Phytochemical, Antioxidant, and Anticancer Evaluation of *Passiflora edulis* Leaf Extract Using *In-vitro* and *In-silico* Approaches

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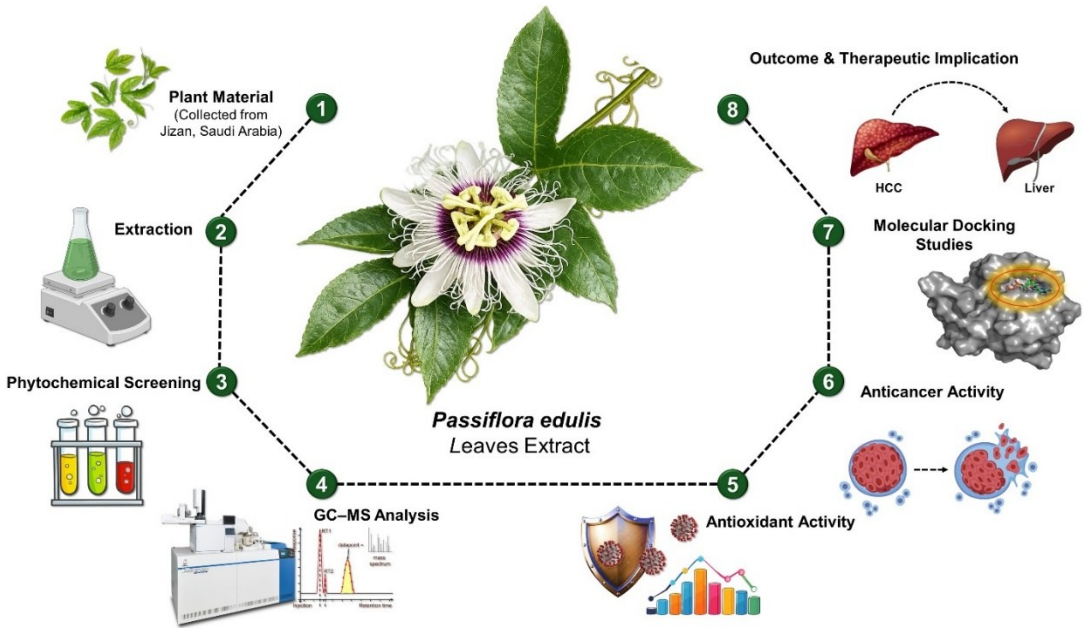
GRAPHICAL ABSTRACT



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Methanolic leaf extract of *Passiflora edulis* was characterized by its phytochemical composition and biological activities in an integrated *in vitro* and *in silico* approach. Qualitative phytochemical screening indicated that bioactive secondary metabolites such as flavonoids, saponins, and steroids were present. The DPPH activity increased in a dose-dependent manner, reaching 81% at the highest concentration, without implying a complete or maximal endpoint. The cytotoxic study in HepG2 human liver cancer cells revealed a moderate antiproliferative effect with an IC_{50} value of 251 µg/mL. Gas chromatography–mass spectrometric analysis showed different phytoconstituents and 13-docosenamide (Z) as predominant, however hexadecane and N-isopropyl-3-phenylpropanamide were also present. Molecular docking studies showed moderate binding affinities (−6.5 to −7.8 kcal/mol) of selected compounds against the target protein, which represented PCSK9–LDLR complex. Significantly, N-isopropyl-3-phenylpropanamide demonstrated the most favorable binding interactions by engaging in both hydrogen bonding and hydrophobic interactions with key amino acid residues, indicating potential modulation of protein function. These results suggest that the biological activity of *P. edulis* extract may be mediated through multi-target interactions with cancer-associated metabolic pathways. Nonetheless, moderate cytotoxic potency and basic extract evaluation justify further studies.

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Keywords: Passiflora edulis; GC–MS; Antioxidant activity; HepG2; Cytotoxicity; Molecular docking; Anticancer activity

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INTRODUCTION

Hepatocellular carcinoma (HCC) accounts for approximately 75% to 85% of primary liver cancers and is one of the most common malignant tumors in the world, contributing to cancer-related death (Sung *et al.* 2020). The prognosis of patients with HCC remains grim, and the five-year survival rate is under 20% in most regions despite progress in diagnostic and therapeutic modalities (Amin *et al*. 2025). This poor prognosis is due to late-stage diagnosis, high tumor recurrence rates and the limited success of currently available therapies. Traditional systemic therapies, including multikinase inhibitors (*e.g.*,

sorafenib and lenvatinib) and immune checkpoint inhibitors result in limited survival with the common drawbacks of acquired resistance, off-target toxicity, as well as significant economic costs (Sadrinasab *et al*. 2025). These constraints highlight a pivotal demand for new therapeutic agents with both potency and mechanistic diversity. The molecular pathogenesis of HCC is extremely complex and involves a complex network of interactions among chronic inflammation, oxidative stress, metabolic dysregulation and genetic and epigenetic alterations. The constant generation of reactive oxygen species (ROS) leading to DNA damage, mitochondrial impairment, and oncogenic pathways activation is at the heart of hepatocarcinogenesis (An and Li 2025). Several key molecular cascades have been implicated in the progression of HCC, including the PI3K/Akt/mTOR, MAPK/ERK and Wnt/β-catenin as well as NF-κB pathways that coordinately regulate cellular proliferation apoptosis resistance angiogenesis and metastasis. Dysregulation of these pathways facilitates tumor initiation and progression and increases therapeutic resistance. Therefore, simultaneous targeting of multiple signaling nodes is a promising strategy to enhance treatment outcomes in HCC (Xue *et al*. 2024).

The significant number of anticancer drugs developed from natural sources with a plant source origin is a well-known fact, and natural products have always played an essential role in the process of discovery for anticancer drugs. Essentially, phytochemicals (flavonoids, alkaloids, terpenoids, as well as phenolic compounds) demonstrate diverse biological activities such as antioxidative activity, anti-inflammatory action, and antiproliferative effects (Chunarkar-Patil *et al*. 2024). It is noteworthy that these compounds achieve their anticancer activity mainly *via* a multi-target mechanism that modulates crucial signaling pathways implicated in tumor advancement, while reducing toxicity to healthy tissue. Therefore, this polypharmacological nature of many plant-derived compounds makes them particularly appealing candidates in the next-generation approaches for anticancer therapeutics development most especially complex malignancies like HCC (Khan *et al*. 2019).

Passiflora edulis Sims (family Passifloraceae), popularly known as passion fruit, is a tropical medicinal plant widely utilized in many traditional medicine systems. Although considerable studies with reported bioactive metabolites have been found in the fruit, peel, and seeds of *P. edulis* (such as flavonoids, phenolic acids, alkaloids and glycosides which exhibited antioxidant, anti-inflammatory, and antimicrobial activities), relatively fewer studies have yet been done on determining the pharmacological activity of leaves of this plant (He *et al*. 2020). A growing body of evidence reflects that due to the defensive mechanisms activated in plant leaves, these organs generally aggregate a higher concentration of secondary metabolites. Therefore, they represent a promising but still under-explored source of bioactive compounds (Gulcin 2025).

