Phyton-
International Journal of
Experimental Botany

Tech Science Press

Doi:10.32604/phyton.2025.063403

ARTICLE

Chemical Composition, Antioxidant and Anticancer Activities of *Thymus capitatus* Essential Oil: Experimental and Computational Approaches

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Received: 14 January 2025; Accepted: 21 February 2025; Published: 31 March 2025

ABSTRACT: Traditional Palestinian medicine uses *Thymus capitatus* (*T. capitatus*), a plant recognized for its therapeutic properties due to its high concentration of essential oils such as thymol and carvacrol, to treat skin diseases, gastrointestinal disorders, and respiratory infections. The present study was conducted to evaluate the antioxidant and anticancer activities of *T. capitatus* essential oil (EO). Moreover, this study employed computational methods including ADMET analysis and molecular docking. Using Gas chromatography-mass spectrometry (GC-MS) analysis, the phytochemical composition of *T. capitatus* essential oil was identified. The DPPH scavenging method was used to assess antioxidant activity. The Michigan Cancer Foundation-7 (MCF-7) and human colorectal carcinoma (HCT-116) cell lines were used to test for cytotoxic and cytostatic effects. The results of GC/MS analysis revealed 21 chemicals, accounting for 95.82% of their content, with carvacrol (61.23%), p-Cymene (9.49%) and γ-Terpinene (9.4%) being the most abundant. With an IC50 value of 0.27 ± 0.009 mg/mL, the DPPH assay demonstrated a robust scavenging capacity when compared to the IC50 value of butylated hydroxytoluene (BHT), which was 0.37 ± 0.007 mg/mL. *T. capitatus* EO showed potent anticancer activity on HCT-116 and MCF cell lines. The ADMET *in-silico* investigations revealed satisfying physicochemical and pharmacokinetics profiles, justified by good human intestinal absorption (HIA exceeding 93%), good permeabilities to the blood-brain barrier (BBB) and central nervous system (CNS), without any inhibition effect on 1A2, 2C9, 2C19, 2D6, and 3A4 cytochromes. Furthermore, all ligands were determined to be non-toxic, with no Ames mutagenicity detected based on toxicity predictions, making them ideal candidates for further drug development.

KEYWORDS: *Thymus capitatus*; gas chromatography–mass spectrometry (GC-MS); docking; cytostatic; cytotoxic

1 Introduction

Medicinal and aromatic plants (MAPs) have emerged as a major source of natural chemicals, exhibiting a wide range of biologically validated effects in recent decades. The potential of these plants to provide natural remedies in fields including antibacterial, anticancer, anti-inflammatory, and antioxidant therapy has attracted a lot of attention [1,2]. The increasing demand for safer, more cost-effective, and environmentally friendly alternatives for synthetic medications in the treatment of contemporary health issues is reflected in the rising reliance on MAPs. In fact, the development of civilizations is linked with the history of medicinal and aromatic plants [3,4].

The history of various cultures worldwide demonstrates that these plants have always played a significant role in healing, the creation of fragrances, and food preparation [5]. In addition, the use of plants for

medicinal purposes is ingrained in all civilizations' traditions. Natural products are an important source of novel and chemically diverse molecules for drug discovery [6]. Nevertheless, the necessity of developing new natural substances with therapeutic properties persists [7]. These substances consistently offer novel remedies as alternatives to existing pharmaceuticals since they have the advantage of being less harmful than their counterparts made of synthetic materials. Furthermore, for many thousands of years, essential oils (EOs) have been a source of bioactive compounds and are being extensively researched for their potential use as a synthetic product alternative for treating infectious illnesses and numerous pathological ailments linked to oxidative stress [5,8]. Oxidative stress has been linked to many diseases such as cancer, diabetes, cardiovascular, and neurological [9,10]. As a result, antioxidants are recommended, with synthetic ones being the most commonly used. Since synthesized antioxidants are currently being questioned for use because of their potential health risks and toxicity, the investigation of plant antioxidant activity is crucial [11]. Today, the investigation of plant antioxidant activity is becoming crucial. The potent antioxidant properties of medicinal plants are due to their richness of polyphenols, such as phenolic acids, flavonoids, carotenoids, and vitamins (E, C, and A) [12].

The Lamiaceae family, which includes *Thymus capitatus* L., represents one of the most botanically and pharmacologically significant plant groups due to its rich diversity of secondary metabolites and widespread medicinal applications. *Thymus capitatus*, an endemic Mediterranean plant, is distributed across regions such as Albania, Algeria, Morocco, Palestine, and Türkiye, among others, reflecting its ecological adaptability and cultural significance in traditional medicine [13,14]. This plant's therapeutic potential lies in its bioactive compounds, particularly phenolic acids, flavonoids, and essential oils, which are responsible for its potent antioxidant, antiviral, and antibacterial properties [15,16]. These properties have made it a cornerstone in the treatment of various ailments, including diabetes, cancer, heart disease, and gastrointestinal disorders, in regions where it is naturally found. The high concentrations of carvacrol, thymol, and p-Cymene in its essential oil not only contribute to its biological activities but also enhance its pharmacological relevance, bridging its historical use in traditional medicine with its potential in modern pharmaceutical and nutraceutical development [17–19]. This convergence of geographical distribution, biochemical composition, and therapeutic applications underscores the holistic importance of *T. capitatus*.

