Texila International Journal of Public Health
ISSN: 2520-3134
DOI: 10.21522/TIJPH.2013.13.02.Art091

In-silico and Cytotoxicity Assessment of Persea Americana Fruit Extract on MDA-MB-231 Breast Cancer Cells

Sanjana Dandapani, Vishnu Priya*
Centre of Molecular Medicine and Diagnostics (COMManD), Department of Biochemistry, Saveetha Dental College & Hospitals, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India

Abstract

Avocados, or Persea americana, have been traditionally used to treat a variety of illnesses. Breast cancer in women is a serious health issue that requires early detection and timely intervention for effective management and treatment. In silico investigations speed up and lower the cost of medication development by analyzing natural products using computational models. The phytochemicals in Persea americana fruit extract are examined in this work, along with their potential inhibitory effects on C-MET in triple-negative breast cancer. In contrast to the conventional, expensive, and complicated drug discovery procedures, these techniques are successful and economical. Investigating the phytochemicals' potential for C-MET inhibition and triple-negative breast cancer treatment was the aim of the study. Using molecular docking, this study found potent C-MET inhibitors, providing fresh information for the creation of therapeutic drugs. The extraction of bioactive chemicals from Persea americana pulp involved heating, boiling, and filtering. A comparison was made between the potential anticancer activity of 98 chosen chemicals and carboplatin. The SwissADME online service was used to analyze drug-likeness and pharmacokinetics, and the C-MET protein was used for molecular docking to measure compound affinity. With the help of BIOVIA Discovery Studio Visualizer, docked positions were examined. ProTox II was used for toxicological screening, which produced a list of lead chemicals that showed promise. The molecular targets of interest, toxicity profiles, and bioavailability were predicted by SwissADME and SwissTargetPrediction. The research revealed 5 phytochemicals from Persea americana that can be the potential drugs with strong binding affinities to C-MET and antineoplastic activity, thus treating TNBC and MDA-MB-231 once and for all. In case of Triple Negative Breast Cancer, using a structure-based drug design, 5 of the 98 phytochemicals with high bioactivity against C-MET demonstrated potent anticancer activity, which was confirmed by the MTT test.

Keywords: *Anticancer Activity, C-MET, Docking Analysis, Persea Americana, Phytochemicals, TNBC.*

Introduction

Persea americana, commonly called Avocado or alligator pear, represents a medium to large evergreen tree growing mostly between 9 to 20 meters in height and having a geographical presence in various parts of the world. It has varying leaves and flowers. Its canopy is comparatively low and spreading. Its leaves come in different shapes. It is a berry-type fruit containing one big seed, with buttery pulp containing 3-30% oil. Avocado fruits are variable in color and shape, from spherical to pear-shaped, even on individual trees, and the fruit may weigh up to 2.3 kg. Indigenous to temperate climates, *Persea americana* has a long history of uses for traditional medicine [1]. It has been used for centuries to lower various disorders such as anemia, exhaustion, hypercholesterolemia, hypertension, gastritis, and gastroduodenal ulcers. The leaves are

Received: 15.08.2024 ***Accepted: 15.01.2025*** ***Published on: 30.06.2025***
****Corresponding Author: vishnupriya@saveetha.com***

considered of value for their purported therapeutic effects, which include relieving coughs, controlling diabetes, and reducing the pain of arthritis. Avocado is also cultivated in most of the tropics for its very nutritious and palatable fruit. The seeds are greatly valued in traditional Mexican medicine for a wide variety of problems, such as skin rashes, dysentery, diarrhea, infections, asthma, hypertension, and rheumatism provoked by helminths, amoebas, fungi, and bacteria [2].

Breast cancer is the most common cancer in women and often follows a latent and insidious course. Current therapies can slow the growth of tumours, but recurrence is high and related mortality is significant [3]. Breast cancer behaviour has its roots in its origin from embryonic mammary cells, characterized by their ability to move and invade tissues and alter cellular interactions. It is one of the most common diseases in the world, causing 570,000 deaths in 2015. Every year, over 1.5 million new cases are diagnosed, and breast cancer contributed to 30% of the new cancer cases in America in the year 2017 [4]. Its ability to metastasize makes it incurable. The outcome can only be best if it is detected early, and this is attested to by the fact that the 5-year relative survival rate in North America is now above 80%. The risk factors for breast cancer are gender, advanced age, exposure to estrogen, family history, genetic mutations, and reprobate lifestyles. Not only is breast cancer at the top of the list of cancers that occur in women, but it is also the most significant female killer globally. According to the WHO, early detection and prompt treatment are ways to deal with recent increases in cases of breast cancer. The current modality of therapy ranges from targeted therapy to hormonal treatment, radiation, surgery, and chemotherapy, which is being targeted at the patient's needs [3].

Triple-negative breast cancer is an aggressive subtype of breast cancer with no hormone receptor expression and HER2 overexpression. Therefore, in this case, having a good prognosis becomes challenging [5]. Very few patients survive for as long as five years, and there is a higher rate of post-adjuvant therapy recurrence [6]. TNBC refers to a negative status for estrogen receptors, Progesterone receptors, and HER2 protein expression in cancer cells. This is a subtype characterized by characteristic molecular features and aggressive phenotype, accompanied by unique metastatic patterns, with limited directed treatment options [7]. The MDA-MB-231 Cell line is a widely used model in cancer research for studying triple-negative breast cancer.

