



Phytochemical Screening, Antimicrobial, Anticancer Potentials and Molecular Docking of *Chlorella* Extract Targeting Caspase-9

Nahed Ahmed Hussien¹, Aisha Ehab Tantawy²

10.18805/IJAR.BF-2108

ABSTRACT

Background: *Chlorella*, a green alga, is rich in nutrients and bioactive compounds that support health and has potential as a source of natural therapeutic agents.

Methods: *Chlorella*'s phytochemicals were analyzed using GC-MS and its antimicrobial and anticancer properties were evaluated. Molecular docking was performed with the major active compounds of *Chlorella* targeting caspase-9.

Result: *Chlorella* extract contains trimethylsilyl palmitate (20.22%), oleic acid (9.87%) and arachidic acid methyl ester (6.12%). It exhibits antimicrobial activity against *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Bacillus cereus* and *Candida albicans*, with minimum inhibitory concentrations (MIC) and bactericidal concentrations (MBC) above 1000 µg/mL, indicating relatively low antimicrobial potency. *Chlorella* extract exhibited inhibitory activity against Huh-7 hepatocellular carcinoma (HCC) cells, with an IC₅₀ above 100 µg/mL. After docking with caspase-9, methyl arachidate is the most effective compound, with a binding affinity of -4.5 kcal/mol. It meets Lipinski's rule, shows drug-like properties via SwissADME and has low predicted toxicity (LD50≈5000 mg/kg). It is stabilized in the binding pocket with three hydrophobic and two hydrogen bonds. This initial finding suggests that *Chlorella* could be a promising candidate for HCC treatment.

Key words: Antimicrobial, Caspase-9, *Chlorella*, Huh-7, *In silico*, Phytochemicals.

INTRODUCTION

Microalgae primarily inhabit aquatic environments and have high growth rates due to efficient sunlight and CO₂ use. They contain bioactive compounds like proteins, lipids and polyphenols, used in food, pharma and cosmetics (Barkia *et al.*, 2019) and support health with antibacterial, anticancer, antioxidant, antifungal and antiviral activities (Haq *et al.*, 2019). Algae extracts contain antioxidants that may reduce oxidative stress and lower the risk of chronic diseases (Sruthy and Baiju, 2025). Reactive oxygen species (ROS), generated internally or externally, can damage cells and DNA, leading to heart, brain diseases and cancers. Antioxidants can help mitigate these risks (Haq *et al.*, 2019).

Chlorella species can be widely cultivated and supplements are now available globally. *Chlorella* cells contain nutrients and bioactives that promote health and may prevent diseases, making them potential sources for therapeutic compounds. These compounds' levels vary by culture conditions and species (Rajeswari *et al.*, 2026). Since their cell walls are cellulose, they are poorly digestible intact, requiring mechanical disruption in supplements. Commercial *Chlorella* products also include essential vitamins and minerals (Bito *et al.*, 2020).

Hepatocellular carcinoma (HCC) causes high cancer mortality worldwide, with low survival rates despite advances in technology, screening and treatment (Mustafa *et al.*, 2023). Challenges include late diagnosis, complex gene networks and drug side effects, leading to treatment failures. Effective care for end-stage liver disease and new

¹Department of Biology, College of Science, Taif University, Taif 21944, Saudi Arabia.

²Department of Computer Engineering, College of Computers and Information Technology, Taif University, Taif, Saudi Arabia.

Corresponding Author: Nahed Ahmed Hussien, Department of Biology, College of Science, Taif University, Taif 21944, Saudi Arabia. Email: n.nahed@tu.edu.sa

ORCID: <https://orcid.org/0000-0001-7019-9682>

How to cite this article: Hussien, N.A. and Tantawy, A.E. (2026). Phytochemical Screening, Antimicrobial, Anticancer Potentials and Molecular Docking of *Chlorella* Extract Targeting Caspase-9. *Indian Journal of Animal Research*. **60(7)**: 1174-1186. doi: 10.18805/IJAR.BF-2108.

Submitted: 21-01-2026 **Accepted:** 15-02-2026 **Online:** 02-04-2026

anti-HCC drugs are vital. Molecular docking studies how phytochemicals interact with proteins at the atomic level, aiding in drug development (Kattan *et al.*, 2020). Key protein targets in cancer therapy include transcription factors, apoptotic proteins, growth factor receptors, cell division kinases, MAP kinases and serine/threonine kinases. Caspases are crucial in apoptosis, initiating via two main pathways: the extrinsic and intrinsic pathways. The extrinsic pathway triggers when ligands bind to receptors, activating adaptor molecules that lead to caspase 8 activation. The intrinsic pathway involves mitochondrial signals, like membrane depolarization, causing caspase 9 to bind apoptosome complexes. Both pathways converge to activate caspases 3 and 7 (Anand *et al.*, 2020).