The aim of the present work was to use gas chromatography-mass spectrometry (GC-MS) to thoroughly analyzes the chemical composition of *T. capitatus* essential oil and quantify their antioxidant capacity, via 2,2′-diphenyl-1-picrylhydrazyl (DPPH) assay, to evaluate their biological activities. Additionally, the essential oil of *T. capitatus* was tested on HCT-116 and MCF-7 to examine its anti-cancer properties. Finally, *in silico* receptor-ligand docking experiments were used to further examine the antioxidant and anticancer properties of *T. capitatus* essential oil. By fanticipating the interactions between the oil's bioactive components and certain molecular targets, these computer analyses attempted to offer comprehensive insights into the mechanisms of action that underlie its therapeutic effects.

2 Material and Methods

2.1 Chemicals

2,2′-diphenyl-1-picrylhydrazyl (DPPH), butylated hydroxytoluene (BHT), acidic isopropanol, ethanol, and hexane were purchased from Sigma-Aldrich, Germany. Human colorectal carcinoma (HCT-116, ATCC® CCL-247™) and breast cancer (MCF-7, ATCC® HTB-22™) were obtained from the American Type Culture Collection (Manassas, VA, USA). 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) DMEM-5671, fetal calf serum, glutamine, penicillin, formazan crystals, Streptomycin, formazan crystals and were purchased from Sigma-Aldrich, St. Louis, MO, USA.

2.2 Plant Sample

Thymus capitatus aerial parts, including the leaves and flowers, were gathered from the Hebron region of Palestine to extract the plant's essential oil. The integrity of the plant material and its medicinal ingredients were preserved by careful harvesting. In order to minimize the loss of volatile chemicals during the drying process, the aerial parts were allowed to air dry naturally at room temperature after collecting.

2.3 Essential Oil Extraction

100 g of the plant's aerial parts were weighed and utilized immediately for the distillation process. 1000 mL of distilled water and the plant material were added to a distillation flask. A Clevenger-type device was then used to hydrodistillate the mixture, which is a common and dependable technique for extracting essential oils. Steam and volatile oils were co-distilled, condensed, and collected over the course of the four-hour distillation process. This time frame guarantees the oil's complete extraction while maintaining its chemical makeup. Following the distillation process, the essential oil was meticulously separated and dried over anhydrous sodium sulphate to eliminate any remaining moisture that would have risked its stability and purity. After that, the dried essential oil was placed in an airtight container and kept at a regulated temperature of 2 to 4°C in a dark atmosphere to preserve its chemical integrity and stop oxidative deterioration [20].

2.4 2,2-Diphenyl-1-Picryl-Hydrazyl-Hydrate (DPPH) Assay

The DPPH reagent, which was made by dissolving 0.005 g of DPPH in 200 mL of 96% ethanol, was combined with a 50 µL aliquot of the *T. capitatus* essential oil (EO) dilution series, which had initial concentrations ranging from 15.62 to 500 mg/mL and 0.84 to 104 mg/mL, respectively [20]. The sample and the DPPH solution were added to the total reaction volume, which was set at 1 mL. A spectrophotometer was used to measure the absorbance at 517 nm after the reaction mixture had been incubated for an hour.

The scavenging activity of the DPPH radical was calculated as a percentage of inhibition using the following formula:

$$\%\,\text{Inhibition} = ((\text{control absorbance} - \text{sample absorbance})/\text{control absorbance}) \times 100$$

A positive control, which contained 25 µL of Butylated Hydroxytoluene (BHT), a common antioxidant, and a negative control, in which water was used in place of the sample, were compared to the absorbance readings. Furthermore, using the graph of the inhibition percentage plotted against the oil concentrations, the concentration of the essential oil needed to attain 50% radical inhibition (IC_{50}) was determined.

2.5 Anticancer Activity

2.5.1 Cell Culture

Cell lines from breast cancer (MCF-7, ATCC® HTB-22™) and human colorectal carcinoma (HCT-116, ATCC® CCL-247™) were cultivated in DMEM-5671 medium with 4.5 g/L of glucose. The medium was supplemented with 10% (vol/vol) heat-inactivated fetal calf serum (FCS), 1% glutamine, 1% nonessential amino acids, 100 U/mL penicillin, and 10 µg/mL streptomycin. A humidified environment containing 5% CO_2 and a medium pH of 7.4 were used to incubate the cells at 37°C.

2.5.2 Cytotoxic and Cytostatic Assays

For cytotoxic and cytostatic assays, cells were placed into 96-well microtiter plates at densities of 20,000 and 5000 cells/100 µL, respectively. After one day, the cells were exposed to increasing concentrations of

essential oil solutions for a 24-h cytotoxic assay and a 72-h cytostatic assay. The MTT test was used to determine cell viability, which was then expressed as a percentage of treated cells' absorbance in comparison to untreated control cells.

