calcium and potassium, and organic matter levels directly affect essential oil biosynthesis. Temperature and altitude are also crucial; warmer climates can enhance the production of phenolic monoterpenes like carvacrol and thymol. Studies show that both altitude and temperature affect the essential oil yield in related species, emphasizing the importance of these factors in oil composition[43,47]. Furthermore, research is needed to study the influence of each factor separately on the chemical composition of the EOs.

Antioxidant activity

DPPH assay

Antioxidant activity was assessed using DPPH (2,2-diphenyl-1-picrylhydrazil), one of the earliest free radicals utilized in research on antioxidant activity[48]. Table 3 shows the percent inhibition of EOs of the districts at different concentrations ranging from (0–100 g/mL), in addition to the values of IC_{50}.

Analysis of Table 2 showed that the three districts had nearly the same IC50. However, the antioxidant activity of gallic acid and Trolox was less potent.

Statistical results were analyzed using SPSS software and the *Kruskal-Wallis Test*. The results showed a significant value ($P < 0.05$), which concludes that there are significant differences between districts according to the DPPH assay.

Conc (µg/mL)	% Inhibition		
	Hebron	Ramallah	Jenin
Control	0	0	0
1000	87.8 ± 0.2082	88 ± 0.2081	88.3 ± 0.2091
500	86.5 ± 0.2646	88.7 ± 0.3055	89 ± 0.1732
250	87.9 ± 0.1528	88.7 ± 0.3606	88.4 ± 0.4041
125	85.6 ± 0.3000	84.4 ± 0.3058	88.5 ± 0.3606
62.5	85.9 ± 0.5686	82.5 ± 0.3215	87.1 ± 0.3786

Table 3. % Inhibition of *T. capitatus* EOs in three districts against hepatic G2 cancer cells.

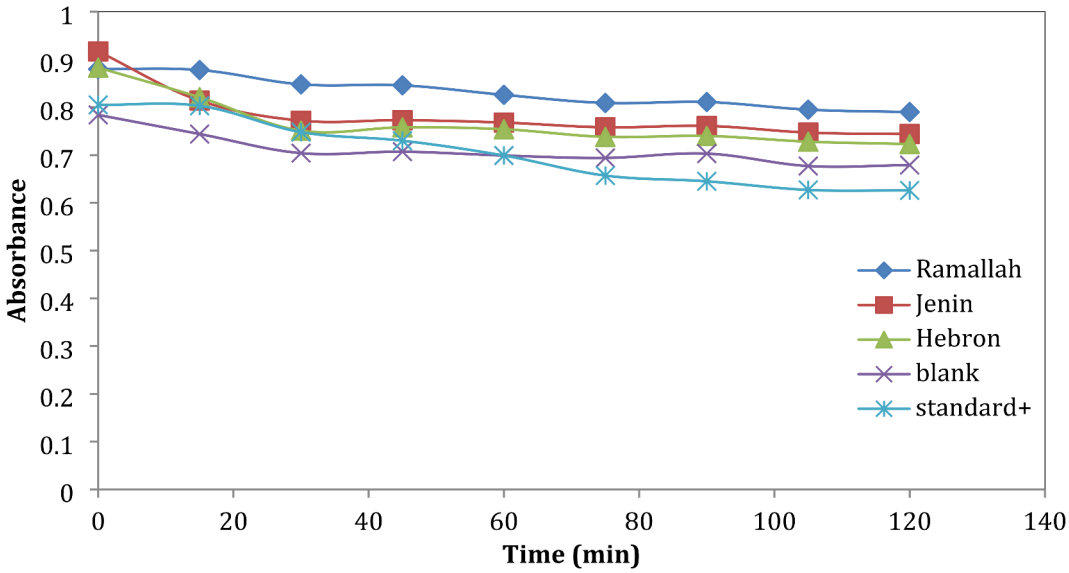

Fig. 1. β -Carotene assay of *T. capitatus* EOs in three districts.