In silico studies are cutting-edge ways of research methods that allow one to carry out analysis devoid of the barriers of conventional laboratory experiments. Such methodologies have become core in the analysis of natural products and in discovering new drug candidates, greatly revolutionizing the process of drug development in terms of cost and time [8]. The term "*in silico*" designates computational models that investigate pharmacological and biological hypotheses using databases, analysis tools, data mining, machine learning, and network analysis. These techniques are usually complemented by the generation of *in vitro* data for the validation of models and hence embed computational analysis into a wide range of research areas. Particularly in toxicity studies, cytotoxicity of these natural products and their structural and pharmacological implications are well elaborated through *in silico* approaches, which are easier and more effective to perform compared to the traditional chemical analysis methods. The main aim of the study was to explore the phytochemicals present in the fruit extract of *Persea americana* and the evaluation of their drug-like properties with pharmacokinetic attributes, which may be inhibitors of C-MET in Triple Negative Breast Cancer. Thus, the phytochemicals were analyzed for their interactions and binding affinities with the active site of C-MET using

molecular docking. Identifying an effective C-MET inhibitor within *Persea americana* could significantly advance the development of innovative therapeutic drugs for triple-negative breast cancer.

Materials and Methods

Preparation of Fruit Extract: For the extraction of bioactive compounds from avocado, 5 grams of *Persea americana* pulp was mixed with 100 ml of distilled water and heated to a temperature of 80°C. Afterwards, it was boiled for one hour and then cooled. Upon cooling, the extract was filtered and stored at -20°C for a minimum of six hours and -80°C overnight, to enhance its stability. The next day, 500 mg of lyophilized avocado extract powder was dissolved in a solution of 5 ml of phosphate-buffered saline (PBS).

Proteins and Ligands: The phytochemicals of *Persea americana* were taken from Dr. Duke's Phytochemical and Ethnobotanical Database, which consists of comprehensive information on a wide variety of plants regarding their composition, including *Persea americana*. 2D and 3D structures for some selected phytochemicals with their canonical SMILES were extracted from PubChem. Only phytochemicals whose structure and property information are available at PubChem have been considered. 98 compounds were selected for the current study after reviewing relevant literature that showed potential for anticancer properties. Carboplatin, an approved drug for TNBC treatment, was prepared as a control. The three-dimensional structure of the C-MET protein was retrieved from the Research Collaboratory for Structural Bioinformatics Protein Data Bank.

Drug Likeness and Pharmacokinetics: The online web server SwissADME was used to test not only 98 compounds for drug likeness and pharmacokinetics groups, but also the control drug. The SwissADME web server was used to evaluate drug likeness through the Lipinski rule of five: (a) molecular weight (MW) not more than 500 Dalton, (b) five or fewer H-bond donors, (c) ten or fewer H-bond acceptors, and (d) log P (less than 5). Compounds that can be administered by the oral route designation i.e. oral bioavailability, and with druggable features were our preferred class of compound. Pharmacokinetics (PK) parameters were computed using our generated absorption data in the gastrointestinal tract, permeability across the blood-brain barrier, potential substrates for P-glycoprotein, and potential cytochrome P450 isoenzymes, which were tested for interaction. The attractive antidiabetic, anti-inflammatory, and anti-tumour modules were verified through these *in vitro* assays, giving reliable evidence in support of drug safety with relation to the tested substances and thus enabling a partial view of their pharmacokinetics. Prediction of drug likeness and pharmacokinetics was done using the PubChem SMILES of the compounds, and the SMILES were the SMILES that were resubmitted for the SwissADME web server for analysis.

Molecular Docking: This is one of the main means for determining the affinity of a drug to its target, which in turn helps new drugs to be discovered. In this case, C-MET was made to be the target protein. The docking was executed using AutoDockTools version 1.5.7 software from The Scripps Research Institute. The target C-MET was made by first deleting water molecules, then adding hydrogen atoms, and then computing Gasteiger charges in the ligand docking step. The ligands were treated as flexible to move through conformational changes during the docking process. Grid coordinates were used for the binding and set using the inhibitor that was already bound to the C-MET receptor. The ligands were docked to the C-MET complex, and various conformations were spun off. The conformation with the least binding to the receptor was then selected as the most likely to be the binding location. These docked poses were then visualized and analyzed using

BIOVIA Discovery Studio Visualizer version 21.1.0.20298, from Dassault Systèmes, France.

Toxicity Analysis: The binding affinity of the compounds within a range of -5 to -8.6 kcal/mol was screened for their toxicological properties using the ProTox II online web server. This was done to find out any risks that the compound would cause to the body. The assessment comprised hepatotoxicity, carcinogenicity, immunogenicity, mutagenicity, and cytotoxicity as results. Compounds that did not have any predicted toxicity were selected as the lead compounds, and the identified lead compounds were considered for further analysis.

Bioavailability Radar and Molecular Target Prediction: The SwissADME web server was used to determine the *in silico* toxicological profile and bioavailability of all lead compounds. Moreover, the web server SwissTargetPrediction was harnessed to identify potential molecular targets for these lead compounds and infer their overall pharmacological activities and therapeutic uses.