2.5.3 3-[4,5-Dimethylthiazol-2-yl]-2,5 Diphenyl Tetrazolium Bromide (MTT) Assay

The MTT assay was performed according to the protocol described by [21,22]. After being incubated with essential oil solutions, the media were taken out of each well, and the cells were then rinsed with phosphate-buffered saline (PBS). Following that, the cells were cultured for four hours in the dark in serum-free media containing MTT (0.5 mg/mL). Following incubation, the cells were rinsed with PBS, and 100 µL of acidic isopropanol (0.08 N HCl) was added to each well to dissolve the formazan crystals that had developed in the mitochondria. With an ELISA reader, the absorbance of the dissolved MTT formazan was determined at 570 nm. The absorbance of treated cells divided by that of untreated control cells, given as a percentage, was used to determine cell viability.

2.6 Gas Chromatography–Mass Spectrometry (GC-MS) Analysis

The GC-MS system (GC ULTRA S/N 20062969; Polaris QS/N 210729) with a nonpolar fused silica HP-5MS column (60 m × 0.32 mm, 0.25 µm film thickness) was used to analyze the volatile components of the sample. A 1:10 dilution of the sample was made in hexane, and 1 µL of the diluted oil was introduced into the apparatus. The column temperature was planned to rise from 40°C to 260°C at a rate of 2°C per minute, while the injector temperature was kept at 250°C. With a steady flow rate of 1 mL/min, helium was employed as the carrier gas. The identification of the volatile components was performed by determining their Kovats retention indices (Ki), which were calculated relative to a series of n-alkanes (C_8–C_{20}). The obtained retention indices were compared with those reported in the literature. Additionally, the mass spectra of the components were matched against those in the NIST MS Library to confirm their identity [23,24].

2.7 Molecular Docking

Physicochemical and pharmacokinetics features of absorption, distribution, metabolism, excretion, and toxicity (ADMET) were properly predicted for three major compounds of the thymus plant using Swiss ADME and pKCSM servers [25]. With the help of Protein Data Bank (PDB), four targeted proteins coded 6GUE, 2CDU, 1OG5, and 4JK4 have been extracted using the X-ray diffraction method with good resolutions of 1.99, 1.8, 2.55, and 2.65 Å, respectively. After that, we used AutoDock 4.2 software to start the docking calculation. We organized the grid box using the AUTOGRID method, setting the sizes 100, 100, and 100 in its three-dimensional structure with a 0.375 Å spacing between them. Ten adaptive algorithms were then run, a total of 25 million evals. Ultimately, we obtained the strongest complex of the fifty conformations that were acquired and then used Discovery Studio 2021 to illustrate the protein-ligand interactions in both two and three dimensions.

3 Results and Discussion

3.1 Antioxidant Activity

The identification of antioxidant activity *in vitro* of the EO of *T. capitatus* was determined through the method of the scavenger effect against DPPH radical. Results of the antioxidant activity of *T. capitatus* EO are summarized in Fig. 1. The purple DPPH radical transforms into a stable yellow molecule. The level of discoloration can be a sign of free radical scavenging capacity. According to the IC_{50} values, the IC_{50} (mg/mL) of *T. capitatus* EO by DPPH radical scavenging assay was found to be 0.27 ± 0.009 mg/mL, while

the reference BHT was 0.37 ± 0.007 mg/mL. Our results showed an agreement with previous reports on the oil of *T. capitatus* [5]. A study conducted by Amarti et al. showed that *T. capitatus* EO exhibited remarkable antioxidant activity [26]. In the same context, Bounatirou et al. and Zaïri et al. reported that *T. capitatus* EO demonstrated a high antioxidant power [27,28]. Furthermore, our results were in agreement with studies in which the phenolic compounds were responsible for the antioxidant activity [29,30]. The high concentration of phenolic compounds, such as carvacrol, in the examined thyme species, is associated with the plants' antioxidant capacities.

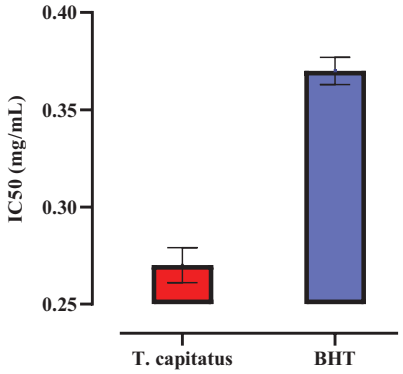

Figure 1: Antioxidant results of *T. capitatus* essential oil by DPPH assay. The data are shown as mean ±SD, and the experiment was conducted at three repetitions

3.2 Anticancer Activity

The cytotoxic potential of *T. capitatus* EO was performed against colorectal carcinoma cells HCT and MCF human breast cancer cell lines. Results of the cytostatic and cytotoxic activity of tested essential oil are summarized in Fig. 2 and Table 1. As can be seen, EO presented a higher anti-proliferative activity/cytotoxicity on HCT-116 and MCF cell lines. Comparing cell survival in the presence of different concentrations of EO indicated higher sensitivity of carcinogenic cell lines in all tested tumor cell lines, although each culture showed different sensitivity, in accordance with determined IC_{50}. It is depicted in this table that *T. capitatus* EO exhibited a potent cytostatic and cytotoxic activity on HCT-116 with IC_{50} values equal to 178.85 and 301.05 µg/mL, respectively. For the MCF cell lines, the EO exhibited a cytotoxicity effect with an IC_{50} value of 464.0.4 µg/mL. Consistent with our results are numerous literature data concerning the chemotherapeutic potential of different *Thymus* species EO [31,32].