β-Carotene assay

The β-carotene linoleic acid test is another antioxidant test performed on *T. capitatus*. To evaluate the antioxidant activity of the EOs, an emulsion system consisting of β-carotene and linoleic acid was used. Figure 1 shows the behavior of essential oils (EOs) from three districts over two hours. All samples exhibited higher antioxidant efficiency than the water control and the synthetic antioxidant α-tocopherol, which caused the highest β-carotene degradation. Higher absorbance values indicate greater β-carotene protection and antioxidant activity. After two hours, the absorbance values for Ramallah, Jenin, and Hebron were 0.79, 0.744, and 0.723, respectively, all exceeding the positive control and demonstrating strong antioxidant activity.

Numerous studies have found a correlation between monoterpenes and their oxygenated monoterpenes, which include phenols and alcohols, and the presence of antioxidant activity. Carvacrol (the main component in Ramallah EO sample) was discovered to have the most significant antioxidant activity. As the results of β the carotene assay showed, the Ramallah sample gave the highest antioxidant activity[49,50]. Other components of EOs, such as α-terpinene and γ-Terpinene, are also responsible for antioxidant activity[51]. The second potent antioxidant activity was the Jenin sample rich in γ-Terpinene (67%). Sabinene and other non-phenolic terpenoids have been shown to have significant antioxidant properties[52].

Anti-lipase activity

In terms of metabolic illnesses, obesity is one of the fastest-growing. One way to treat obesity is to limit the number of calories consumed and to take medicines that stop the body from absorbing fat from food, speed up metabolism, and prevent it from storing fats[53].

Lipases cannot hydrolyze dietary fat in the presence of orlistat. It forms a covalent bond with the active serine site, rendering them ineffective. Flatulence, fecal urgency, deficiency in fat-soluble vitamins, steatorrhea, and abdominal cramping are possible side effects of the drug[54].

The anti-lipase activity of *T. capitatus* against the positive control anti-lipase commercial medication Orlistat was evaluated using the porcine pancreatic lipase inhibitory assay.

Orlistat was used as an active, positive control; the results shown in Fig. 2 revealed that all tested extracts have anti-lipase action at a concentration of > 200 µg/mL. The three district EOs demonstrated anti-lipase activity greater than Orlistat.

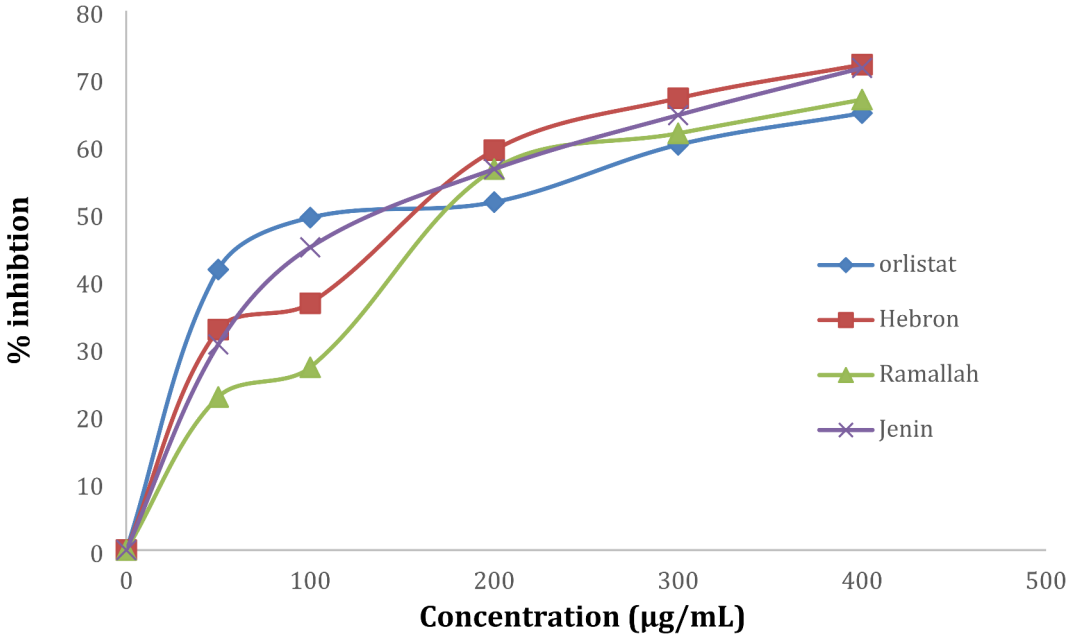

Fig. 2. Anti-lipase activity of EOs in three districts.