Results

Table 1 evaluates the phytochemicals from Persea americana (avocado) that have the potential to be drugs by examining important metrics like bioavailability ratings, Lipinski violations, and Log P. For substances like myristic acid (4.45), log P values, which represent the lipophilicity essential for cell membrane permeability, are favourable, suggesting strong absorption potential. While molecules like Ferulic acid, which has one violation because of a higher TPSA of 66.76, may have decreased bioavailability, most compounds, like Jasmone and p-Cymene, have zero Lipinski violations, indicating great adherence to Lipinski's rule of five and likely oral bioavailability. With high scores of 0.85 for myristic acid and palmitic acid, which indicate a strong likelihood of oral bioavailability, and scores of 0.55 for compounds like gamma-Terpinene and beta-Farnesene, which indicate moderate bioavailability, the bioavailability scores further support the potential therapeutic efficacy of these compounds. With good Log P values, few Lipinski violations, and high bioavailability ratings, avocado phytochemicals show promise as drugs. These attributes underscore their potential as potent therapeutic agents.

Table 1. Drug Likeness of Phytochemicals in Persea Americana

S. No	Compound	MW	Rotatable Bonds	NHBA	NHBD	MR	TPSA	Consensus Log P	Lipinski Violations	Bioavailability Score
1	Myristic acid	228.37	12	2	1	71.18	37.3	4.45	0	0.85
2	Jasmone	164.24	3	1	0	52.13	17.07	2.74	0	0.55
3	Tetradecanal	212.37	12	1	0	69.61	17.07	4.67	0	0.55
4	Bicyclogermacrene	204.35	0	0	0	68.78	0	4.15	1	0.55
5	gamma-Terpinene	136.23	1	0	0	47.12	0	3.35	0	0.55
6	Guaiol	222.37	1	1	1	70.72	20.23	3.46	0	0.55
7	p-Cymene	134.22	1	0	0	45.99	0	3.5	1	0.55
8	Perillene	150.22	3	1	0	47.24	13.14	2.84	0	0.55
9	Palmitic acid	256.42	14	2	1	80.8	37.3	5.2	1	0.85

10	Bulnesol	222.37	1	1	1	70.72	20.23	3.41	0	0.55
11	beta-Gurjunene	204.35	0	0	0	67.14	0	4.33	1	0.55
12	beta-Elemene	204.35	3	0	0	70.42	0	4.65	1	0.55
13	beta-Farnesene	204.35	7	0	0	72.32	0	4.97	1	0.55
14	Humulene	204.35	0	0	0	70.42	0	4.26	1	0.55
15	Anethole	148.2	2	1	0	47.83	9.23	2.79	0	0.55
16	Farnesyl acetate	264.4	9	2	0	83.7	26.3	4.73	0	0.55
17	Ferulic acid	194.18	3	4	2	51.63	66.76	1.36	0	0.85
18	alpha-Farnesene	204.35	6	0	0	72.32	0	4.96	1	0.55
19	(+)-delta-Cadinene	204.35	1	0	0	69.04	0	4.12	1	0.55
20	alpha-Pinene	136.23	0	0	0	45.22	0	3.44	1	0.55
21	beta-Pinene	136.23	0	0	0	45.22	0	3.42	1	0.55
22	(-)-alpha-Cadinol	222.37	1	1	1	70.72	20.23	3.43	0	0.55
23	Levomenol	222.37	4	1	1	72.36	20.23	3.76	0	0.55
24	Caryophyllene oxide	220.35	0	1	0	68.27	12.53	3.68	0	0.55
25	(Z)-beta-Ocimene	136.23	3	0	0	48.76	0	3.4	0	0.55
26	beta-Caryophyllene	204.35	0	0	0	68.78	0	4.24	1	0.55
27	Limonene	136.23	1	0	0	47.12	0	3.37	0	0.55
28	Linoleic acid	280.45	14	2	1	89.46	37.3	5.45	1	0.85
29	alpha-Copaene	204.35	1	0	0	67.14	0	4.3	1	0.55
30	Allo-Aromadendrene	204.35	0	0	0	67.14	0	4.34	1	0.55

MW: molecular weight

NHBD: number of hydrogen bond donors

NHBA: number of hydrogen bond acceptors

RB: rotatable bond

MR: molar refractivity

TPSA: topological polar surface area

According to the pharmacokinetic profile of the phytochemicals found in Persea americana (avocado) **(Table 2),** substances with high GI absorption and BBB permeability, such as jasmone and myristic acid, may have systemic and/or central nervous system activity. P-glycoprotein (Pgp) substrates are not found in most substances, which promotes drug

retention. Nevertheless, CYP enzymes such as CYP1A2, CYP2C9, and CYP3A4 are inhibited by alpha-Farnesene and Ferulic acid, indicating potential drug-drug interactions. Several of the phytochemicals found in avocados have good pharmacokinetic qualities overall, including high absorption and BBB permeability. However, their metabolic stability and interaction patterns may be impacted by CYP inhibition.