Volatile compounds contained in *T. capitatus* such as carvacrol and thymol demonstrated anticancer properties on several cancer cell lines. The mechanisms underlying these effects involve the arrest of cell cycle progression in the S phase and G0/G1 transition, and inducing of apoptosis [14]. Importantly, our findings support the hypothesis that a mixture of phytochemicals with a plethora of biological effects found in EO may have synergistic effects against cancer cell lines and may be more potent against cancer than an isolated compound. Within this context, numerous pre-clinical and clinical studies have suggested lower effectiveness of single phytochemicals compared to whole plant foods against cancer [33].

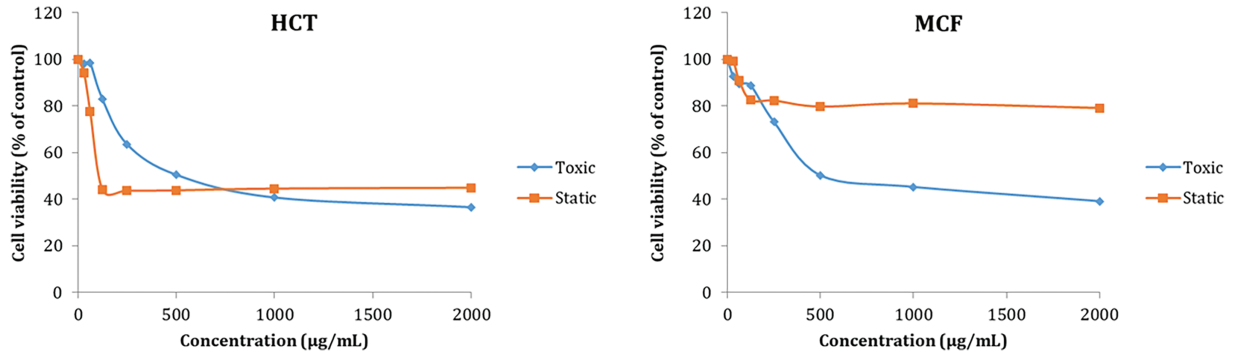

Figure 2: Effects of *T. capitatus* essential oil on HCT-116 and MCF-7 cells, both cytostatic and cytotoxic, at concentrations ranging from 0 to 2000 µg/mL. The data are presented in the form of mean ± standard deviation (SD)

Table 1: IC_{50} values (µg/mL) of *T. capitatus* essential oil on HCT-116 and MCF-7 cells

	HCT (µg/mL)	MCF (µg/mL)
Static	178.85	–
Toxic	301.05	464.0.4

3.3 Phytochemical Profile of T. capitatus Essential Oil

The gas chromatography–mass spectrometry (GC-MS) method was used to determine the chemical makeup of *T. capitatus*'s essential oil. The chemical components of *T. capitatus* essential oil are detailed in Table 2. GC/MS identified 21 chemicals in total, which accounted for 95.82% of the total content. The most abundant components found were carvacrol (61.23%), which was followed by p-Cymene (9.49%) and γ-Terpinene (9.4%). It should be mentioned that, in comparison to the other essential oils, carvacrol was the one with the highest content. α-Thujene (3.81%), Caryophyllene (1.51%), Camphene (1.23%), Tricyclene (4.23%), and α-terpinene (1.09%) were the minor constituents. For the same plant, El Ouariachi et al. confirmed the same composition in their work [15]. It was found that the main constituents in *T. capitatus* EO from Tunisia were carvacrol (61.6%–83%), p-Cymene (5%–17%), γ-Terpinene (2%–14%), and caryophyllene (1%–4%), but this composition changes depending on the location and the growing season [28]. Similarly, *T. capitatus* EO from Greece, Portugal [34], Sicily [35,36], and Albania [37] are characterized by a high percentage of carvacrol. However, Italian researchers detected that thymol (29.3%) was considered one of the primary molecules present in *T. capitatus* while carvacrol represents only (10.8%) [38]. Furthermore, in another recent study from the south of Tunisia, thymol (89%) was found to be one of the major compounds in the EO of *T. capitatus*. The oil from Türkiye showed carvacrol (35.6%), p-Cymene (26.4%), and thymol (18.6%) as the principal components [18]. In general, carvacrol and p-Cymene are the main chemicals that can be identified in *T. capitatus* based on our findings and these earlier investigations. The origin of the plant, various environmental factors (such as sunlight, salinity, crop effects, harvest time, and seasonal and climatic conditions), genetic background, and the extraction technique of the plants could all be responsible for the variation observed in the percentages of the main components of the essential oils examined in this and other studies [24,39]. Consequently, the impact of these factors on biosynthesis pathways results in variations in the qualitative and quantitative characteristics of the majority of compounds, leading to the existence of many chemotypes that differentiate essential oils from different sources [40,41].