The Kruskal-Wallis statistical result for the anti-lipase activity of the EOs in the three districts was ($P = 0.946$), which is higher than (0.05), indicating no significant differences between the districts.

literature on the anti-lipase activity of Thymus species is limited. One study on Thymus serpyllum indicated potential benefits in managing obesity parameters in mice on a high-fat diet[55]. Recent studies have also highlighted the anti-lipase activity of various plant extracts, with aqueous extracts of Vitis vinifera and Rhus coriaria showing significant effects, demonstrating IC_{50} values of 14.13 and 19.95 µg/mL, respectively[56].

α-Amylase

A diabetic is a metabolic condition due to insulin resistance or insulin shortage. Chronic hyperglycemia can create a wide range of problems that affect numerous cells and organs, resulting in several fatal disorders[57]. Oral anti-diabetic medicines and/or parental insulin are used to treat diabetes. A significant number of these pharmaceuticals are associated with a variety of significant adverse effects and potentially hazardous contraindications. Patients have strongly preferred herbal supplements and pharmaceuticals combining high therapeutic efficacy with low adverse effects in recent years. Anti-diabetic medicines derived from herbs show great promise, and the anti-diabetic potential of plants that have been used in traditional medicine for a long time is currently the subject of research[58]. Numerous plants have been confirmed to exhibit hypoglycemic capabilities, as evidenced by literature sources from multiple databases; these planets tend to reduce blood glucose either as an insulin mimetic or through insulin secretory activity. Most hypoglycemic plants are found in the following families: (*Leguminoseae*, *Lamiaceae*, *Liliaceae*, *Cucurbitaceae*, *Asteraceae*, *Moraceae*, *Rosaceae*, and *Araliaceae)*. Anti-diabetic properties are attributed to polyphenols, flavonoids, terpenoids, coumarins, and other components in these medicinal plants[59].

Figure 3 demonstrates α-amylase inhibitory activity for the EOs of the three districts contrasted with Acarbose, which is utilized therapeutically in a concentration-dependent manner for its inhibitory action. Samples at a 400 µg/mL concentration showed a 50% inhibition compared to Acarbose.

Statistical analysis tests of the three districts' results showed a significant value ($p = 0.041$). The effect is ranked according to the following sequence: Jenin 11.3, Ramallah 12.21, Hebron 12.14, Acarbose 22.21. The results confirmed differences in the EO activities between the districts. Research on aqueous extracts from thyme leaf powder showed that higher concentrations increased inhibition of α-amylase in Trogoderma granarium larvae. A 10% extract concentration resulted in an 87 mg/mL inhibition rate, demonstrating a dose-dependent effect[60].

Antimicrobial assay

Microbial infections are a global concern that poses a life-threatening threat to humanity. Owing to the abuse of antibiotics, antibacterial and antifungal resistance has emerged as an urgent global health issue. With an estimated 2 million patients afflicted with medication-resistant germs each year, an alternate strategy to combat drug resistance is required. As a result, there is a proclivity to employ traditional or unusual approaches to solve the problem and prevent the spread of infectious diseases[27].

Gram-negative and gram-positive bacteria were both shown to be highly susceptible to *T. capitatus* EO, even though it was previously stated that EOs are less effective against gram-negative bacteria[61]. A possible explanation for this is that the EOs contain hydrophobic components, which may damage the cell membranes of bacteria and hence impede their ability to operate[51]. Further research has explained that *T. capitatus* EOs