This study identified phytochemical compounds in a commercial *Chlorella* supplement using GC-MS and examined its antimicrobial properties. While previous research has explored *Chlorella*'s health benefits, this work focuses on its anticancer effects against hepatocellular carcinoma in Huh-7 cells. *In silico* molecular docking was used to analyze how *Chlorella*'s active compounds bind to caspase-9, a key protein in apoptosis. This approach supports the compounds' anticancer potential and predicts their interactions, enhancing understanding of their mechanisms.

MATERIALS AND METHODS

Crude extract preparation

The experiment at Taif University's Biology Department in 2025 involved suspending 100 mg of *Chlorella* powder (Organic Traditions®, Canada) in 10 ml of 95% methanol. The mixture was vortexed on ice for 20 minutes, shaken at 25°C for 1 hour, then centrifuged at 4°C for 10 minutes at 1209 g. The supernatant was collected, re-extracted twice and combined supernatants filtered through No.1 Whatman paper. The extract was dried with a rotary evaporator (BÜCHI Rotavapor R-200, Switzerland) and stored at -20°C for future analysis (Ferdous *et al.*, 2023).

Gas chromatography-mass spectroscopy analysis

The 95% methanolic *Chlorella* extract was analyzed using an RTX-5 MS capillary column (Restek) with a GC-MS-QP2010 Plus system (Shimadzu, Japan), covering 1.5-1000 m/z. Helium at 1 mL/min was the carrier gas. Detection employed an electron ionization source at 70 eV, with a split ratio of 1:10 to control helium flow at 14.1 mL/min and a 1.00 µL injection volume. The injector was at 250°C and the ion source at 200°C. Mass spectra from 45 to 450 Da were recorded at 70 eV during a 0.5-second scan. The run lasted 50 minutes with a solvent delay of 0 to 4.5 minutes. The oven started at 80°C for 4 minutes, then increased by 10°C per minute to 280°C. GC separates compounds by retention time, while MS identifies them. The software generates a chromatogram of peak abundance vs. retention time (Mohamed and Hussien, 2024). Comparing spectra with the Wiley Registry 8e library (similarity index ≥70%) helps identify compounds.

Antimicrobial evaluation

The antimicrobial activity of *Chlorella* extract was tested against five microbes: four bacteria and one fungus from Nawah Scientific Inc. (Mokatam, Cairo, Egypt): *Escherichia coli* ATCC 8739, *Enterococcus faecalis* ATCC 19433, *Staphylococcus aureus* ATCC 29213, *Bacillus cereus* ATCC 9634 and *Candida albicans* ATCC 10231. A two-fold serial dilution of *Chlorella* from 1000 to 1.953 µg/mL in Mueller-Hinton broth was used for treatment, as described by Hussien *et al.* (2025). Ciprofloxacin (1.953-1000 g/mL) served as a positive control. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Chlorella* were evaluated against various strains,

with MIC representing the lowest concentration that prevents growth and MBC the concentration that kills 99.9% of bacteria. Three replicates were performed for each strain and treatment and a microplate ELISA reader (TECAN, Switzerland) was used to measure turbidity at an optical density (OD) of 600 nm.

Sulforhodamine B (SRB) cytotoxicity

The Huh-7 hepatocellular carcinoma (HCC) cell line was obtained from Nawah Scientific Inc. (Mokatam, Cairo, Egypt). Cells were cultured in DMEM containing 100 mg/mL streptomycin, 100 U/mL penicillin and 10% heat-inactivated fetal bovine serum and maintained in a humidified atmosphere with 5% CO₂ at 37°C. Cells were treated with media containing various concentrations of *Chlorella* extract (100 µL/well; 0.001-100 µg/mL), as described by Allam *et al.* (2018). Media without *Chlorella* served as a negative control, while cisplatin (0.01-100 µg/mL) served as a positive control. Absorbance was measured at 540 nm and the extract was tested in triplicate at each concentration to ensure accuracy.

Statistical analysis

Data were presented as mean ± SE of replicates and analyzed using non-linear regression in OriginLab to generate a sigmoidal dose-response curve of Log₁₀ *Chlorella* extract concentration versus cell viability. IC₅₀ value was interpolated from the curve.

Molecular docking study

Based on GC-MS results, we selected the most abundant compounds for molecular docking with caspase-9 as the receptor. The 2D and 3D structures of these compounds (ligands) were obtained from the PubChem database. The 3D crystal structure of dimeric caspase-9 bound to ligand (D-Malate) (PDB ID: 2ar9, resolution 2.80 Å) was downloaded from RCSB PDB (<http://www.rcsb.org/pdb/home/home.do>). We used PyMOL to remove D-Malate and water molecules from the receptor. SwissADME was employed to predict their physicochemical properties, drug-likeness, pharmacokinetics and medicinal chemistry features (<http://www.swissadme.ch/>) (Daina *et al.*, 2017). The top-docked compounds were selected according to Lipinski's rule of five, a guideline for ideal drug candidates. Lipinski suggests that a compound demonstrates drug-like properties if it meets certain criteria: a molecular weight of 500 or less, no more than 5 hydrogen bond donors, no more than 10 hydrogen bond acceptors, lipophilicity below 5 and molar refractivity between 40 and 130 (Lipinski *et al.*, 2001). Predicted LD₅₀ values and toxicities for the seven compounds were determined using the ProTox-II web server (https://tox-new.charite.de/protox_II/) (Banerjee *et al.*, 2024). Molecular docking of selected compounds into the receptor protein's binding pockets was carried out with SMINA/Anaconda. Additionally, re-docking was performed to validate the accuracy of the docking protocol. Docking validation involved re-docking the co-crystallized ligand