Table 2. Pharmacokinetic Properties of the Phytochemicals in Persea Americana

S.No	**Compound**	**GO absorption**	**BBB permeant**	**Pgp substrate**	**CYP1A2 inhibitor**	**CYP2C19 inhibitor**	**CYP2C9 inhibitor**	**CYP2D6 inhibitor**	**VYP3A4 inhibitor**
1	Myristic acid	High	Yes	Yes	Yes	No	No	No	No
2	Jasmone	High	Yes	Yes	No	No	No	No	No
3	Tetradecanal	High	Yes	Yes	Yes	No	No	No	No
4	Bicyclogermacrene	Low	No	No	No	Yes	Yes	No	No
5	gamma-Terpinene	Low	Yes	Yes	No	No	No	No	No
6	Guaiol	High	Yes	Yes	No	No	No	No	No
7	p-Cymene	Low	Yes	Yes	No	No	No	Yes	No
8	Perillene	High	Yes	Yes	Yes	No	No	No	No
9	Palmitic acid	High	Yes	Yes	Yes	No	Yes	No	No
10	Bulnesol	High	Yes	Yes	No	No	No	No	No
11	beta-Gurjunene	Low	Yes	Yes	Yes	Yes	Yes	No	No
12	beta-Elemene	Low	No	No	Yes	Yes	Yes	No	No
13	beta-Farnesene	Low	No	No	No	No	Yes	No	No
14	Humulene	Low	No	No	No	No	Yes	No	No
15	Anethole	High	Yes	Yes	No	No	No	No	No
16	Farnesyl acetate	High	Yes	Yes	No	No	Yes	No	No
17	Ferulic acid	High	Yes	Yes	No	No	No	No	No
18	alpha-Farnesene	Low	No	No	No	No	Yes	No	No
19	(+)-delta-Cadinene	Low	No	No	Yes	Yes	Yes	No	No
20	alpha-Pinene	Low	Yes	Yes	No	No	Yes	No	No

21	beta-Pinene	Low	Yes	Yes	No	No	Yes	No	No
22	(-)-alpha-Cadinol	High	Yes	Yes	Yes	Yes	No	No	No
23	Levomenol	High	Yes	Yes	No	No	Yes	No	No
24	Caryophyllene oxide	High	Yes	Yes	Yes n	Yes	Yes	No	No
25	(Z)-beta-Ocimene	Low	Yes	Yes	No	No	No	No	No
26	beta-Caryophyllene	Low	No	No	Yes	Yes	Yes	No	No
27	Limonene	Low	Yes	Yes	No	No	Yes	No	No
28	Linoleic acid	High	Yes	Yes	No	No	Yes	No	No
29	alpha-Copaene	Low	Yes	Yes	Yes	Yes	Yes	No	No
30	Allo-Aromadendrene	Low	Yes	Yes	Yes	Yes	Yes	No	No

p-gp: P-glycoprotein

GIA: absorption in the gastrointestinal tract

BBB: blood-brain barrier

With carboplatin serving as a control, **Table 3** displays the amino acid interactions and binding affinity between the C-MET protein and several phytochemicals from Persea americana. Notably, Bulnesol formed conventional hydrogen bonds with Asp1222, demonstrating the greatest binding affinity at -8.6 kcal/mol. Additionally, Guaicol showed substantial binding (-7.5 kcal/mol), engaging through multiple van der Waals contacts as well as Tyr1230. Carboplatin, on the other hand, displayed a lower binding affinity of -5.1 kcal/mol. The stability of these contacts is dependent on a variety of van der Waals forces as well as hydrogen bonding, such as that between ferrulic acid and Tyr1230. According to the research, there may be therapeutic value for some avocado phytochemicals, including guaicol and bulbensesol, which exhibit positive binding interactions with C-MET.

Table 3. Binding Affinity and Amino Acid Interactions between C-MET and the Phytochemicals

S. No	**Target**	**Binding Affinity (Kcal/mol)**	**NHB**	**Conventional Hydrogen Bond**	**Carbon Hydrogen / Pi-anion / Pi-Pi stacked / Pi-Sulfur / Pi-Alkyl Bond**	**Van der Waals**
1	Myristic acid	-5.9	2	Asn1209(3.26), Arg1208(5.35)	Met1211, Val1092, Ala1226, Ala1221, Lys1157, Met1160, Ile1084, Leu1140, Ala1108, Tyr1230	

2	Jasmone	-6.7	0		Tyr1230, Ala1108, Met1211,Val1092	eu1157, Leu1140, Ile 1084, Ala1226, Ala1221, asp1164, Asp1222, Arg1208, Asn1209
3	Tetradecanal	-5.6	0		Leu1157, Ala1226, Met1160, Leu1140,Ala1108, Met1211, Val1092, Ile1084, Lys1110	Tyr1230, Tyr1159, Gly1085, Ala1221, Asn1209, Arg1208, Asp1222, Asp1164
4	gamma-Terpinene	-6.7	0		Tyr1230, Ala1221, Met1211	Asp1222, Ala1226, Lys110, Leu1157, Leu1140, Val1092, Asp1164, Asn1209, Arg1208
5	Guaiol	-7.5	0		Tyr1230, Ala1108, Leu1157, Ala1226, Lys1110, Met1211, Val1092	Ile1084, Asp1164, Gly1085, Arg1208, Leu1140, Ala1221, Asp1222, met1160
6	Perillene	-6.1	1	Asp1222(3.81)	Ala1221, Met1211, Tyr1230, Val1092, Ile1084, Ala1108	Met1160, Arg1208, Asn1209
7	Bulnesol	-8.6	0		Met1211, Ala1221, Tyr1230, Val1092	Gln1085, Ile1084, Asp1164, Asn1209, Arg1208, Asp1222, Ala1226, Leu1140, Leu1157, Ala1108, Lys1110
8	Anethole	-6.2	1	Asp1222(3.95)	Ala1221, Leu1140, Ala1226, Tyr1230, Met1211	Asn1209, Arg1208, Asp1231, Asn1167, Asp1164, Asn1209, Leu1140, Asp1222, phe1223
9	Ferulic acid	-6.6	2	Tyr1230(5.79), Ala1226(3.30)	Val1092, Ala1108, Met1211, Ala1221	Leu1157, Arg1208, Asp1231, Asn1167, Asp1164, Asn1209, Leu1140, Asp1222, Phe1223
10	(Z)-beta-Ocimene	-5.9	0		Ala1108, Ala1221, Leu1157, Met1211, Val1092, Tyr1230, Ile1084	Met1160, leu1140, Aa1226, Asp1222, Asn1209
C	Carboplatin	-5.0	1	Tyr1230(5.59)	Asp1222, Met1211, Val1092	Ala1221, Phe1223, Leu1140, Ala1226, Leu1157, Lys1110