However, several knowledge gaps remain that limit interest in *P. edulis*. Firstly, there is a need for comprehensive and systematic studies on *P. edulis* leaf extracts anticancer activity against specific HCC models. Second, most previous studies have been restricted to individual bioactivity evaluations and lack an integrated phytochemical profiling to functional and mechanistic investigations (Villada Ramos *et al*. 2023). Third, there is a need for cytotoxicity/phytochemical work on *in silico* docking specifically for *Passiflora edulis*. The authors are aware of no such work in the context of hepatocellular carcinoma (HCC), which is precisely the gap our manuscript aims to address. The closest related work, Huang and Huang (2024), evaluated acetone-extracted *P. edulis* rind for cytotoxic effects on oral carcinoma cells alongside antibacterial activity, but that work did not extend to HCC cell lines or incorporate molecular docking. Similarly, a 2025 study

integrating metabolomic profiling and molecular docking on *P. edulis* by-product fractions focused on anti-aging/collagenase targets rather than anticancer mechanisms, again confirming the absence of a combined *in vitro–in silico* HCC-specific framework for this species. For the broader HCC angle, the most directly comparable integrated study that was found in the literature search used a different plant: a study on *Lepidium sativum* combined *in vitro* cytotoxicity testing against HuH-7 and HepG2 hepatocellular carcinoma cell lines, GC-MS-based chemical characterization, apoptotic gene expression analysis, and molecular docking of the major identified metabolites against predicted biological targets. The search results suggest that this type of fully integrated workflow remains rare, and it has, to our knowledge, not previously been applied to *P. edulis* in an HCC context. The current study aimed to enhance insights on *P. edulis* leaves as a promising source of bioactive compounds for anticancer drug development through phytochemical profiling combined with antioxidant, antimicrobial, cytotoxic, and molecular docking evaluation through phytochemical profiling combined with antioxidant, antimicrobial, cytotoxic, and molecular docking evaluation.

MATERIALS AND METHODS

Chemicals and Reagents

Throughout the study, all analytical grade chemicals and reagents were employed. DMSO, methanol, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were obtained from standard suppliers. Gibco (USA) supplied Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), penicillin–streptomycin and trypsin. MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) was procured from Sigma-Aldrich (USA) (Dechayont *et al*. 2021).

Plant Material and Authentication

Fresh leaves of *Passiflora edulis* Sims were collected from the private farm in the Jizan region, Saudi Arabia. Plant material was taxonomically authenticated by a qualified taxonomist and a voucher specimen (Ref. no. MU-BIO-0112) was deposited in the herbarium of the Department of Biology, College of Science Zulfi, Majmaah University for future reference.

Preparation of Extract

The collected leaves were washed, followed by shade drying at room temperature (25 to 28 °C) for 15 to 20 days. Then they were ground to coarse powder. About 10 g of powdered sample was extracted with a Soxhlet apparatus (200 mL methanol, 1:20 w/v) for around 8 h (~25 to 30 cycles). The extract was evaporated using a rotary evaporator at 40 °C under reduced pressure and stored at 4 °C in amber-colored bottles until required. The extract was obtained and the percentage yield were determined (Ahmed *et al*. 2026). Similarly, the other three solvents (ethanol, acetone, and ethyl acetate) were used for extraction following the same procedure, and these extracts were employed in the antimicrobial assay, whereas the methanolic extract was used for all other assays.

Phytochemical Screening

Qualitative phytochemical analysis was carried out to screen the major secondary metabolites notably alkaloids, flavonoids, saponins, steroids, phenols, terpenoids, and carbohydrates according to standard protocols (Dubale *et al*. 2023).

DPPH Radical Scavenging Assay

The antioxidant activity for the extract was determined according to the DPPH free radical scavenging assay by Brand-Williams *et al*. (1995). Dilutions of the extract (25 to 400 µg/mL) were prepared and mixed with DPPH solution (Baliyan *et al*. 2022). Absorbance at 517 nm was measured for the reaction mixture, incubated in the dark for 30 min, using a UV–Vis spectrophotometer. Ascorbic acid was used as the standard. Percent inhibition was calculated and IC_{50} values were obtained. Each experiment was performed in triplicate (n=3) (Basu and Maier 2016).

GC–MS Analysis

The methanolic extract was analyzed with GC–MS (PerkinElmer Clarus 500) system, which was fitted with an Elite-1 capillary column (30 m × 0.25 mm × 1 µm). Helium served as the carrier gas at a flow rate of 1.0 mL/min (Kanthal *et al*. 2014; Viji *et al*. 2025a). From 45 to 200 °C oven temperature was programmed in increasing increments. Injections of 2 µL with a split ratio of 25:1 were used. Mass spectra were acquired at 70 eV over a scan range of 40 to 550 *m*/*z* and compounds were identified by matching observed spectra to those available on the NIST library database (Znidersic *et al*. 2020).

Antimicrobial Activity

The antibacterial and antifungal activities were determined by using the agar well diffusion method as described by Bauer *et al*. (1966). *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Candida albicans* microbial suspensions were inoculated onto Mueller–Hinton agar plates and wells end-loaded with extract solutions (Hossain 2024; Ramya *et al*. 2026). Plates were incubated for 24 h at 37 °C, and zones of inhibition were measured (in mm). Positive controls were standard antibiotics (tetracycline and fluconazole). All assays performed in triplicate (n = 3) (Alex *et al*. 2025; Viji *et al*. 2025b). The minimum inhibitory concentration (MIC) was assessed by broth dilution method in the range 0.125 to 128 mg/mL (Saravanakumar *et al*. 2014).

Cell Culture and Cytotoxicity Assay

The human liver cancer cell line HepG2 was purchased from a reputable cell bank and cultured in DMEM supplemented with 10% FBS and 1% penicillin–streptomycin at 37 °C in a humidified atmosphere containing 5% CO_2 (Xie *et al*. 2017). The MTT assay (Mosmann 1983) was used to measure cytotoxicity. Cells were seeded in 96-wells plates (5×10^3 cells/well) and incubated for 24 hours. Cells were incubated for 24 to 48 h with extract at the indicated concentrations (0 to 800 µg/mL). Following the treatment with MTT solution (0.5 g/L), all wells were incubated for 4 h at 37 °C. Formazan crystals were dissolved in DMSO, and the absorbance was measured at 540 nm using a microplate reader. Cell viability in percentages and IC_{50} values were calculated. Three independent experiments were performed (n = 3) (Ghasemi *et al*. 2021).

In-silico Toxicity Prediction

The selected phytocompounds were screened for the prediction of toxicity through the on-line predictive platform pkCSM based on graph-based signatures calculated for estimating pharmacokinetic and toxicity parameters. The chemical structures of hexadecane, N-isopropyl-3-phenylpropanamide, 13-docosenamide and other identified constituents were acquired in SMILES format from the PubChem database and input for analysis. The pkCSM tool was used to predict several toxicity endpoints, mutagenicity (AMES toxicity), cardiotoxicity (hERG I and II inhibition), hepatotoxicity, and skin sensitization potential (Pires *et al*. 2015).

Finally, these studies included quantitative toxicity parameters, including maximum tolerated dose in humans (log mg/kg/day), oral rat acute toxicity (LD_{50}), oral rat chronic toxicity (LOAEL), and aquatic toxicity indicators such as *Tetrahymena pyriformis* lethality and minnow lethality. Predictions were interpreted according to established toxicological thresholds, with absence of AMES toxicity or hERG inhibition interpreted as low mutagenic and cardiotoxic risk (Selvaraj *et al*. 2024). Potential safety issues, namely those related to hepatotoxicity and skin sensitization prediction were particularly scrutinized as potential obstacles for liver-targeted anticancer drugs development. Provisional acute (LD_{50}) and chronic toxicity profiles (LOAEL) were estimated using quantitative parameters, and predictions of aquatic toxicity lent further insight into environmental safety considerations.

By taking these parameters into consideration, a concise *in silico* analysis was performed to assess the safety of the phytoconstituents identified from our library and rank them based on desirable toxicity profiles for further pharmacological profiling (Ezhilarasan *et al*. 2023; Niyomchan *et al*. 2023).

Molecular Docking Studies

Molecular docking studies were performed to study the interaction of identified phytocompounds with our target protein (PDB ID: 3GCW) (Selvaraj *et al*. 2021). The protein structural model was acquired from the Protein Data Bank and prepared by removing water molecules and adding hydrogen atoms (Basu *et al*. 2020; Suguna and Mowna Sundari 2026).