(D-Malate) into the active site using AutoDock's fast scoring function. The most favorable conformations were chosen by comparing RMSD values between the protein and the co-crystallized ligand (Hevener *et al.*, 2009). After docking, the resulting complexes were examined with the Protein Ligand Interaction Profiler (PLIP, <https://plip-tool.biotech.tu-dresden.de/plip-web/plip/index>) to identify non-covalent interactions, such as hydrogen bonds, hydrophobic contacts, water bridges and ionic interactions and to generate their 2D visualizations (Adasme *et al.*, 2021).

RESULTS AND DISCUSSION

GC-MS analysis of the *Chlorella* extract identified phytochemicals, including fatty acids, hydrocarbons, phenolics, terpenes and their derivatives (Fig 1). The major phytochemical components of *Chlorella* extract are high-oleic algal oil, fatty acids and their derivatives (including arachidic, palmitic, oleic and linolenic) (Table 1).

This confirms that *Chlorella*'s commercial dietary supplement is as rich in fatty acids as cultivated (Pantami *et al.*, 2020). The main phytochemicals in *Chlorella* extract include trimethylsilyl palmitate (20.22%), oleic acid (9.87%), oleic acid trimethylsilyl ester (6.25%), arachidic acid methyl ester (6.12%), linoleoyl chloride (5.81%), monopalmitin (5.80%), 3Beta-hydroxy-5-cholestene 3-oleate (3.64%) and Algal oil (3.36%) (Table 1). Pantami *et al.* (2020) reported that *C. vulgaris* extract contains over 60% fatty acids, rich in omega-6, -7, -9 and -13, with omega-6 (notably 9,12-Octadecadienoic acid, Linoleic acid at 35.1%) being predominant, as determined through GC-MS analysis. Consequently, *Chlorella* species may serve as a valuable nutritional source when added to diets, highlighting its potential as an outstanding dietary supplement.

The study found that trimethylsilyl palmitate, a palmitic acid ester, is the main component, comprising 20.22% of the extract. This ester exhibits various effects, including antioxidant, hypocholesterolemic, nematocidal, hemolytic, 5-alpha-reductase inhibition, antipsychotic, anti-androgenic and anticancer activities (El-Fayoumy *et al.*, 2021). Next are Oleic acid (9.87%) and its derivative (6.25%), which play beneficial roles in diseases such as coronary heart disease, rheumatoid arthritis and cancer (Piccinin *et al.*, 2019).

Arachidic acid methyl ester (6.12%) exhibits antifungal and antioxidant properties (Abdelillah *et al.*, 2013) and helps prevent cholesterol gallstones by acting as a cholesterol solubilizer (Gilat *et al.*, 2001). Phenolics in *Chlorella* sp. combat reactive oxygen and singlet oxygen species and decrease ROS production (Pradhan *et al.*, 2021).

Chlorella extract exhibits antimicrobial effects against Gram-negative bacteria, *E. coli* and Gram-positive bacteria, *E. faecalis*, *S. aureus* and *B. cereus*, as well as the fungus *Candida albicans*. Its MIC and MBC are over 1000 µg/mL, but it's less potent than Ciprofloxacin (Table 2). As previously reported, MIC values for antibiotics range from 0.01 to 10 µg/mL, whereas plant extracts are considered antimicrobials if their MICs fall between 100 and 1000 µg/mL (Silva *et al.*, 2013). This large gap between the antibiotic and the natural extract used could be attributed to several factors. The difference in their nature, solubility and size. Using a crude extract is less effective than using a single, isolated compound (Ben-Khalifa *et al.*, 2021).

The current findings align with previous research reporting *Chlorella* sp.'s antibacterial effects against Gram-positive bacteria, *S. epidermidis*, *S. aureus*, Methicillin-resistant *S. aureus* (MRSA), *E. faecalis* and *B. thuringiensis*, as well as Gram-negative bacteria, *E. coli*, *S. sonnei* and *S. marcescens* (Shaima *et al.*, 2022). They have reported that the inhibition zones ranged from 11 to 13.8 mm, with MIC values of 0.39-6.25 mg/mL. Additionally, antibiotics demonstrated stronger bacterial inhibition across various strains. The antimicrobial activity of *Chlorella* sp. is primarily attributed to its phytochemicals, especially polyphenols, which promote hydrogen peroxide production under aerobic conditions and are linked to hydroxyl groups (Dell'Anno *et al.*, 2019).