Table 2: Phytochemical composition of *T. capitatus* EO

Compound number	KI	Compound name	Formula	Mol. Wt.	RT (min)	Area (%)
C1	927	Tricyclene	$C_{10}H_{16}$	136.23	4.63	4.23
C2	932	α-Thujene	$C_{10}H_{16}$	136.23	4.96	3.81
C3	936	α-Pinene	$C_{10}H_{16}$	136.23	12.52	0.31
C4	1010	δ-3-Carene	$C_{10}H_{16}$	136.23	14.35	0.51
C5	1013	α-terpinene	$C_{10}H_{16}$	136.23	15.25	1.09
C6	1015	p-Cymene	$C_{10}H_{14}$	134.22	15.59	9.49
C7	1024	1,8-Cineol	$C_{10}H_{18}O$	154.25	15.79	0.36
C8	1051	γ-Terpinene	$C_{10}H_{16}$	136.23	16.66	9.4
C9	1123	Camphene	$C_{10}H_{16}$	136.23	17.95	1.23
C10	1150	Borneol	$C_{10}H_{18}O$	154.25	20.21	0.13
C11	1053	trans-Sabinene hydrate	$C_{10}H_{18}O$	154.25	20.46	0.37
C12	1082	Terpinolene	$C_{10}H_{16}$	136.23	20.89	0.08
C13	1082	cis-Sabinene hydrate	$C_{10}H_{18}O$	154.25	21.04	0.18
C14	1215	Thymol methyl ether	$C_{11}H_{16}O$	164.24	22.31	0.11
C15	1215	Pulegone	$C_{10}H_{16}O$	152.23	22.62	0.53
C16	1278	Carvacrol	$C_{10}H_{14}O$	150.22	24.25	61.23
C17	1421	Caryophyllene	$C_{15}H_{24}$	204.36	27.32	1.51
C18	1494	Valencene	$C_{15}H_{24}$	204.35	29.35	0.1
C19	1507	γ-Cadinene	$C_{15}H_{24}$	204.35	29.68	0.09
C20	1520	δ-Cadinene	$C_{15}H_{24}$	204.35	29.85	0.19
C21	1578	Caryophyllene oxide	$C_{15}H_{24}O$	220.35	31.55	0.87
		Total				95.82

Note: KI: Kovats indices.

3.4 ADMET Properties Prediction

In the present study, the major compounds, namely p-Cymene (C6), γ-Terpinene (C8), and Carvacrol (C16) were chosen for ADMET *in-silico* investigations. Based on important rules like Lipinski's Rule of Five, Veber, Egan, Muegge, and Ghose guidelines, the physicochemical characteristics of compounds C6, C8, and C16 were assessed. They looked at variables like molecular weight, molar refractive index, rotatable bonds, logP, and hydrogen bond donors and acceptors. All compounds showed molar refractive indices (45.99–48.01) within Ghose's permissible range (40–130) and complied with molecular weight limits (<500 g/mol). Their favorable membrane permeability and bioavailability were shown by their meeting the requirements for rotatable bonds (<10) and logP (<5). C16 met Lipinski's requirements for increased cell permeability since it possessed one hydrogen bond donor and one acceptor, but C6 and C8 lacked both (Table 3).

To predict their pharmacokinetic and toxicological profiles, the ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties of the main compounds were assessed. The findings from these evaluations are detailed in Table 4. All of the ligands showed high intestinal absorption rates, with C6 at 95.52%, C8 at 95.716%, and C16 at 93.712%, indicating excellent bioavailability potential. Blood-brain barrier (BBB) permeability values suggested limited CNS penetration for all of the compounds, with numeric values below 0.6. Metabolic analysis revealed no interaction with key cytochrome P450 enzymes (CYP1A2, CYP2C19, CYP2C9, and CYP2D6), indicating a low likelihood of metabolic complications. Concerns of drug resistance linked to efflux were also allayed because none of the ligands were P-glycoprotein substrates.

Additionally, all ligands were found to be non-toxic, with no Ames mutagenicity found, according to toxicity predictions, which made them excellent candidates for additional drug development.

Table 3: Physicochemical parameters prediction of C6, C8, and C16 compounds based on Lipinski, Veber, Egan, Muegge, and Ghose regulation

	Physical and chemical properties						Lipinski violations	Veber violations	Egan violations	Ghose violations	Muegge violations
	Molecular weight (g/mol)	Molar refractive index	Rotatable bonds number	Log P (Octanol/ Water)	Hydrogen bond acceptors	H-bond donors number					
Threshold	**≤500**	**40 ≤ MR ≤ 130**	**<10**	**<5**	**<10**	**<5**	**Yes/No**	**Yes/No**	**Yes/No**	**Yes/No**	**Yes/No**
C6	134,22	45,99	1	2.51	0	0	Yes	Yes	Yes	No	No
C8	136,23	47.12	1	2.73	0	0	Yes	Yes	Yes	No	No
C16	150.22	48.01	1	2.24	1	1	Yes	Yes	Yes	No	No