Ligand structures were downloaded from the PubChem database and then minimized energy. Autodock Vina (version 1.1.2) was utilized for docking simulations, while visualization of interactions was done using Biovia Discovery Studio Visualizer (version 2021) (Yadav *et al*. 2020; Vanajothi 2026). The binding affinities were reported in kcal/mol, and hydrogen bond interactions were studied (Iqbal *et al*. 2023; Beema Shafreen *et al*. 2026).

Statistical Analysis

All experimental data were represented as mean ± standard deviation (SD) of three independent experiments (n = 3). Statistical analysis was conducted using GraphPad Prism (version 8.0). Differences between groups were evaluated using one-way analysis of variance (ANOVA) with Tukey's post hoc test. Statistical significance was assumed at the $P < 0.05$ level (Ledolter *et al*. 2020).

RESULTS AND DISCUSSION

Phytochemical Composition and Its Biological Implications

Qualitative phytochemical screening of methanolic leaf extract of *Passiflora edulis* indicated that the flavonoids, saponins, steroids, carbohydrates, and reducing sugars were present, while alkaloids, phenols, and terpenoids were absent (Table 1). The results indicated a range of flavonoids and saponins having potential to play a role in biological activity.

Flavonoids have been widely investigated because of their antioxidant and anticancer potential, but mainly they act as scavengers of free radicals and modulators of signaling pathways related to apoptosis and cell proliferation. Similarly, saponins appeared to induce cytotoxic damage in target cancer cells by damaging the integrity of membranes and causing apoptotic pathways. The phytochemical composition fits with previous studies on *Passiflora edulis*, whereby flavonoid containing extracts have been associated with pharmacological activity.

GC–MS Profiling and Chemical Characterization

Fifteen different phytoconstituents were identified in the methanolic extract through GC–MS analysis (Table 2), among which 13-docosenamide (Z) was the major compound (72.15% peak area). The remaining 14 constituents, in decreasing order of relative peak area, comprised propanenitrile, 3-(propylamino)- (4.35%); 1-carbomethoxycyclohexyl ethyl phosphonate and cyclohexasiloxane, dodecamethyl- (3.42% each); benzoic acid, 2,5-bis(trimethylsiloxy)-, trimethylsilyl ester (3.21%); 1-oxaspiro[2.3]hexan-5-one derivative (2.64%); furo[2,3-c]pyridine, 2,3-dihydro-2,7-dimethyl- (2.24%); N,O-bis(methoxycarbonyl)-N-((1-methylcyclopropyl)methyl)-hydroxyamine (2.16%); hexadecane (1.16%); (3r*,4s*)-3-ethyl-4-methyltetrahydrofuran-3-ol (1.54%); N-isopropyl-3-phenylpropanamide (0.97%); 1,6-octadien-3-ol, 7-methyl-3-(trifluoromethyl)- (0.82%); 2-(2-oxo-2-phenyl-ethyl)-1,3-dioxolane (0.81%); 1,3-diphenyl-1-((trimethylsilyl)oxy)-1(E)-heptene (0.61%); and 4-(phenylthio)-2,4-dimethyl-2-pentene (0.51%), as listed in Table 2.

Fatty acid amide derivatives dominated the extract composition, as indicated by 13-docosenamide. These compounds are known to have membrane-interacting characteristics and have an impact in the modulation of signaling within cells. However, their anticancer function is not well delineated. N-isopropyl-3-phenylpropanamide was found in smaller amounts with structural similarity based on the presence of both aromatic (Ph) and amide functional groups, which are known to be bioactive or possess protein binding activity. However, caution should be taken when interpreting GC–MS results. Siloxane derivates, such as cyclohexasiloxane are likely analytical artifacts from column bleed or sample preparation rather than authentic phytoconstituents.

Furthermore, GC–MS is also aimed at volatile and semi-volatile compounds, and it may fail to identify non-volatile bioactive molecules like high-molecular-weight polyphenols. Thus, although GC–MS offers useful exploratory information, detailed chemical characterization requires complementary analytical approaches. Most importantly, compound identification does not directly indicate biological relevance and subsequent functional and computational analyses are essential to confirm correlations between chemical composition and bioactivity.

Table 1. Phytochemical Screening of *Passiflora edulis*

Test	Qualitative Results of Methanolic Extracts of *P. edulis*
Alkaloids	_
Steroids	+
Terpenoids	-
Flavonoids	+
Saponins	+
Carbohydrates	+
Phenols	-
Reducing Sugars	+

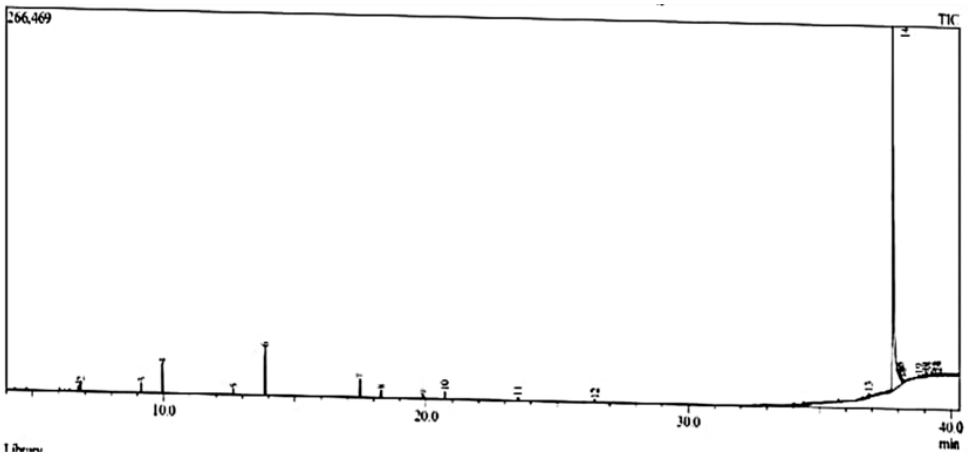

Fig. 1. GC–MS Analysis of *Passiflora edulis*

Table 2. GC–MS Analysis of *Passiflora edulis*

Peak	Retention time (min)	Area (%)	Name of the Compounds
1	6.748	0.81	2-(2-Oxo-2-Phenyl-Ethyl)-1,3-Ioxolane
2	6.827	1.54	(3r*,4s*)-3-Ethyl-4-Methyl tetra-Hydrofuran-3-ol
3	9.143	1.16	Hexadecane
4	9.95	3.21	Benzoic Acid, 2,5-Bis(Trimethylsiloxy)-, Trimethylsilyl Ester
5	13.87	3.42	Cyclohexasiloxane, Dodecamethyl-
6	17.482	0.61	1,3-Diphenyl-1-((Trimethylsilyl)Oxy)-1(E)-Heptene
7	18.281	0.97	N-Isopropyl-3-Phenylpropanamide
8	26.43	2.24	Furo[2,3-C]Pyridine, 2,3-Dihydro-2,7-Dimethyl-
9	37.802	72.15	13-Docosenamide, (Z)-
10	37.94	3.42	1-Carbomethoxycylohexyl Ethyl Phosphonate
11	38.025	4.35	Propanenitrile, 3-(Propylamino)-
12	38.065	2.64	1-Oxaspiro[2.3]Hexan-5-one, 2,2-Bis(1,1-Dimethylethyl)-4,4,6,6-Tetramethyl-
13	38.11	2.16	N,O-Bis(Methoxycarbonyl)-N-((1-Methyl-cyclopropyl)Methyl)Hydroxyamine
14	38.145	0.82	1,6-Octadien-3-ol, 7-Methyl-3-(Trifluoromethyl)-
15	38.173	0.51	4-(Phenylthio)-2,4-Dimethyl-2-Pentene