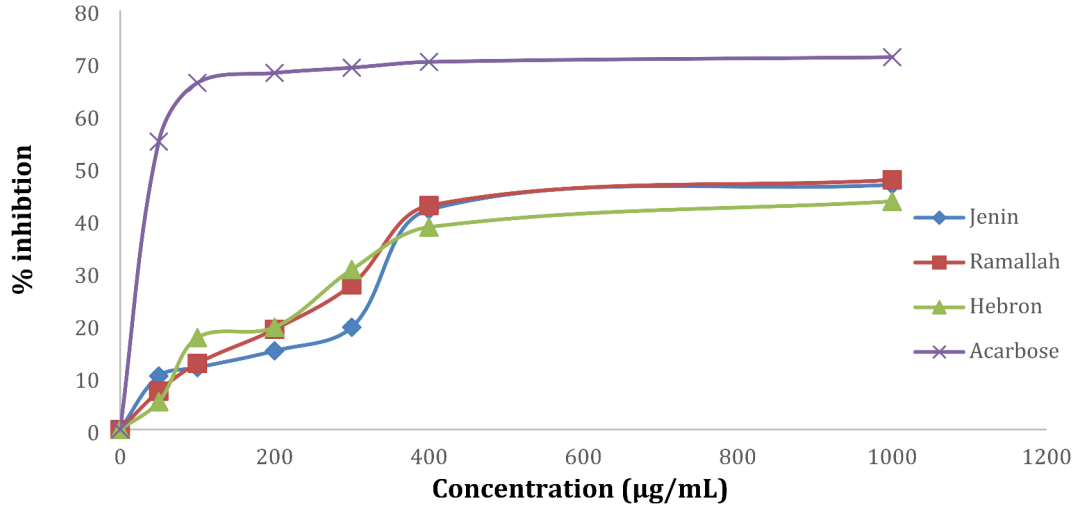

Fig. 3. Alfa amylase percent inhibition of EOs in three districts.

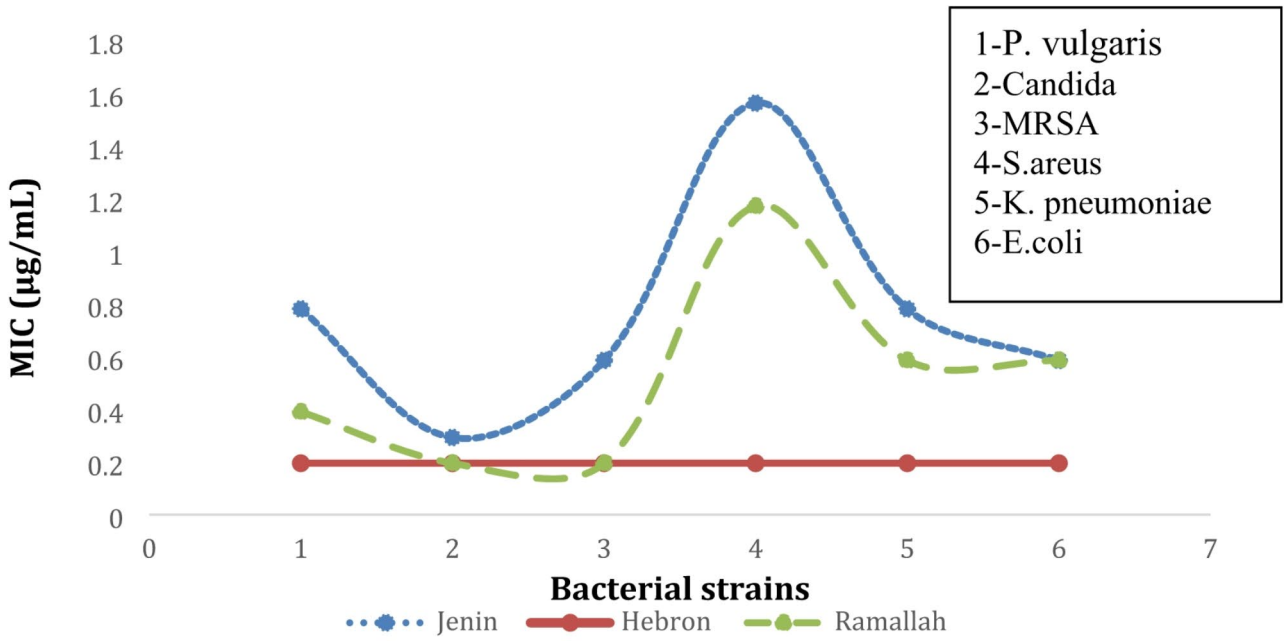

Fig. 4. MIC values of *T. capitatus* EOs in three districts.

effectiveness against fungus may be due to the quantity of phenolic chemicals (carvacrol and thymol), which may interfere with enzymes involved in cell wall synthesis, such as chitin synthase and chitinase, and with α and ß-glucanases. The antifungal impact of *T. capitatus* was previously shown to involve telomerase suppression as it increased the rate of cell senescence and apoptosis, as previously reported. (γ-terpinène and p-cymene) have also been implicated in antifungal action[61].