Table 4: ADMET properties prediction of C6, C8 and C16 compounds

Ligands number	Absorption	Distribution			Metabolism							Excretion	Toxicity
	Intestinal absorption (human)	VDss (human)	BBB permeability	CNS permeability	Substrate		Inhibitor					Total clearance	AMES toxicity
							CYP						
					2D6	3A4	1A2	2C19	2C9	2D6	3A4		
	Numeric (% Absorbed)	Numeric (Log L/kg)	Numeric (Log BB)	Numeric (Log PS)	Categorical (Yes/No)							Numeric (Log mL/min/kg)	Categorical (Yes/No)
C6	95.52	0.539	0.541	−1.348	No	No	Yes	No	No	No	No	0.239	Not toxic
C8	95.716	0.397	0.74	−2.023	No	No	No	No	No	No	No	0.217	Not toxic
C16	93.712	0.351	0.381	−1.438	No	No	No	No	No	No	No	0.243	Not toxic

3.5 Molecular Docking Simulation

A powerful computer method that is widely used to gain a crucial understanding of the molecular mechanisms underlying pharmacologically active medications is molecular docking. In this section, the major compound namely carvacrol was chosen for molecular docking toward four targeted proteins to explore the inhibition mechanisms responsible for antioxidant and anticancer activities.

Due to the overexpression of CDK2 and cyclins A and E in patients with ovarian, breast, colorectal, breast, prostate, and lung cancer, [42–44], protein 6GUE.pdb was selected to assess the anticancer mechanism of carvacrol in *T. capitatus* essential oil. The crystal structure of human Cyclin-Dependent Kinase 2 (CDK2) in association with Cyclin A2 and the inhibitor AZD5438 is represented by the protein 6GUE. A member of the cyclin-dependent kinase family, CDK2, plays a critical role in regulating the cell cycle, particularly the passage through the S phase and the change from the G1 phase to the S phase. Since dysregulation of CDK2 activity can lead to unregulated cell proliferation and genetic instability, it is commonly associated with cancer. Because of this, CDK2 is a prospective therapeutic target for the treatment of cancer. Inhibitors like AZD5438 are being researched for their ability to reduce tumor growth by stopping CDK2 activity [45]. The obtained results demonstrate that the carvacrol compound was docked to 6GUE.pdb with a binding energy of −5.34 kcal/mol, revealing various intermolecular interactions including two Pi-Sigma bonds detected with Leu134 and Phe80 amino acids residues, more than one Hydrogen bond created towards Leu83 amino acid residue and several Alkyl and Pi-Alkyl bonds, as presented in Fig. 3A.