Antimicrobial Activity and its Relevance

The leaf extracts showed moderate antimicrobial activity against a panel of Gram-positive, Gram-negative, and fungal strains, with an observed solvent-dependent inhibitory profile. Antimicrobial screening was performed using four solvent extracts (acetone, methanol, ethanol, and ethyl acetate), in contrast to the other bioassays, GC–MS profiling, and docking analyses, which were restricted to the methanolic extract; this broader solvent screen was undertaken specifically for the antimicrobial assay to determine whether a solvent system other than methanol might yield superior antimicrobial efficacy, consistent with conventional practice in antimicrobial bioprospecting. The methanolic fraction showed measurable antibacterial activity against *Staphylococcus aureus* (10.33 ± 0.94 mm), *Escherichia coli* (10.00 ± 1.41 mm), *Klebsiella pneumoniae* (10.00 ± 1.00 mm), and *Pseudomonas aeruginosa* (9.00 ± 0.81 mm), among extracts tested. Methanolic extract was found to have no antifungal activity against *Candida albicans*. The ethanolic extract showed a relatively higher activity against *E. coli* (12.33 ± 0.47 mm) and *C. albicans* (15.00 ± 0.81 mm), indicating that solvent polarity is an important factor in the recovery of antimicrobial constituents.

Bacillus subtilis was not inhibited by either the methanolic or ethanolic extracts but showed the strongest susceptibility among all organisms tested to the acetone extract (11.66 ± 0.47 mm) and moderate susceptibility to the ethyl acetate extract (9.33 ± 0.00 mm) (Table 3), reinforcing that the antimicrobial spectrum of *P. edulis* leaf extracts is strongly solvent- and organism-dependent. Minimum inhibitory concentration (MIC) values, determined by the broth microdilution method (range 0.125–128 mg/mL), The inactivity observed for some solvent fractions (e.*g.*, ethyl acetate fraction against several bacterial strains) reinforces the concept of selective extraction of bioactive compounds, as well suggesting that individual classes of antimicrobial constituents might be heterogeneously distributed in solvent systems.

The average antimicrobial effect observed can probably be described as mechanistic, due to the presence of flavonoids and saponin detected through a phytochemical screen. Flavonoids are reported to be anticancer agents through various routes, including disruption of microbial cell membranes; inhibition of nucleic acid synthesis; and interference with energy metabolism. Likewise, saponins can increase membrane permeability through their interaction with sterols, resulting in cell lysis. The lack of uniformity in cell wall composition and structure means that the effectiveness of such a mechanism would vary between different microorganisms, particularly Gram-negative *versus* Gram-positive bacteria. While the antimicrobial activity is not a focus of this study, it supports the bioactivity and chemical diversity shown in the extract. Crucially, some mechanisms of antimicrobial action, such as membrane disturbance and inhibition of enzyme activity, are shared with anticancer effects, indicating a common underpinning of biological activities. However, because moderate inhibition was observed and no strong broad-spectrum activity noted, the antimicrobial findings are incidental to *P. edulis* therapeutic potential, rather than essential.

These results taken together indicate that *P. edulis* leaf extracts do display an antimicrobial effect, but that further optimization with bioactivity-guided fractionation and compound isolation would be required to enhance the potency and identify specific antimicrobials.

Table 3. Antimicrobial Activity of *P. edulis* against Selected Microorganisms

Organism	Acetone	Methanol	Ethanol	Ethyl Acetate	Antibiotic
Staphylococcus aureus	8.00±0.00	10.33±0.94	10.00±0.81	0.00±0.00	29 mm (T)
Bacillus subtilis	11.66±0.47	0.00±0.00	0.00±0.00	9.33±0.00	30 mm (T)
Escherichia coli	0.00±0.00	10.00±1.41	12.33±0.47	0.00±0.00	32 mm (T)
Pseudomonas aeruginosa	0.00±0.00	9.00±0.81	9.00±0.81	0.00±0.00	30 mm (T)
Klebsiella pneumoniae	0.00±0.00	10.00±1.00	0.00±0.00	11.33±0.94	24 mm (T)
Candida albicans	8.33±0.47	0.00±0.00	15.00±0.81	0.00±0.00	18 mm (F)

Note: A value of 0.00 ± 0.00 denotes the complete absence of an observable zone of inhibition around the well (*i.e.*, no antimicrobial activity detected at the tested concentration) and is not a measured zone diameter. T = bacterial standard antibiotic (tetracycline); F = standard antifungal (fluconazole).

Antioxidant Activity and Redox Modulation Potential

The DPPH radical scavenging assay was performed to evaluate the antioxidant potential of methanolic leaf extract of *Passiflora edulis*, and this study revealed that the free radical inhibition was significantly increased in a concentration dependent manner. Extracts showed gradual activity from 21.4 ± 1.2% at the concentration of 20 µg/mL up to 81.0 ± 2.3% at the highest concentration tested (100 µg/mL). This revealed that radical scavenging capacity was steadily reinforced with increasing dose, without reaching a true plateau or maximal endpoint. By non-linear regression of percent inhibition against concentration, the IC_{50} for DPPH scavenging by the methanolic extract was calculated as [59.97 µg/mL], compared with [39.03 µg/mL] for the ascorbic acid standard (Table 4). As a control, the conventional antioxidant ascorbic acid demonstrated greater activities (35.6 ± 1.0% - 91.2 ± 1.9%) at all concentrations tested in comparison with the synthesized antioxidants, validating their superior effectiveness over synthesized antioxidants.

The tendency of doses to increase in such extracts is due to the presence of bioactive constituents with antioxidant ability: the main mechanism for this activity is their capacity to donate electrons or hydrogen atoms needed to stabilize DPPH radicals, as stated above (the standard would have a higher efficiency). The steep increase without a sudden inflection in activity is also consistent with mild antioxidant strength that may have developed from crude extracts (characterized by heterogeneous profiles of phytochemicals). Statistical consistency (low standard deviation values) asserts the reproducibility of the observed effects.

From a mechanistic standpoint, antioxidant activity is particularly relevant in hepatocellular carcinoma, where oxidative stress represents a key tumorigenic driver. High concentrations of reactive oxygen species (ROS) induce DNA damage, lipid peroxidation, and activation of oncogenic signaling pathways including NF-κB, PI3K/Akt, and Wnt/β-catenin. DPPH is a stable free radical which has been used since it was well-known for measuring the antioxidant activity of molecules. In this perspective, the results of DPPH scavenging activities implied that the extract could reduce oxidative stress through free radical neutralization and subsequently inhibit ROS-mediated signaling pathways involved in cancer development.

The high antioxidant activities can be attributed to the presence of flavonoids, as evidenced in phytochemical screening. These motifs are established to efficiently act as radical scavengers *via* hydrogen atom transfer (HAT) and single electron transfer (SET)

processes, stabilizing the reactive species by resonance structures. However, the undetectable level of these phenolic compounds could justify that the extract did not reach an activity close to ascorbic acid because they are serious contributors to high antioxidant capacity. Flavonoids are phytochemicals that are naturally and widely present in plants, so they may still be detectable but limited in concentration or structural diversity. This is significant, since this antioxidant activity may be associated with the moderate cytotoxic effects previously reported, suggesting a possible link between redox modulation and anticancer activity; however, no direct correlation analysis between the DPPH and MTT assay data was performed in this study, and this association should therefore be regarded as a hypothesis rather than an established relationship. Nevertheless, it should be noted that results obtained from DPPH assays are an overly simplistic chemical model that would not match what occurs in the intracellular oxidative environment. Thus, additional validation by cellular ROS assays would be required to determine the biological importance of these observations. The methanolic extract of *P. edulis* possess moderate to biologically significant antioxidant activity in a clear dose–response manner. These results underscore the usefulness of the extract as a potential source of redox-active compounds while emphasizing that bioactivity-guided fractionation and isolation might be used to find and concentrate more bioactive antioxidant constituents.