The 2D docked structures of several ligands interacting with the C-MET protein are shown in **Figure 1**. Important interactions between the ligands and particular amino acid residues of C-MET, including hydrogen bonds, van der Waals forces, and pi-stacking, are highlighted in the picture. These interactions point to a consistent binding affinity, especially for ligands that exhibit strong interactions with key residues, such as guaicol and bulbenesol. The potential therapeutic importance of these phytochemicals is supported by this image.

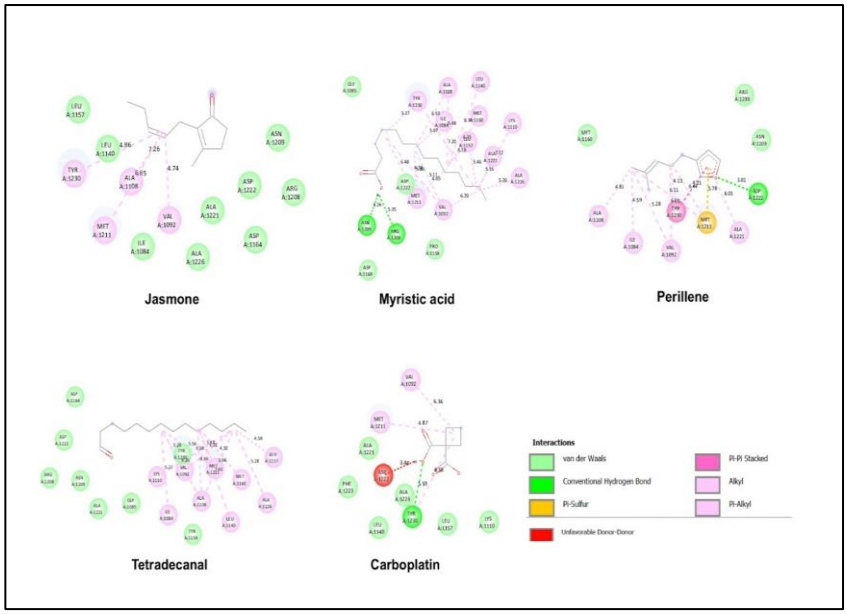

Figure 1. 2D Docked Structure of the Ligands Interacting with C-MET

The 2D docked structures of ligands interacting with the C-MET protein are shown in **Figure 2**, which highlights important interactions such as hydrogen bonds and van der Waals forces. The ligand-protein complexes must be stabilized by these interactions. Interestingly, ligands such as Guaicol and Bulnesol show notable affinity with critical amino acids in C-MET, supporting their potential as therapeutic agents. The graphic illustrates the high interaction characteristics that these phytochemicals have with C-MET and validates the docking results.

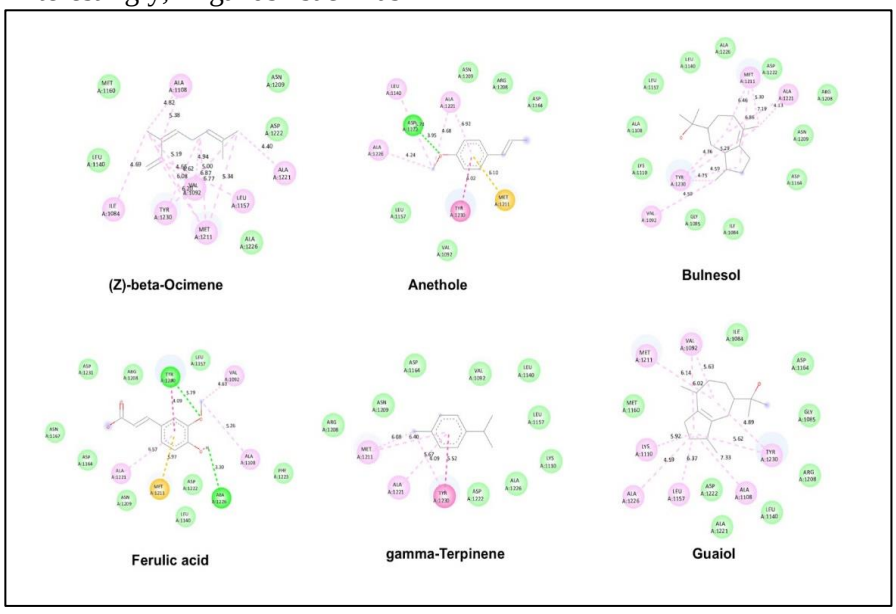

Figure 2. 2D Docked Structure of the Ligands Interacting with C-MET

The study's noteworthy conclusions are collectively illustrated by the figures and graphs. To comprehend how the phytochemicals from Persea americana interact with C-MET's active site, **Figure 3** (Protein Data Bank ID: 3ZZE) depicts the 3D structure of C-MET. The C-MET protein-protein interactions are illustrated in **Figure 4**, which also showcases the protein's interaction network.