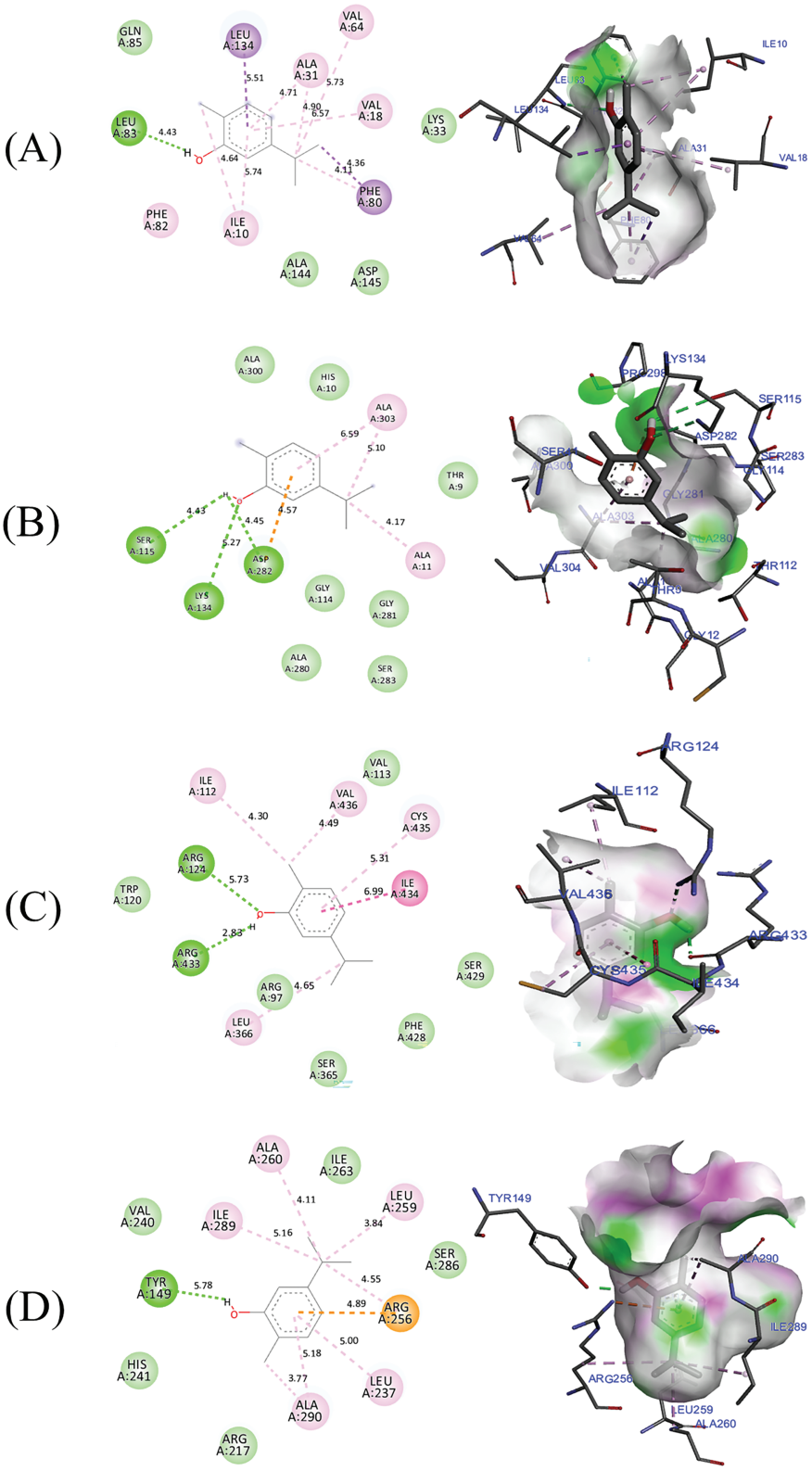

Figure 3: The intermolecular interactions between carvacrol and its target proteins, 6GUE **(A)**, 2CDU **(B)**, 1OG5 **(C)**, and 4JK4 **(D)**, in both two and three dimensions

The mechanism by which carvacrol suppresses free radicals was examined using the remaining three proteins (2CDU, IOG5, and 4JK4). A water-forming NAD(P)H oxidase from *Lactobacillus sanfranciscensis*, an enzyme that is a member of the NAD(P)H oxidase family, has a crystal structure represented by the 2CDU. By catalyzing the oxidation of NADH or NADPH, which reduces oxygen to water and controls the amounts of reactive oxygen species (ROS), this enzyme is essential for maintaining cellular redox equilibrium. 2CDU helps the cellular antioxidant defense system by regulating ROS concentrations, which stops oxidative stress and shields cellular constituents from harm [46,47]. As shown in Fig. 3B, carvacrol also complexed to the active sites of the NADPH oxidase protein encoded 2CDU.pdb, exhibiting three hydrogen bonds fixed to Ser115, Lys134, and Asp282 amino residues, more than the Alkyl and Pi-Alkyl bonds found towards Ala11 and Ala303 active sites. Second, 1OG5, a human cytochrome P450 2C9 (CYP2C9) complexed with warfarin, an anticoagulant medication, is shown. A member of the cytochrome P450 superfamily, CYP2C9 is an enzyme that is essential to the oxidative metabolism of several endogenous and exogenous substances, such as steroids, fatty acids, and medications. The liver is the primary site of expression for this enzyme, which metabolizes 15% of pharmaceuticals used in clinical settings. The 1OG5 structure offers comprehensive information about the binding interactions between warfarin and CYP2C9, exposing a new binding pocket and pointing to possible allosteric mechanisms during substrate metabolism [47–49]. As shown in Fig. 3C, the candidate molecule was once more docked to the structure of human cytochrome P450 CYP2C9 encoded as 1OG5.pdb, with a binding energy of −5.09 kcal/mol. This revealed multiple Alkyl and Pi-Alkyl bonds, two hydrogen bonds fixed to the residues of the amino acids Arg124 and Arg433, and one Pi-Pi Stacked bond with the residue of Ile434. Ultimately, the main compound was docked to the crystal structure of bovine serum albumin (4JK4.pdb), which is coded as 4Jk4.pdb. The lowest binding affinity, which was −5.54 kcal/mol, revealed various intermolecular interactions, including those found towards the Tyr149 hydrogen bond, the Arg256 anion bond, and a number of Alkyl and Pi-Alkyl bonds, as shown in Fig. 3D. The 4JK4.pdb of bovine serum albumin (BSA) is important for antioxidant defense because it may bind free radicals and scavenge reactive oxygen species (ROS). By trapping dangerous reactive molecules and halting cell and tissue damage, BSA functions as a molecular buffer in the bloodstream, lowering oxidative stress. Furthermore, BSA can neutralize ROS through thiol-based redox processes because of its high cysteine concentration, especially the free thiol group in Cys-34. Because of its antioxidant properties, BSA is essential for preserving redox equilibrium and shielding biological systems from oxidative stress [46,47,49].

The inhibition mechanisms produced by molecular docking simulations were successfully examined using the validation protocol, in which the resulting intermolecular contacts were compared to the experimental interactions in complex the native ligand bound to each protein target. Thereafter, the superposition process of docked and co-crystallized ligands was done with the root mean square deviations (RMSDs) not exceeding the 2 Å threshold, as presented in Fig. 4.