Table 4. DPPH Radical Scavenging Activity of Methanolic Extract

Concentration (µg/mL)	% Inhibition (Mean ± SD, n = 3)	Ascorbic Acid (% Inhibition)
20	21.4 ± 1.2	35.6 ± 1.0
40	34.7 ± 1.5	52.3 ± 1.3
60	49.8 ± 1.8	65.9 ± 1.5
80	63.2 ± 2.0	75.4 ± 1.7
100	81.0 ± 2.3	91.2 ± 1.9

Cytotoxicity Assessment in HepG2 Cells

The MTT assay was used to measure the cytotoxicity of methanolic leaf extract of *P. edulis* on HepG2 human HCC cells. The extract caused a concentration-dependent reduction in cell viability. This was shown by the dose–response curve (Fig. 4), which had an IC_{50} = 251 µg/mL. The progressive drop in viability at increasing concentrations suggests a broad antiproliferative effect, but the slope of the curve indicates low cytotoxic potency and not a steep high-efficacy response.

Microscopic examination (Fig. 3) further supported these results, showing morphological changes consistent with cytotoxic stress *i.e.*, cell shrinkage, decreased cell density, and detachment as well as possible membrane blebbing at highest concentrations. The phenotypic manifestations are reflective of loss in cellular integrity (note the membrane blebbing), and these changes are often associated with apoptosis or other forms of programmed cell death. The precise mode of cell death cannot, however, be definitively established in the absence of specific staining or molecular assays. In this context, the IC_{50} value of 251 µg/mL classifies the extract as having moderate cytotoxicity activity, especially in comparison to classical chemotherapeutic agents with IC_{50} values commonly in the low micromolar range. Such a high IC_{50} indicates that the crude extract is not very potent in this form and would need further purification to be considered therapeutic, to some extent. It can be correlated to the presence of bioactive phytochemicals (flavonoids and saponins) observed in the phytochemical screening. These compounds ameliorate different cellular pathways, such as apoptosis induction, *via* mitochondrial dysfunction,

activation of caspase cascades (czx), arrested under specific checkpoints that can be regulated through a cell cycle. Furthermore, the antioxidant activity observed with the extract may indirectly play a role by regulating intracellular redox balance and, thus, ROS-mediated signaling pathways critical for the survival of cancer cells.

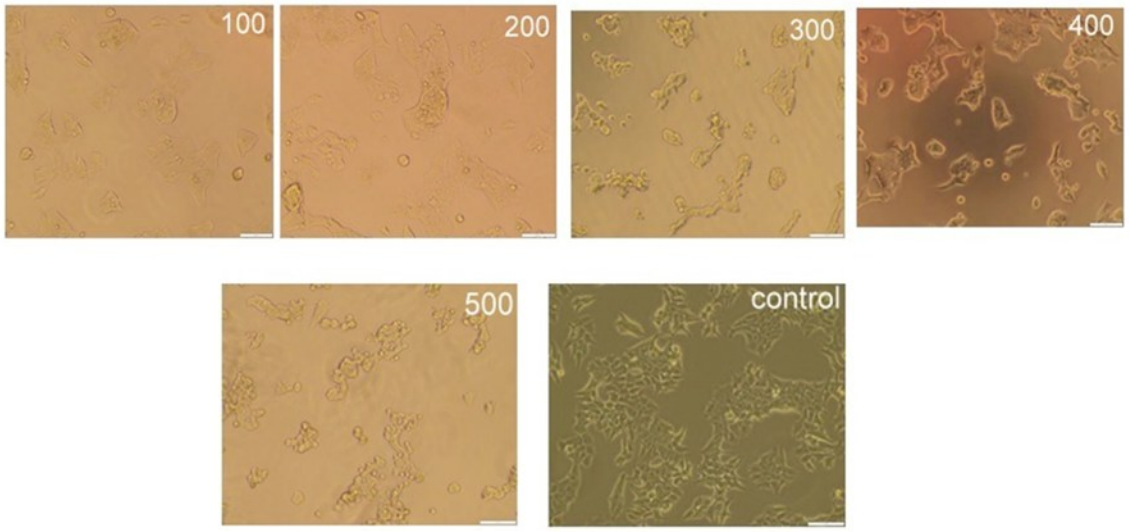

Fig. 2. Microscopic evaluation of cytotoxicity of *Passiflora edulis* extract

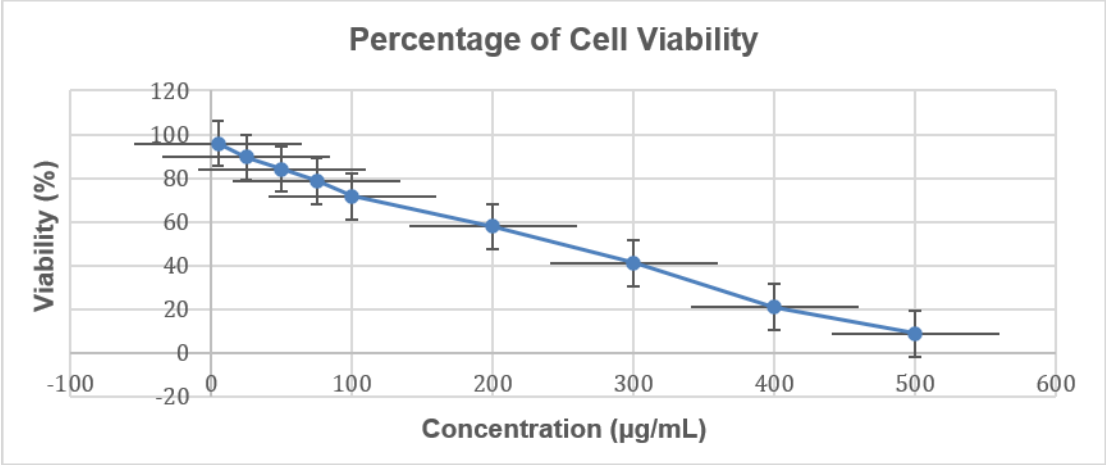

Fig. 3. Dose-dependent decrease in cell viability following treatment with *Passiflora edulis* extract

Crude plant extracts are complex mixtures, containing both bioactive and inactive constituents, which can lead to diminished overall potency because of attenuation or dilution effects. Thus, the moderate cytotoxicity seen here may be a result of cumulative but unoptimized interactions among multiple compounds rather than single high-potency molecules. Moreover, the lack of a selectivity evaluation on normal (non-cancer) cell lines is another limitation, since cytotoxic activity alone does not necessarily translate into clinical relevance. Selectivity index evaluation was not performed in this commentary, and future studies are encouraged to implement mechanistic assays (including induction of apoptosis detection, ROS quantification, and cell cycle analysis) that will better elucidate the extract's biological effects and safety profile. These results reveal that *P. edulis* leaf

extract can inhibit HepG2 growth in a concentration dependent manner and although our results indicate measurable antiproliferative activity against this cell line the effect is moderate which may lend support toward its reserve as an anticancer lead source. Nevertheless, bioactivity-guided fractionation, isolation of active constituents and exploration of detailed distinct mechanistic involvement will be required to increase potency and establish translational relevance.