Various chemical groups, such as phlorotannins, fatty acids, peptides, terpenes and polysaccharides, have been found to inhibit bacterial growth in algae. Nonetheless, the exact mechanisms behind this inhibition remain unclear (Shannon and Abu-Ghannam, 2016). Algal free fatty acids inhibit the bacterial electron transport chain and oxidative phosphorylation, disrupting ATP transfer. They inhibit enzymes like bacterial enoyl-acyl carrier protein reductase, crucial for fatty acid synthesis, causing cell lysis and

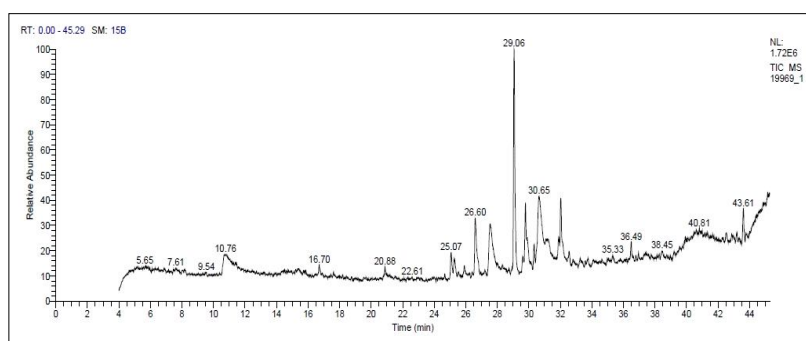
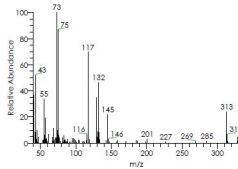
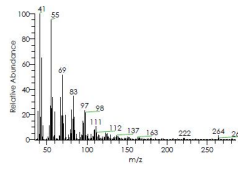
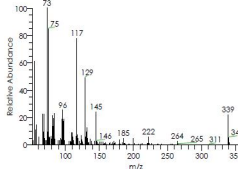
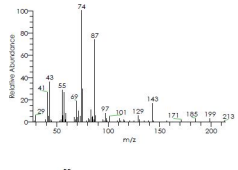
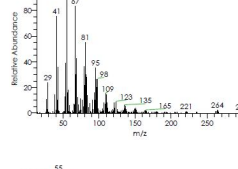
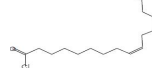
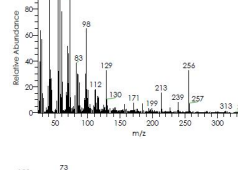
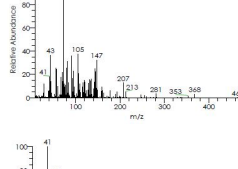
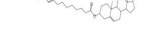
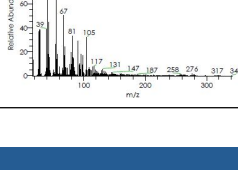


Fig 1: Chromatogram of *Chlorella* methanolic extract.

degradation from peroxidation and auto-oxidation (Pradhan *et al.*, 2014). Algal and sulfated polysaccharides interact with glycoprotein receptors, bind to bacterial walls, membranes and DNA and increase permeability,

causing protein leakage and DNA binding (Pereira and Valado, 2025). Amphipathic peptides bind to polar and nonpolar membrane regions, disrupting bacterial processes and replication (Nguyen *et al.*, 2011).

Table 1: Major compounds of *Chlorella* and their chemical structures.

Compound name	Synonyms	Area %	Molecular formula	Mass spectrum	Structure
Hexadecanoic acid, Trimethylsilyl ester	Trimethylsilyl palmitate	20.22%	$C_{19}H_{40}O_2Si$		
9-Octadecenoic acid (z)-	Oleic acid	9.87%	$C_{18}H_{34}O_2$		
Oleic acid, (Z)-, TMS derivative	Oleic acid, trimethylsilyl ester	6.25%	$C_{21}H_{42}O_2Si$		
Eicosanoic acid, methyl ester	Arachidic acid methyl ester; methyl arachidate	6.12%	$C_{21}H_{42}O_2$		
9,12-Octadecadienoyl chloride, (Z,Z)-	Linoleoyl chloride	5.81%	$C_{18}H_{31}ClO$		
Hexadecanoic acid, 2,3-dihydroxypropyl ester	Monopalmitin	5.80%	$C_{19}H_{38}O_4$		
Cholest-5-en-3-yl (9z)-9-octadecenoate	3Beta-hydroxy-5-cholestene 3-oleate	3.64%	$C_{45}H_{78}O_2$		
Hi-Oleic algal oil	Algal oil	3.36%	$C_{21}H_{42}O_{11}$		This structure is not available because it is not discrete.