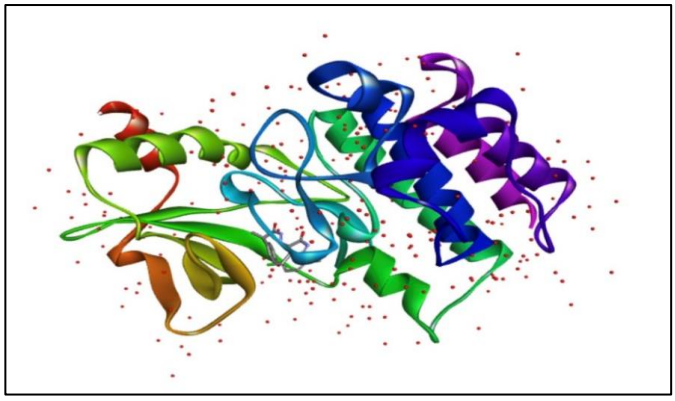

Figure 3. 3D Structure of C-MET (Protein Data Bank ID: 3ZZE)

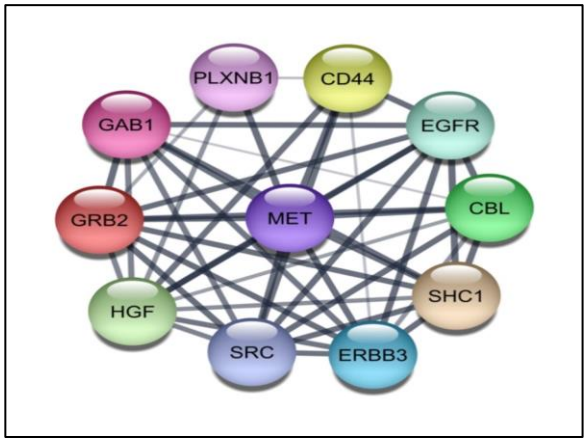

Figure 4. Protein-Protein Interaction

The expression profile of C-MET, which is crucial for determining how the phytochemicals affect its levels, is shown in **Figure 5**.

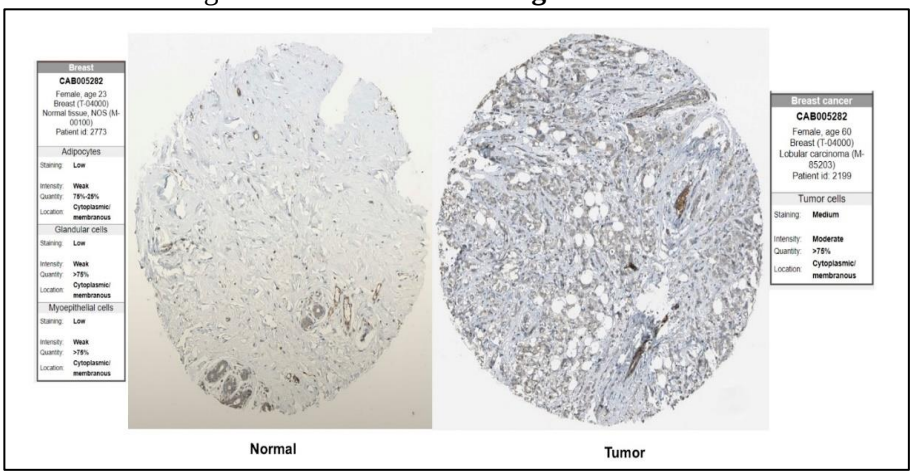

Figure 5. Expression Profile Analysis of C-MET

The preliminary insights into the possible effectiveness of the compounds are provided by the in silico cytotoxicity predictions, as illustrated in **Table 4**. The phytochemicals' biological activity spectrum, which supports their potential for therapeutic use, is shown in **Table 5**. The morphology of MDA-MB-231 cells treated with 31.25 µg/mL and 125 µg/mL of Persea americana fruit extract is compared with control cells in **Figure 6,** indicating the cytotoxic effects of the extract.