In-silico ADME and Toxicity Profiling: Integrated Analysis

Drug-likeness, bioavailability, and safety profiles of selected phytoconstituents from *P. edulis* leaf extract were evaluated using pkCSM platforms to determine their pharmacokinetic and toxicity characteristics. Evaluation of the predicted ADME (Absorption, Distribution, Metabolism, and Excretion) properties indicated that the studied compounds have a variable compliance with the drug-likeness criteria. For instance, smaller and more balanced structures such as N-isopropyl-3-phenylpropanamide and furo[2,3-c]-pyridine derivatives have been shown to satisfy Lipinski's rule of five indicating their favorable oral bioavailability owing to the acceptable physicochemical properties including molecular weight, lipophilicity (LogP), and hydrogen bonding capacity (Supplementary Table S1). Conversely, long-chain aliphatic values such as hexadecane and 13-docosenamide (Z) have high lipophilicity and low polarity, possibly restricting solubility in aqueous medium as well gastrointestinal absorption. Higher lipophilicity increases permeability across cellular membranes but introduces the risk for bioaccumulation and less metabolic clearance with changes to pharmacokinetic properties. Gastrointestinal (GI) prediction indicates that structurally simple, moderately polar compounds will be absorbed more efficiently while highly hydrophobic compounds may have poor oral bioavailability despite a membrane permeability benefit. Furthermore, interactions with cytochrome P450 enzymes suggest potential metabolic liabilities for compounds that can act as enzyme inhibitors and therefore cause drug–drug interactions or changing the metabolic stability.

Evaluation of the compounds done using pkCSM showed that all analyzed compounds were non-mutagenic (AMES negative) and did not show hERG I or II inhibitions, suggesting very low risk of genotoxicity and cardiotoxicity. These results constitute a potent safety advantage, especially during early-phase drug discovery. In contrast to hexadecane, several compounds, such as benzoic acid derivatives, furo[2,3-c] pyridine derivatives, and cyclohexasiloxane were predicted to have hepatotoxicity (Supplementary Table 1). This is a key consideration for hepatocellular carcinoma where agents used to treat the disease need selective cytotoxicity against cancerous cells while sparing normal liver activity. Predicted MTD values differed among compounds, suggesting differential tolerability of sequence to systemic interaction. Acute toxicity (LD_{50}) and chronic toxicity (Loael) estimates indicated that the compounds possess moderate toxicity properties, which do not appear to be markedly toxic or harmless, highlighting the need for additional dose refinement in future studies. Non-genotoxic skin sensitization predictions suggested allergenic potential in some of the compounds, associated with the presence of reactive functional groups that mediate or contribute to adverse biological responses.

In summary, green chemistry principles evaluated including ecotoxicology showed a wide ecological impact from the environmental toxicity indicators of *Tetrahymena pyriformis* and minnow toxicity. This indicated that some constituents may need to be handled in a way where disposal is cared for in a more advanced way. The ADME and

toxicity prediction vectors together suggest that the overall pharmacokinetic and safety properties of phytoconstituents of *P. edulis* are mixed, with moderate drug-likeness and acceptable safety in some parameters but non-negligible limitations. Molecules with high lipophilicity suffer from low solubility and possible accumulation, whereas the compounds possessing balanced physicochemical characteristics have the best pharmacokinetic potential. Notably, the absence of mutagenicity and cardiotoxicity indicates a favorable baseline safety profile, while hepatotoxicity signals represent an important limitation (especially so for liver-targeted therapeutic applications). These findings are coherent with the moderate cytotoxic and docking results obtained in this study, suggesting that the biological activity reflects multi-component, moderate-intensity action rather than the effect of a single highly active compound. These findings emphasize the importance of bioactivity-guided fractionation as well as structure optimization to improve pharmacokinetic properties and reduce toxicity. Additional validation, most notably metabolic stability and toxicity assays involving *in vitro* and *in vivo* studies of these compounds are still needed before using these compounds as potential therapeutic agents.

Molecular Docking and Interaction Analysis

Molecular docking analysis of selected phytoconstituents against the target protein (PDB ID: 3GCW), representing the interaction complex of proprotein convertase subtilisin/kexin type 9 (PCSK9) with low-density lipoprotein receptor (LDLR), showed binding energies between −6.5 and −7.8 kcal/mol which indicated that these interactions were moderately stable but functionally relevant. Out of the compounds, N-isopropyl-3-phenylpropanamide had the highest binding affinity (−7.8 kcal/mol), which bound two hydrogen bonds with ARG-66 and ARG-73, π–π interaction with PHE-64 as well as hydrophobic interactions with LEU-131 and LEU-135. These interactions imply that the binding interface is occupied consistently, and hydrogen bonds give rise to β-agonist specificity with aromatic/hydrophobic interactions bolstering ligand stabilization in the pocket. Biologically, PCSK9 is important when it comes to cholesterol homeostasis because its interaction with the LDLR results in receptor degradation (instead of recycling), which leads to impaired lipid clearance.

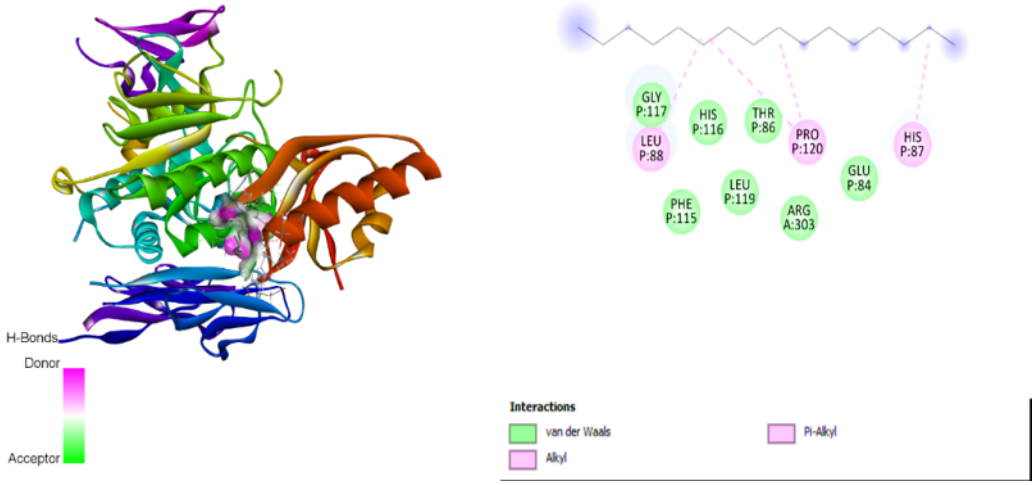

Fig. 4a. Binding interaction of hexadecane with 3GCW protein obtained through molecular docking analysis

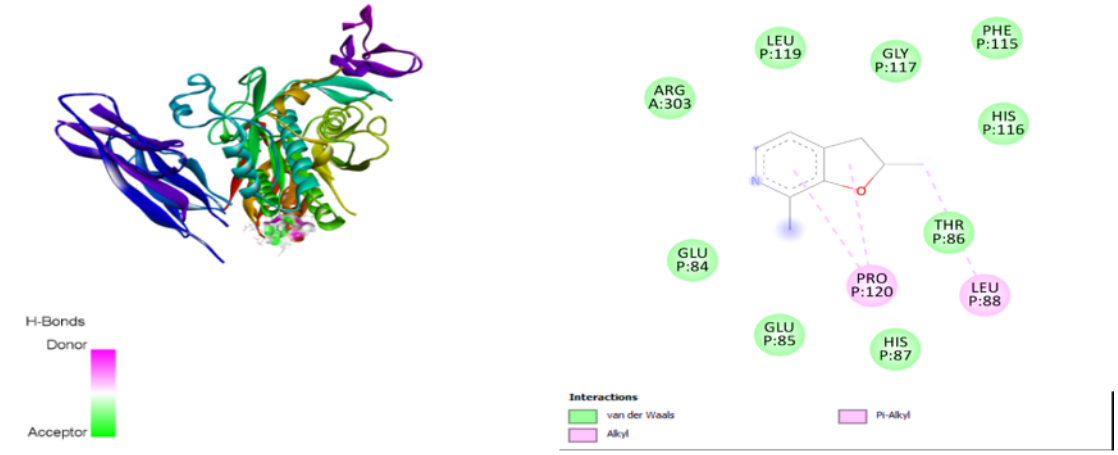

Fig. 4b. Binding interaction of furo[2,3-c]pyridine, 2,3-dihydro-2,7-dimethyl- with 3GCW protein as revealed by molecular docking analysis