All proposed antimicrobial mechanisms of algae are outlined in Fig 2.

HCC is among the most prevalent primary liver cancers and ranks as the fourth leading cause of cancer-related deaths worldwide (Mustafa *et al.*, 2023). Results shown in Fig 3 indicate that *Chlorella* demonstrated a dose-dependent anticancer effect from 0.01 to 100 µg/mL. After 72 hours, cell viability was significantly reduced, with an IC₅₀ exceeding 100 µg/mL, suggesting relatively lower cytotoxicity than cisplatin. These findings align with Shanab *et al.* (2012), who reported the anticancer potential of aqueous *Chlorella* extract against Ehrlich Ascites Carcinoma cells and HepG2 cells at 100 µg/mL. *Chlorella*'s anticancer effects are attributed to polar compounds such as phycobilins, phenolics and polysaccharides that induce apoptosis (Aboul-Enein *et al.*, 2011). Oleic acid in *Chlorella* promotes apoptosis and differentiation in colorectal HT-29 cells (Llor *et al.*, 2003) and decreases Her-2/neu expression in breast cancer cells (Menendez *et al.*, 2005). Studies show *Chlorella* sp. has anticancer effects by activating apoptosis through increased P53, Bax and caspase-3, decreased Bcl-2 and suppressed AKT/mTOR pathways, causing DNA damage and apoptosis (Sawasdee *et al.*, 2023).

Seven phytocomponents-trimethylsilyl palmitate; oleic acid; oleic acid trimethylsilyl ester; arachidic acid methyl

ester (methyl arachidate); linoleic acid chloride (linoleoyl chloride); 2,3-Dihydroxypropyl hexadecanoate (monopalmitin) and 3Beta-hydroxy-5-cholestene 3-oleate (cholesteryl oleate)-were examined for Caspase-9 docking studies. Tables 3-6 present data on the pharmacokinetics, physicochemical properties, medicinal chemistry and drug-likeness of these compounds. Linoleoyl chloride was omitted because its calculated parameters were unavailable. Cholesteryl oleate does not meet Lipinski's rules, with two violations: MW>500 and MLOGP>4.15. Only methyl arachidate and monopalmitin were selected for Caspase-9 docking because they meet these criteria, which indicate potential drug-like properties and lower toxicity, with a predicted LD₅₀ of 5000 mg/kg. Both are expected to be non-mutagenic, non-tumorigenic and non-irritant. Methyl arachidate is highly lipophilic, resulting in low gastrointestinal absorption and limited blood-brain barrier permeability, whereas monopalmitin is less lipophilic, with good gastrointestinal absorption and blood-brain barrier permeability. These two compounds were docked into the Caspase-9 chain A docking pocket, replacing the co-crystallized ligand (Malate), with a binding affinity of -4.5 kcal/mol.

Based on the data in Fig 4 and Table 7, methyl arachidate appears more stable within the docking site, forming three hydrophobic contacts and two hydrogen

Table 2: The minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC) of *Chlorella* sp.

Bacterial strain	<i>Chlorella</i> (µg/mL)		Ciprofloxacin (µg/mL)	
	MIC	MBC	MIC	MBC
<i>Escherichia coli</i>	>1000	>1000	<1.95	<1.95
<i>Enterococcus faecalis</i>				250
<i>Staphylococcus aureus</i>				31.25
<i>Bacillus cereus</i>				<1.95
<i>Candida albicans</i>				<1.95

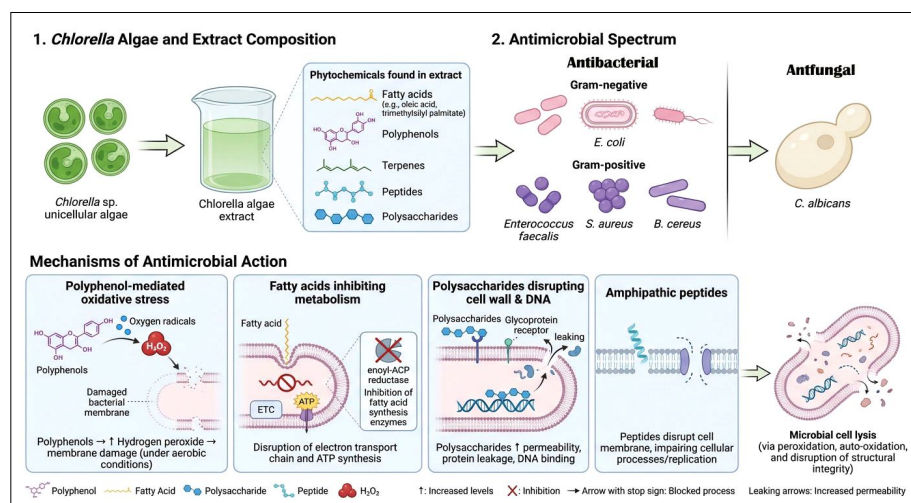
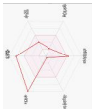
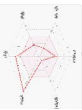
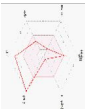
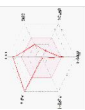
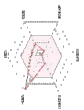
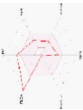
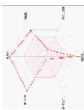


Fig 2: Schematic of the proposed antibacterial mechanism of *Chlorella* extract's active components.