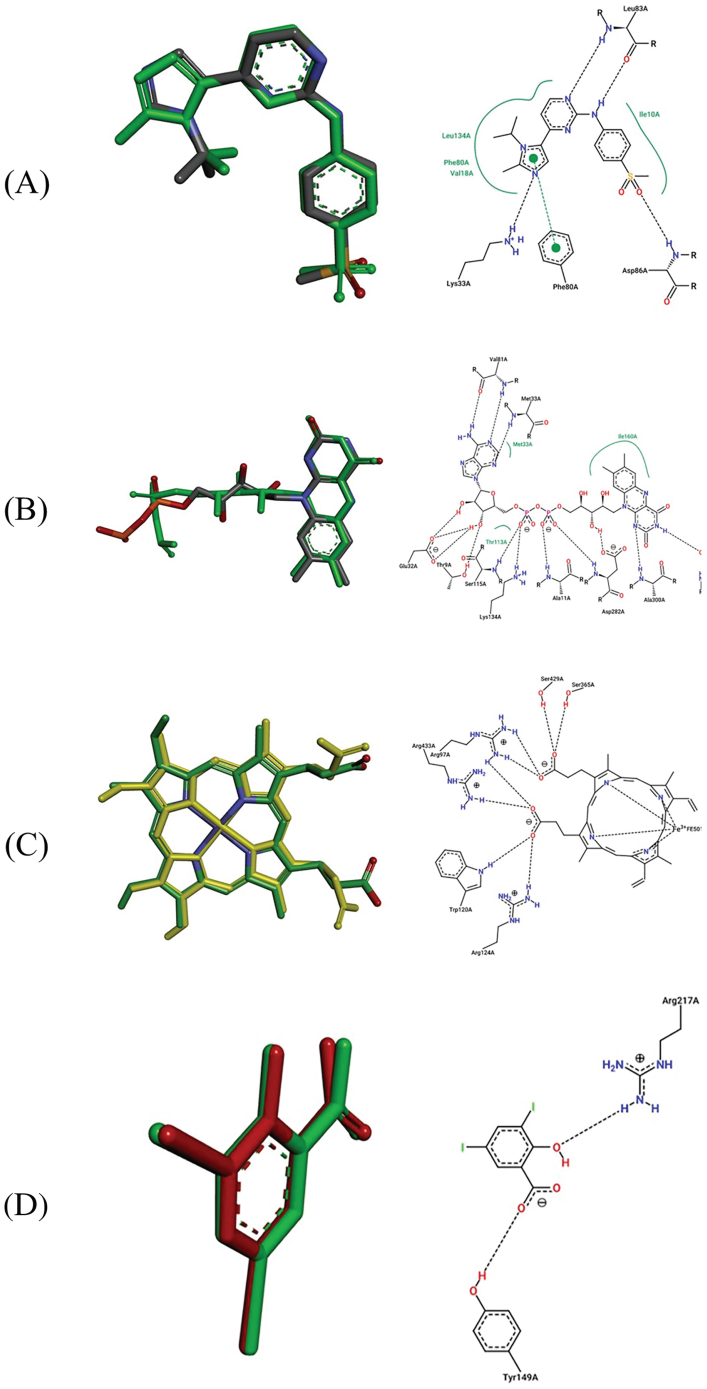

Figure 4: Results of docking validation protocol (Re-docking methodology). 6GUE (A), 2CDU (B), 1OG5 (C), and 4JK4 (D)

4 Conclusion

The study highlights the significant antioxidant and anticancer properties of *Thymus capitatus* essential oil, showing its medicinal potential. Carvacrol, p-Cymene, and γ-Terpinene were the main constituents of the 21 compounds that were discovered by GC-MS analysis. Compared to the synthetic antioxidant BHT,

the EO showed a stronger antioxidant capacity. Additionally, *T. capitatus* EO has strong anticancer effects on MCF-7 and HCT-116 cell lines. Advantageous physicochemical and pharmacokinetic profiles were validated by computational ADMET calculations. These profiles include high intestine absorption in humans, good blood-brain barriers and central nervous system permeability, and non-toxicity with no Ames mutagenicity or inhibition of important cytochrome enzymes. These results make *T. capitatus* EO a viable option for medication development, especially for uses that target cancer and oxidative stress.

Acknowledgement: Not applicable.

Funding Statement: The authors received no specific funding for this study.

Author Contributions: The authors confirm their contribution to the paper as follows: Conceptualization: Hamada Imtara and Feras Abujaber; methodology: Hamada Imtara, Feras Abujaber and Faady Siouri; software: Aziz Tumeh; validation: Aziz Tumeh and Bashar Saad; formal analysis: Hamada Imtara, Feras Abujaber, Faady Siouri, Aziz Tumeh and Bashar Saad; data curation: Hamada Imtara and Aziz Tumeh; writing—original draft preparation: Hamada Imtara; writing—review and editing: Feras Abujaber, Faady Siouri, Aziz Tumeh and Bashar Saad. All authors reviewed the results and approved the final version of the manuscript.

Availability of Data and Materials: The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Ethics Approval: Not applicable.

Conflicts of Interest: The authors declare no conflicts of interest to report regarding the present study.

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