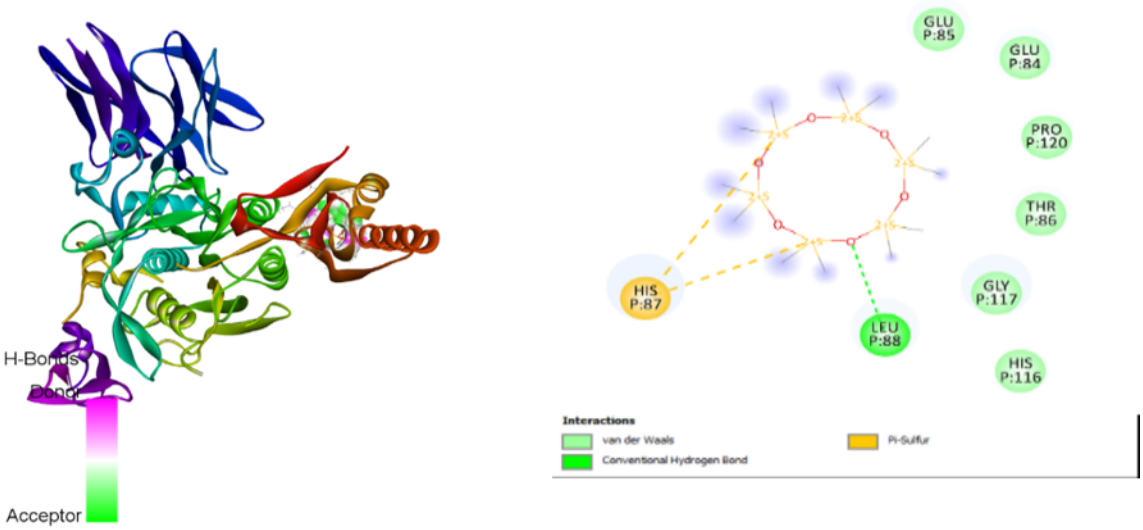

Fig. 4c. Binding interaction of cyclohexasiloxane, dodecamethyl with 3GCW protein as revealed by molecular docking analysis

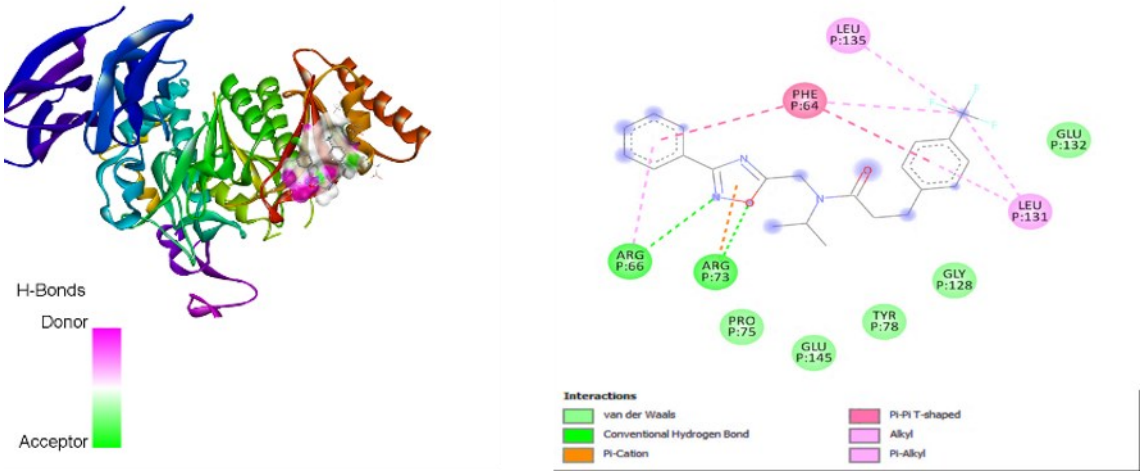

Fig. 4d. Binding interaction of N-isopropyl-3-phenylpropanamide with 3GCW protein as revealed by molecular docking analysis

Reprogramming lipid metabolism is also intimately linked with tumor growth, membrane biosynthesis, and activation of oncogenic pathways (such as PI3K/AKT and MAPK) in hepatic biology in hepatocellular carcinoma. Thus, the addition of a ligand to key residues in the PCSK9–LDLR interface (*e.g.*, ARG-66 and ARG-73) will potentially disrupt this interaction, leading to reduced LDLR degradation and receptor recovery. This, in turn, could hinder the availability of lipids needed for rapid tumor proliferation. Compounds such as hexadecane and the furo[2,3-c]pyridine derivative engaged mainly in hydrophobic π–alkyl interactions with residues such as PRO-120. By contrast, the residues THR-86, and LEU-88 were associated with non-specific binding, likely affecting function at poorer rates as judged by weak regulatory impact. Indeed, while the binding of 1,3-docosenamide involved a greater number of hydrogen bonding interactions (SER-91, LEU-88 PHE-115, and LEU-119), it also revealed lower overall binding affinity, which is likely to result from less rigid conformational orientation. Cyclohexasiloxane exhibited weak interactions and is probably an abiotic contaminant.

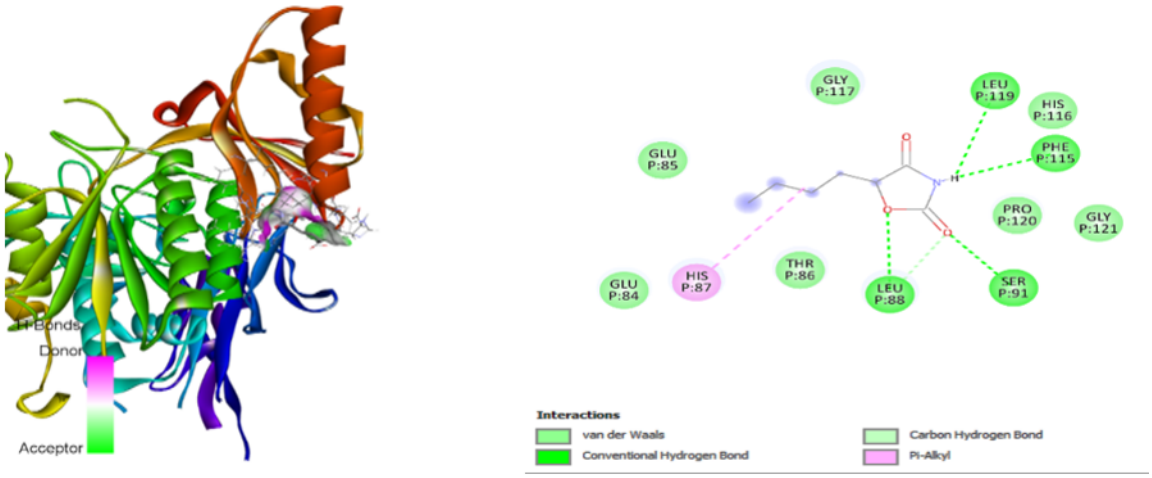

Fig. 4e. Binding interaction of 3-docosenamide with 3GCW protein as revealed by molecular docking analysis

Overall, these findings indicate that the anticancer properties of *Passiflora edulis* leaf extract may be partially mediated *via* modulation of the PCSK9–LDLR axis through targeting lipid metabolic pathways enabling hepatocellular carcinoma progression. Nonetheless, because of the moderate binding affinities, such effects are most likely a symptom of multi-compound synergistic interactions rather than ascribed to one high-affinity inhibitor and will need to be validated in functional studies/pathway-specific analysis.