Table 4. In Silico Cytotoxicity Prediction

S No.	Leads	Pa	Pi	Cell-line	Cell line full name	Tissue	Tumor type
	Jasmone	0.43	0.42	MDA-MB-231	Breast adenocarcinoma	Breast	Adenocarcinoma
	Jasmone	0.317	0.274	MDA-MB-453	Breast adenocarcinoma	Breast	Adenocarcinoma
	gamma-Terpinene	0.091	0.068	Bcap37	Breast adenocarcinoma	Breast	Adenocarcinoma
	Guaiol	0.32	0.268	MDA-MB-453	Breast adenocarcinoma	Breast	Adenocarcinoma
	Guaiol	0.093	0.060	Bcap37	Breast adenocarcinoma	Breast	Adenocarcinoma
	Ferulic acid	0.422	0.059	MDA-MB-453	Breast adenocarcinoma	Breast	Adenocarcinoma

Table 5. Biological Activity Spectrum

S. No.	Leads	Pa	Pi	Activity
	Jasmone	0,211	0,089	Antineoplastic (breast cancer)
	gamma-Terpinene	0,257	0,181	Antineoplastic
	Guaiol	0,101	0,095	Antineoplastic antimetabolite
	Bulnesol	0.266	0,174	Antineoplastic
	Ferulic acid	0,467	0,023	Antineoplastic (breast cancer)

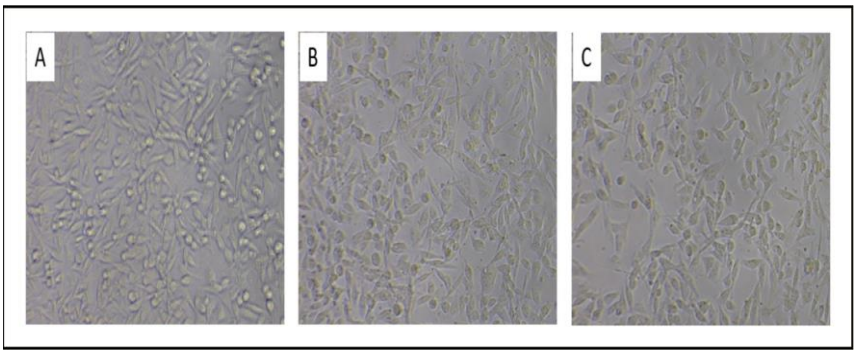

Figure 6. Morphology of Control MDA- MB-231 cells (A), MDA- MB-231 Cells Treated with 31.25 µg/mL (B) and 125 µg/mL (C) of Persea Americana Fruit Extract

The proportion of MDA-MB-231 cells impacted by different extract concentrations is quantified in **Figure 7**, which supports the extract's potential as a novel therapeutic agent against triple-negative breast cancer and confirms the morphological changes that have been seen.

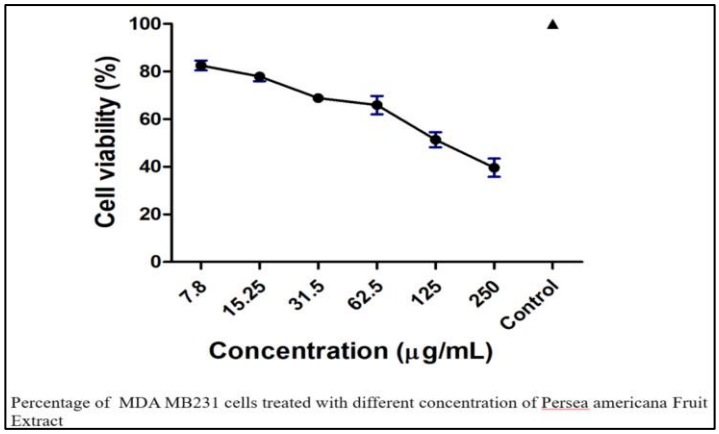

Figure 7. Percentage of MDA- MB -231 Cells Treated with Different Concentrations of Persea Americana Fruit Extract

Discussion

Persea americana, or avocado, has been the subject of recent scientific advancements that suggest it may contain novel anticancer drugs, especially for the treatment of triple-negative breast cancer (TNBC). According to Şüküroğlu (2023) [9] and Awaad et al. (2023) [10], this study emphasizes the phytochemical richness of avocado and its prospective biological activities, including cytotoxic effects against cancer cell lines like MCF-7 and MDA-MB-231. Because avocados have anti-inflammatory, antioxidant, and anticancer capabilities, using avocado-derived nanoparticles and extracts shows great promise in the creation of new treatment approaches [11, 12]. These results highlight the necessity of more research into avocado's potential as a cancer treatment [4].

Table 1 represents the drug likeness of the phytochemicals. The lead candidate must be selected based on the Lipinski rule in such a way as to increase the oral drug delivery potential. During compound analysis, it was considered that a single violation is acceptable for a compound. There are 15 compounds with one violation. In all, 30 compounds passed the criteria of Lipinski's rule for drug likeness. This selection ensures that the compounds have favourable physicochemical properties, thereby maximizing their chances of becoming lead drug candidates. Table 2 provides an overview of the pharmacokinetic properties of the phytochemicals, highlighting their absorption, BBB permeability, p- glycoprotein substrate status, and interactions with various cytochrome P450 isoenzymes.

On screening of 98 phytochemicals extracted from *Persea americana* for their drug candidate potential, all the compounds adhered to Lipinski's rule of 5, suggesting their drug likeness. This rule examines parameters such as molecular weight, lipophilicity, and hydrogen bonding, very important in the pharmacokinetic profile of a molecule and oral bioavailability. The fact that they passed these criteria is what makes these phytochemicals very promising for further pharmaceutical exploitation. Inhibition of cytochrome P450 isoenzymes by compounds noted in the screening process was excluded from further studies. Cytochrome P450 enzymes represent a superfamily of catalytically active proteins involved in sequential electron transfer to a variety of substrates and play a critical role in drug metabolism. Since the inhibition of this enzyme can cause dangerous interactions of drugs and unpredictable pharmacokinetics, avoidance of such compounds at an early stage of the study kept the focus on those with potentially safer and more predictable pharmacological profiles [13-16].