In this research, the antimicrobial activity of EO samples from three regions of Palestine was assayed by using the broth micro-dilution method according to a previous protocol (Balouiri M. et al.2016; Bariş, Ö. et al.2006) with some modifications.

Figure 4 demonstrates the antimicrobial activities of the EOs on different types of bacteria. EOs from all districts showed MIC values between (0.1953 and 1.5625 µg/mL) except for *pseudomonas.* The results also showed that the Hebron EO sample had the highest activity of 0.1953 g/mL against all types of bacteria, which was explained by the high percentage of thymol (40.35%). Moreover, the results demonstrate the efficiency of the EOs against the MRSA strain, which is resistant to most antibiotics. Similar work testing the antimicrobial efficacy of Thymus vulgaris essential oil showed significant effects in combating various bacterial strains[62].

Anticancer activity

Cytotoxic chemotherapy refers to chemicals and medicinal plants that kill various cancer cells. Cytotoxic medications include both plants and pharmaceuticals. They prevent the division of cells, which ultimately results in the death of cancer cells. Additionally, they can improve the results of radiotherapy and surgery and decrease the number of metastases[63]. The cytotoxic and anticancer activities of Thymus EOs and monoterpenes have been extensively studied. Carvacrol, thymol, terpinene, and p-cymene are the main parts of Thymus EOs that give them their healing effects[64].

In this research, HepG2 cells were used to predict the anticancer assay of *T. capitatus* EO.

For a day, the Hep-G2 cells were exposed to increasing quantities of the investigated samples from three districts (1000, 500, 250, 125, and 62.5 µg/mL). The MTS test was used to obtain a quantitative reading on the cell viability.

Table 3 shows the percent inhibition of thymus EOs from three sites in Palestine on Hep-G2 cells. They showed almost similar cytotoxic activity with more than 85% inhibition at concentrations greater than 62.5 µg/mL. Hoverer, Ramallah EO samples showed 82.5% inhibition at 62.5 µg/mL. The results demonstrate that the cytotoxic effects against Hep G2 cells are different in the districts with superiority in Hebron and Jenin. More phytochemical and in vivo pharmacological research is required to validate these exceptional results.

Many researches on Thymus species exhibit potential anticancer properties due to compounds like thymol and carvacrol. Research shows significant antitumor effects in mammary carcinoma models, with in vitro studies on MCF-7 and MDA-MB-231 breast cancer cell lines supporting these findings[65].

Conclusion

Tested samples of *T. capitatus* were collected from three regions of Palestine (Hebron, Jenin, and Ramallah). *T. capitatus* extraction was performed using hydrodistillation. About 21 compounds were separated and identified by GC-MS analysis of EO components from the three samples of three districts. Apparent variations in their percentage between districts, mainly (γ-Terpinene, carvacrol, and thymol), were noticed due to environmental factors.

In vitro assessment of the antioxidant activity of the EOs was carried out using the β-Carotene assay and DPPH assay. The samples showed potent antioxidant activity. Similar results were demonstrated for Alfa amylase and Anti-lipase, showing the activity of EOs against both enzymes when compared with the positive control. The antimicrobial activity of *T. capitatus* EO was effective against all tested bacteria and fungi except *pseudomonas.* The Hebron sample gave distinguishable results at low concentrations against all bacterial and fungal strains put to the test. Moreover, the cytotoxic activity against Hep G2 cells showed more than 85% inhibition at concentrations greater than 62.5 µg/mL for Hebron and Jenin samples. Differences in the active consistency in different districts explain the variation in results.

Data availability

All data generated or analyzed during this study are included in this published article.

Received: 3 January 2025; Accepted: 3 March 2025
Published online: 10 May 2025

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Author contributions

M.Abualhasan and Nidal Jaradat made a substantial contribution to the concept, and design of the article and also approved the version to be published. Alaa Barakat collected the data. Ahmed Khasati and Alaa Barakat analyzed the data and wrote the first draft. All the authors revised the manuscript.

Declarations

Ethics approval and consent to participate

This study complies with all relevant institutional, national, and international guidelines and legislation for collecting and studying plant materials. All necessary permissions and licenses for *T. capitatus* collection and research were obtained from the appropriate authorities.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-025-92835-7.

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