Overall, the findings suggest that *P. edulis* leaf extract has quantitate antioxidant, antimicrobial and moderate anticancer activities, which could result from synergistic action of several phytoconstituents on multiple molecular targets. But the comparatively higher IC_{50} value and moderate docking affinities indicate that the crude extract is limited, highlighting a lack of strong target-specific activity. Hence, additional investigations that incorporate bioactivity-guided fractionation, as well as the isolation and structural characterization of bioactive components along with detailed mechanistic studies, apoptosis induction, oxidative stress modulation and pathway specific analyses, are necessary. Moreover, *in vivo* validation and pharmacokinetic profiling will be pivotal to evaluate therapeutic significance and clinical relevance of *P. edulis*–derived compounds.

To progress this plant from a source of preliminary bioactivity to a candidate for the development of anticancer drugs, such studies will be essential.

Table 5. Molecular Interaction of Target Protein 3GCW with Selected Compounds

S. No.	Compound Name	Binding Energy (kcal/mol)	Interacting Residues	Type of Bond
1	Hexadecane	-7.5	PRO-120 HIS-87 THR-86 GLY-117	Pi-Alkyl Pi-Alkyl Pi-Alkyl Pi-Alkyl
2	Furo[2,3-c]pyridine, 2,3-dihydro-2,7-dimethyl-	-7.3	THR-86 LEU-88 PRO-120	Pi-Alkyl Pi-Alkyl Pi-Alkyl
3	Cyclohexasiloxane, Dodecamethyl	-6.9	LEU-88 HIS-87	H-Bond Pi-Sulphur
4	N-Isopropyl-3-phenylpropanamide	-7.8	ARG-66 ARG-73 PHE-64 LEU-131 LEU-135	H-Bond H-Bond Pi-Pi Pi-Alkyl Pi-Alkyl
5	13-Docosenamide (z)	-6.5	HIS-87 LEU-88 SER-91 PHE-115 LEU-119	Pi-Alkyl H-Bond H-Bond H-Bond H-Bond

CONCLUSIONS

The current study is the first integrated assessment of methanolic leaf extract of *Passiflora edulis*, its phytochemical composition, and biological activities pointing toward a promising source of bioactive compounds with therapeutic applications.

1. Qualitative phytochemical analyses verified the presence of important secondary metabolites such as flavonoids, saponins, and steroids, which together are strongly connected with antioxidant and anticancer activities.
2. A concentration dependent antioxidant activity was observed in the extract, where the maximum 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging reached 81%, which shows a considerable capability of free metabolic products neutralization.
3. The *in vitro* cytotoxicity study showed a moderate antiproliferative activity against HepG2 hepatocellular carcinoma cells (IC_{50} = 251 µg/mL), which suggests the occurrence of compounds that can influence cancer cell viability, but with low potency when analyzed as crude.
4. Gas chromatography–mass spectrometry (GC–MS) analysis showed phytoconstituents such as 13-docosenamide (Z) and other bioactive compounds including hexadecane and N-isopropyl-3-phenylpropanamide.

5. The results were then further verified through molecular docking analysis demonstrating moderate binding affinities of the selected compounds toward the target protein (PDB ID: 3GCW) correlating to the PCSK9–LDLR complex. Interestingly, N-isopropyl-3-phenylpropanamide displayed the best interaction pattern with hydrogen bonding and hydrophobic interactions with functionally important residues, indicating potential in modulating protein activity.

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Conflict of Interest

The authors declare no conflict of interest.

Use of Generative AI

The authors confirm that no generative AI tools or AI-assisted technologies were used in the preparation of the text, data analysis, or collation of references.

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APPENDIX
Supplementary Information

Table S1. Drug-Likeness and Safety Profiles of Selected Phytoconstituents from *P. edulis* Leaf Extract Evaluated Using pkCSM

HEXADECANE			
Property	**Model Name**	**Predicted Value**	**Unit**
TOXICITY	AMES toxicity	**No**	Categorical (Yes/No)
Toxicity	Max. tolerated dose (human)	**0.141**	Numeric (log mg/kg/day)
Toxicity	hERG I inhibitor	**No**	Categorical (Yes/No)
Toxicity	hERG II inhibitor	**No**	Categorical (Yes/No)
Toxicity	Oral Rat Acute Toxicity (LD50)	**1.521**	Numeric (mol/kg)
Toxicity	Oral Rat Chronic Toxicity (LOAEL)	**1.307**	Numeric (log mg/kg_bw/day)
Toxicity	Hepatotoxicity	**No**	Categorical (Yes/No)
Toxicity	Skin Sensitisation	**Yes**	Categorical (Yes/No)
Toxicity	*T. pyriformis* toxicity	**1.825**	Numeric (log ug/L)
Toxicity	Minnow toxicity	**-1.409**	Numeric (log mM)

Benzoic Acid, 2,5-Bis(Trimethylsiloxy)-, Trimethylsilyl Ester			
Property	**Model Name**	**Predicted Value**	**Unit**
Toxicity	AMES toxicity	**No**	Categorical (Yes/No)
Toxicity	Max. tolerated dose (human)	**0.304**	Numeric (log mg/kg/day)
Toxicity	hERG I inhibitor	**No**	Categorical (Yes/No)
Toxicity	hERG II inhibitor	**No**	Categorical (Yes/No)
Toxicity	Oral Rat Acute Toxicity (LD50)	**2.809**	Numeric (mol/kg)
Toxicity	Oral Rat Chronic Toxicity (LOAEL)	**0.596**	Numeric (log mg/kg_bw/day)
Toxicity	Hepatotoxicity	**Yes**	Categorical (Yes/No)
Toxicity	Skin Sensitisation	**No**	Categorical (Yes/No)
Toxicity	*T. pyriformis* toxicity	**1.763**	Numeric (log ug/L)
Toxicity	Minnow toxicity	**0.407**	Numeric (log mM)

Furo[2,3-c]pyridine, 2,3-dihydro-2,7-dimethyl-			
Property	**Model Name**	**Predicted Value**	**Unit**
Toxicity	AMES toxicity	**No**	Categorical (Yes/No)
Toxicity	Max. tolerated dose (human)	**1.01**	Numeric (log mg/kg/day)
Toxicity	hERG I inhibitor	**No**	Categorical (Yes/No)
Toxicity	hERG II inhibitor	**No**	Categorical (Yes/No)
Toxicity	Oral Rat Acute Toxicity (LD50)	**2.266**	Numeric (mol/kg)
Toxicity	Oral Rat Chronic Toxicity (LOAEL)	**2.366**	Numeric (log mg/kg_bw/day)
Toxicity	Hepatotoxicity	**Yes**	Categorical (Yes/No)
Toxicity	Skin Sensitisation	**Yes**	Categorical (Yes/No)

Toxicity	*T. pyriformis* toxicity	**-0.019**	Numeric (log ug/L)
Toxicity	Minnow toxicity	**1.564**	Numeric (log mM

Cyclohexasiloxane, dodecamethyl-			
Property	**Model Name**	**Predicted Value**	**Unit**
Toxicity	AMES toxicity	**No**	Categorical (Yes/No)
Toxicity	Max. tolerated dose (human)	**0.304**	Numeric (log mg/kg/day)
Toxicity	hERG I inhibitor	**No**	Categorical (Yes/No)
Toxicity	hERG II inhibitor	**No**	Categorical (Yes/No)
Toxicity	Oral Rat Acute Toxicity (LD50)	**2.809**	Numeric (mol/kg)
Toxicity	Oral Rat Chronic Toxicity (LOAEL)	**0.596**	Numeric (log mg/kg_bw/day)
Toxicity	Hepatotoxicity	**Yes**	Categorical (Yes/No)
Toxicity	Skin Sensitisation	**No**	Categorical (Yes/No)
Toxicity	*T. pyriformis* toxicity	**1.763**	Numeric (log ug/L)