Subsequently, the binding affinities of 10 selected phytochemicals against C-MET, a protein involved in triple-negative breast

cancer, were assessed through molecular docking studies. Those results yielded strong binding affinities, ranging from -5 to -8.6 kcal/mol, showing strong interactions of these phytochemicals with C-MET. Since C-MET has been identified to play a role in cancer development, progression, and metastasis, this makes these findings are promising for targeting TNBC [5, 6]. These phytochemicals demonstrated antineoplastic activity in subsequent *in silico* analysis. Antineoplastic activity acts to prevent the growth or proliferation of malignant cells. Taken together, computational simulations suggest that these compounds would show characteristics that could potentially hinder the growth and proliferation of TNBC cells, hence their therapeutic potential.

It is further shown by *in silico* cytotoxicity analyzes that the compounds were efficacious against TNBC. Computational models used in this study simulated scenarios predicting how these phytochemicals interplay with TNBC cells at the molecular level. In summary, the analysis revealed that 5 compounds were endowed with remarkable cytotoxic effects against TNBC, further showing their potential as lead candidates for further preclinical and clinical investigations [7, 17]. Besides the individual phytochemicals, the aqueous extract of the plant species *Persea americana* portrayed outstanding cytotoxic activity against MDA-MB-231, one of the most common cell lines that represents TNBC. An IC50 of 125 micrograms/mL was computed. This is the concentration at which the extract can inhibit the growth of those cancer cells by half. This result supports, therefore, the computational experimental findings and echoes the potential therapeutic value of *Persea americana* against TNBC [9, 10].

A recent study observed the separation and classification of a triterpenoid compound from an ethanol extract of avocado seeds. This triterpenoid with a molecular weight of 505 g/mol was the first ever component of this class that has been identified from avocado seeds. Extracts, as well as one fraction and the isolated compound, were analyzed for cytotoxic activity. Results showed pronounced cytotoxic activity against MCF-7 cells with IC50 values below 100 μg/mL. Subsequent purification of the extract was conducted, as a result, its activity against MCF-7 cells increased, and through this, a potential novel drug for chemotherapy to the inhibition of the growth of tumours and cancer cells proliferation was found [16, 18]. Previous research has also assessed the avocado oil for its cytotoxic and genotoxic effects on MCF-7 cells regarding its safety as a food supplement. In the study, MCF-7 cells were exposed to different concentrations of avocado oil for 24, 48, and 72 hours. Results of this study showed a significant decrease in cell proliferation and an increase in micronucleus frequency compared with the control group. The results showed that avocado oil caused cytotoxicity and genotoxicity depending on the time and concentration applied [9]. Another study assessed the effect of organically coated *Persea americana* leaves nanoparticles on cervical cancer and breast adenocarcinoma cells, respectively. Gene expression and cell viability were investigated. Analysis by UV-Vis spectroscopy showed that the nanoparticles are partially stable in cell culture conditions. HeLa cells remained viable after nanoparticle treatment. It produced a dose-dependent, significant cytotoxic response on the breast cancer cell line above the concentration of 50 μM. The study suggested that the selective toxicity of the biosynthesized avocado AgNPs on the MCF-7 cells may have value in novel therapeutics [11, 19].

Apart from the strong evidence indicating avocado's (Persea americana) potential to prevent cancer, studies also demonstrate avocado's several medicinal uses. According to studies, avocado fruit and seed extracts have strong anti-inflammatory, antioxidant, and anticancer effects, which support avocado's potential as a medicinal agent [20]. These

results are consistent with the literature's emphasis on the larger need for innovative treatments for triple-negative breast cancer (TNBC) [21]. The significance of investigating alternative treatments is further highlighted by the progress made in comprehending the mechanisms behind TNBC [22, 23]. Notably, it has been discovered that doxorubicin's efficiency is enhanced when mixed with certain chemicals, indicating that avocado may also increase the effectiveness of already available medicines [24, 25]. All things considered, these findings support more investigation into avocado-derived chemicals because of their unique biochemical characteristics and encouraging in vitro results.

Limitations

In silico predictive models by themselves rely only on pre-existing data and algorithms that may not genuinely represent the biological intricate interactions of live cells. Additionally, these models can be non-specific to their targets, which would be cytotoxic to the normal cells and thus not specific enough for non-cancerous tissue. Another point is that translation from *in silico* and *in vitro* studies to clinical trials has rigorous regulatory and ethical steps to pass, making it a bit lengthy and expensive.

Conclusion

The outcomes show how promising these compounds' interactions are with the target. However, to verify the anticancer efficacy of these substances, experimental validation through in vitro or in vivo research is necessary. From a starting selection of 98 naturally occurring compounds with plant origins, the researchers, using a structure-based approach to drug design, found five bioactive compounds that showed strong anticancer efficacy against C-MET in triple-negative breast cancer. Furthermore, the MTT experiment confirmed that Persea americana has cytotoxic effects on MDA-MB-231 breast cancer cell lines.

Conflict of Interest

There is no conflict of interest.

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