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Table 3: Physicochemical properties of selected compounds found in *Chlorella* sp.

Molecule	Radar chart	MW g/mol	Number of heavy atoms	Number of aromatic heavy atoms	Fraction Csp3	Number of rotatable bonds	Number of H-bond acceptors	Number of H-bond donors	MR	TPSA (Topological polar surface area) Å²
Palmitic acid, trimethylsilyl ester		328.61	22	0	0.95	16	2	0	102.60	26.30
Oleic acid		282.46	20	0	0.83	15	2	1	89.94	37.30
Oleic acid, trimethylsilyl ester		354.64	24	0	0.86	17	2	0	111.74	26.30
Methyl arachidate		326.56	23	0	0.95	19	2	0	104.35	26.30
Linoleoyl chloride		298.89	20	0	0.72	14	1	0	92.69	17.07
Monopalmitin		330.50	23	0	0.95	18	4	2	97.06	66.76
Cholesteryl oleate		651.10	47	0	0.89	22	2	0	209.79	26.30

MW: Molecular weight; MR: Molecular refractivity.

bonds with the receptor protein. In contrast, monopalmitin forms two hydrophobic interactions and three hydrogen bonds. Methyl arachidate interacts with caspase-9 at residues TRP (chain A):354, ARG:355, PRO:357 and makes two hydrogen bonds at ARG: 180 and ARG: 355. Meanwhile, monopalmitin interacts with caspase-9 at VALA: 352, TRPA:354 and forms three hydrogen bonds at ARG: 355 amino acids of the active pocket.

Molecular docking of p53, caspase-9 and cyclin D1, which play roles in apoptosis and cell proliferation, is a target for inducing apoptosis in the MCF-7 breast cancer cells (Nurhayati *et al.*, 2022) and HepG2 liver carcinoma cells (Anand *et al.*, 2020). *In silico* and docking results indicate that methyl arachidate may inhibit caspase-9 and could serve as a promising liver anticancer agent. Our findings align with Mustafa *et al.* (2023), who docked the phytochemicals limonin and obamegine with caspase-9. Limonin interacted with amino acids ArgA:355,

TrpA:354, GlyA:238, ArgA:178 and LeuA:177 at the active site. Obamegine bound to ProA:338, ProA:336, LeuA:335, AsnA:265, PheA:246 and LeuA:244 within the binding pocket. Additionally, Siddiqui *et al.* (2021) docked eight *Moringa oleifera* phytochemicals with caspase-3, where benzyl glucosinolate (binding affinity -8.4 kcal/mol) interacted with Phe265, Phe250, Arg207, Trp206, Tyr204 and Thr62. Suganya and Anuradha (2019) also docked astaxanthin, which interacted with amino acids Ile403, Cys402, Met400, Tyr397, Ile396, Phe351 and Phe348 of caspase-9.

Our findings agree with Wanandi *et al.* (2020), who studied the anti-cancer potential of a natural compound by examining its interactions with survivin, caspase-9 and caspase-3 *via* computational methods. In 2023, Mustafa *et al.* (2023) docked plant phytochemicals-liquoric acid (-9.8 kcal/mol), madecassic acid (-9.3 kcal/mol), limonin (-10.5 kcal/mol) and obamegine (-9.3 kcal/mol)-against EGFR and caspase-9. However, the molecular mechanisms behind HCC development, progression and metastasis are not well understood. In the current *in silico* analysis, we targeted caspase-9, a protease that triggers apoptosis *via* the intrinsic or mitochondrial pathway and is activated at sites involving multiple proteins (Kim *et al.*, 2015). Extracellular signal-regulated kinases-1 and 2 (ERK1/2) are part of the mitogen-activated protein (MAP) kinase superfamily and regulate cell proliferation and survival. They inhibit apoptosis by deactivating proapoptotic proteins and supporting cell survival by blocking caspase-9 activity (Boston *et al.*, 2011). Natural chemicals targeting caspase-9 could be promising for HCC treatment. Microalgae are rich in biocompatible compounds, making them valuable nutraceuticals (Sawasdee *et al.*, 2023;

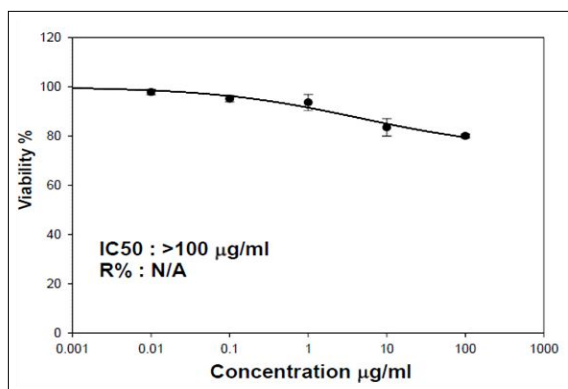


Fig 3: Cell viability (%) of Huh-7 cell line treated with *Chlorella*.

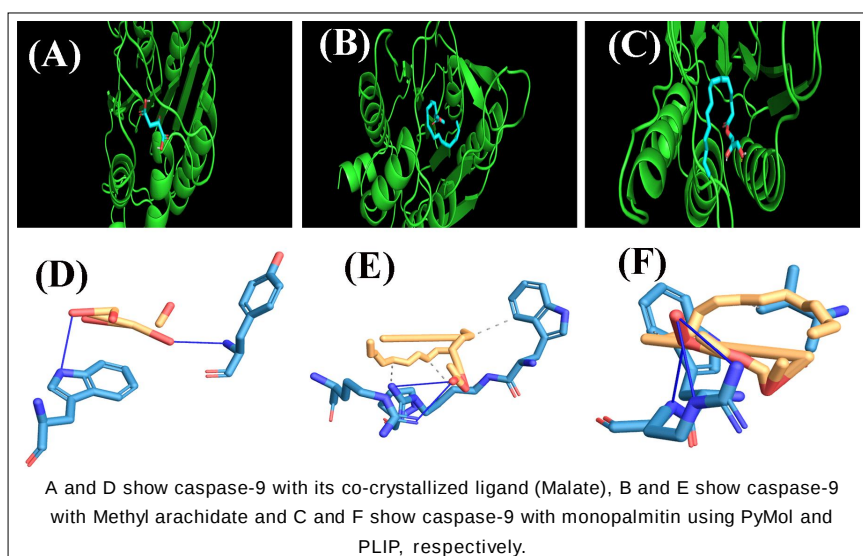


Fig 4: The non-covalent interactions (hydrogen and hydrophobic bonds) between caspase-9 and ligands.

Table 4: Lipophilicity of the selected phytocomponents.

Molecule	Log Po/w (iLogP)	XLogP3	WLogP	MLogP	Silicos-IT Log P	Consensus Log P
Palmitic acid, trimethylsilyl ester	5.33	8.87	6.85	4.91	5.30	6.25
Oleic acid	4.01	7.64	6.11	4.57	5.95	5.65
Oleic acid, trimethylsilyl ester	5.75	9.02	7.40	5.25	6.00	6.68
Methyl arachidate	5.35	9.30	7.20	5.36	7.60	6.96
Linoleoyl chloride	-	-	6.57	-	-	-
Monopalmitin	3.93	6.33	4.36	3.18	5.42	4.64
Cholesteryl oleate	8.35	16.59	13.98	9.24	13.48	12.33

(-) Means unknown data.

Table 5: Water solubility of selected compounds.

Molecule	Log S (ESOL)	Solubility (ESOL) (mg/ml)	Solubility (ESOL) (mol/l)	ESOL class	Log S (Ali)	Solubility (Ali) (mg/ml)	Solubility (Ali) (mol/l)	Ali class	Log S (Silicos-IT)	Solubility (Silicos-IT) (mg/ml)	Silicos-IT solubility (mol/l)	Silicos-IT class
Palmitic acid, trimethylsilyl ester	-6.41	1.28e-04	3.90e-07	Poorly soluble	-9.31	1.62e-07	4.92e-10	Poorly soluble	-6.86	4.50e-05	1.37e-07	Poorly soluble
Oleic acid	-5.41	1.09e-03	3.85e-06	Moderately soluble	-8.26	1.54e-06	5.46e-09	Poorly soluble	-5.39	1.14e-03	4.04e-06	Moderately soluble
Oleic acid, trimethylsilyl ester	-6.60	8.92e-05	2.52e-07	Poorly soluble	-9.46	1.22e-07	3.44e-10	Poorly soluble	-6.94	4.11e-05	1.16e-07	Poorly soluble
Methyl arachidate	-6.47	1.11e-04	3.39e-07	Poorly soluble	-9.75	5.75e-08	1.76e-10	Poorly soluble	-7.61	8.11e-06	2.48e-08	Poorly soluble
Linoleoyl chloride	-	-	-	-	-	-	-	-	-	-	-	-
Monopalmitin	-4.69	6.76e-03	2.05e-05	Moderately soluble	-7.52	9.94e-06	3.01e-08	Poorly soluble	-5.30	1.64e-03	4.95e-06	Moderately soluble
Cholesteryl oleate	-12.88	8.65e-11	1.33e-13	Insoluble	-17.32	3.12e-15	4.80e-18	Insoluble	-11.88	8.62e-10	1.32e-12	Insoluble

(-) Means unknown data.

Table 6: Drug-likeness, pharmacokinetics, and medicinal chemistry of *Chlorella* selected compound.

Molecule	Gastrointestinal (GI) absorption	Blood-brain barrier (BBB) permeant	log Kp (cm/s) (Skin permeation)	Lipinski violations number	Bioavailability score	PAINS alerts	Break alerts	Leadlikeness violations	Synthetic accessibility
Palmitic acid, trimethylsilyl ester	Low	No	-2.01	Yes; 1 violation: MLOGP >4.15	0.55	0 alert	1 alert: heavy _metal	No; 2 violations: Rotors>7, XLOGP3>3.5	4.53
Oleic acid	High	No	-2.60	Yes; 1 violation: MLOGP >4.15	0.85	0 alert	1 alert: isolated _alkene	No; 2 violations: Rotors>7, XLOGP3>3.5	3.07
Oleic acid, trimethylsilyl ester	Low	No	-2.06	Yes; 1 violation: MLOGP >4.15	0.55	0 alert	2 alerts: heavy _metal, isolated _alkene	No; 3 violations: MW>350, Rotors>7, XLOGP3>3.5	4.64
Methyl arachidate	Low	No	-1.69	Yes; 1 violation: MLOGP >4.15	0.55	0 alert	0 alert	No; 2 violations: Rotors>7, XLOGP3>3.5	3.00
Linoleoyl chloride	-	-	-	-	-	0 alert	0 alert	-	-
Monopalmitin	High	Yes	-3.82	Yes; 0 violation	0.55	0 alert	0 alert	No; 2 violations: Rotors>7, XLOGP3>3.5	3.88
Cholesteryl oleate	Low	No	1.51	No; 2 violations: MW>500, MLOGP>4.15	0.17	0 alert	1 alert: isolated _alkene	No; 3 violations: MW>350, Rotors>7, XLOGP3>3.5	7.96

(-) Means unknown data; PAINS, pan-assay interference compounds.

Table 7: The non-covalent interactions (hydrogen and hydrophobic bonds) between Caspase 9 and ligands.

Caspase 9 and malate									
Hydrogen bonds									
Index	Residue	Amino acid	Distance H-A	Distance D-A	Donor angle	Protein donor?	Side chain	Donor atom	Acceptor atom
1	362A	TRP (Tryptophan)	3.20	3.83	113.35	✓	✓	2668 [Nar]	3585 [O3]
2	397A	TYR (Tyrosine)	2.67	3.75	150.15	✓	×	3220 [Nam]	3587 [O3]
Caspase 9 and methyl arachidate									
Hydrophobic interactions									
Index	Residue	Amino acid	Distance	Ligand atom	Protein atom				
1	354A	TRP	3.58	3592	2542				
2	355A	ARG	3.08	3583	2561				
3	357A	PRO (Proline)	3.37	3586	2598				
Hydrogen bonds									
Index	Residue	Amino acid	Distance H-A	Distance D-A	Donor angle	Protein donor?	Side chain	Donor atom	Acceptor atom
1	180A	ARG (Arginine)	3.91	3.79	106.60	✓	✓	611 [Ng+]	3601 [O3]
2	355A	ARG	3.87	3.10	130.40	✓	✓	2567 [Ng+]	3601 [O3]
Caspase 9 and monopalmitin									
Hydrophobic interactions									
Index	Residue	Amino acid	Distance	Ligand atom	Protein atom				
1	352A	VAL (Valine)	3.14	3587	2511				
2	354A	TRP	2.88	3591	2542				
Hydrogen bonds									
Index	Residue	Amino acid	Distance H-A	Distance D-A	Donor angle	Protein donor?	Side chain	Donor atom	Acceptor atom
1	355A	ARG	2.43	3.57	159.56	✓	✓	2564 [Ng+]	3602 [O3]
2	355A	ARG	2.65	3.50	127.33	✓	✓	2567 [Ng+]	3602 [O3]
3	355A	ARG	3.06	3.75	116.77	✓	×	2557 [Nam]	3599 [O3]

Yu *et al.*, 2024). Their phytochemicals may serve as future drug candidates against HCC.

CONCLUSION

This study assesses a commercial *Chlorella* supplement and its potential effects. *In silico* and docking studies suggest that methyl arachidate, a key component, may inhibit caspase-9, but further validation is needed. More research and trials are essential to confirm *Chlorella*'s biological properties. Overall, the findings highlight natural microalgae compounds' potential for developing therapies for hepatocellular carcinoma (HCC) and encourage future medicinal research.

ACKNOWLEDGEMENT

The authors would like to acknowledge the Deanship of Graduate Studies and Scientific Research, Taif University for funding this work.

Research funding

The authors would like to acknowledge the Deanship of Graduate Studies and Scientific Research, Taif University for funding this work.

Conflict of interest

Authors state no conflict of interest.

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