RESEARCH ARTICLE

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In Silico Prediction of Selected Bioactive Compounds Present in *Alpinia elegans* (C.Presl) K.Schum Seed Oil as Potential Drug Candidates Against Human Cancer Cell Lines

Jane Marie N. Caasi[1], Raiza Isabel D.G. Baldoza[1], Mary Sophia C. Bauzon[1], Mirabella Anne F. Odtohan[1], Librado A. Santiago[1,2], Myla R. Santiago-Bautista[1,2]*

Abstract

Objective: *Alpinia elegans* (Zingiberaceae) is a Philippine endemic plant reported to have various folkloric uses. The seed oil of *A. elegans* has been shown to contain a majority of the following bioactive compounds: D-limonene, α-pinene, and caryophyllene oxide. The study sought to determine if the bioactive compounds found in *A. elegans* seed oil would be a good natural, inexpensive, and less-detrimental alternative for cancer treatment. **Methods:** The study utilized *in silico* (Way2Drug predictive services, SwissADME, AutoDock 4) experiment to examine the aforementioned compounds as viable therapeutic candidates against human cancer cell lines. **Result:** Results determined that the compounds D-limonene, α-pinene, and caryophyllene oxide were most potent against thyroid gland carcinoma (8505C) cells, brain glaucoma (Hs 683) cells, and promyeloblast leukemia (HL-60) cells, respectively. Additionally, D-limonene was the only compound to show arrhythmia as an adverse effect. Predictions showed that the compounds could inhibit cellular growth factors and serine/threonine-protein kinase activity. The compounds generated a bioavailability score of 0.55 and exhibited blood-brain barrier (BBB) penetration. D-limonene, α-pinene, and caryophyllene oxide had binding energy of -4.59, -5.43, and -6.92, respectively. **Conclusion:** The binding energy indicated that the ligands could securely dock to the receptors, thus suggesting that interaction between the ligands and receptors was stable. Results have shown that the compounds are promising candidates against human cancer cell lines by inhibiting cell proliferation and inducing apoptosis.

Keywords: *Alpinia elegans*- D-limonene- α-pinene- caryophyllene oxide- cancer- apoptosis

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Introduction

Cancer is a known disease that rapidly proliferates and invades cells beyond normal boundaries (World Health Organization, 2021), affecting people worldwide. Despite the impending causes of side effects due to toxicity in both cancer and healthy cells, synthetic agents are typically used to treat cancer in chemotherapy (Laskar et al., 2020; Hennessy et al., 2009). Because of this, there is a constant demand for drug discoveries that are efficient in combating cancerous cells while being less damaging. Herbal plant usage for cancer treatment can be regarded as an efficient and inexpensive way to promote affordable healthcare and medicine for ordinary people, which provides security for livelihood and advancement in the medical field (Richard et al., 2015). The Philippines, being inhabited by multiple flora species, has limited studies surrounding its potential medical properties.

Alpinia elegans (C. Presl) K. Schum is a plant endemic to the Philippines. It is commonly referred to as "Tagbak", "Bayumbong pula", "de Castanilla", and "Panya pula". In the province of Antique, it is claimed to have a variety of ethnomedicinal uses such as treatment for musculoskeletal diseases, hemoptysis, headache/migraine, stomachache, and anti-relapse for women (Naive et al., 2019). However, claims regarding its ethnomedicinal uses have yet to be investigated. A study by Houdkova et al., (2020) was able to determine the bioactive compound present in the seed oils of *A. elegans*. It was determined that the top three bioactive compounds were: D-limonene, α-pinene, and caryophyllene oxide. The three bioactive compounds are classified under terpenoids, a class of compounds that are known to have a variety of biological activity. Moreover, studies have shown that the bioactive compounds have displayed apoptotic activity, wherein D-limonene triggers apoptosis by promoting autophagy (Yu et al., 2018), α-pinene induces apoptosis by disrupting mitochondrial activity (Salehi et al., 2019), and lastly, caryophyllene oxide inhibits the growth and proliferation of a variety of cancer cells (Francomano et al., 2019).

*[1]Department of Biochemistry, Faculty of Pharmacy, University of Santo Tomas, Manila, Philippines. [2]Research Center for Natural and Applied Sciences, University of Santo Tomas, Manila, Philippines. *For Correspondence: mrsantiago@ust.edu.ph*

If the study's findings are confirmed and deemed valuable, it may result in the plant's production and further research of its therapeutic potential. Evaluating cellular activities, side effects, and binding affinities of ligands with receptors utilizing diverse platform methodologies may lead to advancements in cancer research. Furthermore, data analysis will provide further understanding and information regarding the chemical compounds and their interactions with receptors. Finally, studying and differentiating the characteristics of each ligand-receptor interaction and binding could potentially lead to the development of novel and inexpensive drugs. As such, the study sought to determine if the bioactive compounds found in *A. elegans* seed oil would be a good natural, inexpensive, and less-detrimental alternative for cancer treatment.

Materials and Methods

The 2D structures of the study's bioactive compounds: D-limonene, α-pinene, and caryophyllene oxide, were acquired through the Chemical Entities of Biological Interest (ChEBI): https://www.ebi.ac.uk/chebi/init.do. The MOL files of the compounds were then downloaded from the website of ChEBI, under the European Molecular Biology Laboratory's European Bioinformatics Institute (EMBL-EBI), and the canonical Simplified Molecular Input Line Entry System (SMILES) of each compound was gathered from NCBI PubChem: https://pubchem.ncbi.nlm.nih.gov/. The potency, detrimental effect, protein targets, and cellular activity of the compounds discussed in this paper were determined using each of the PASS online predictive tools.

The receptors used in this study were collected from the generated results from the PASS Target for each of the bioactive compounds. These receptors were retrieved from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB): https://www.rcsb.org/ database in PDB format. On the other hand, the ligands D-limonene, α-pinene, and caryophyllene oxide were downloaded from the NCBI PubChem and then converted into PDB files using BIOVIA Discovery Studio or UCSF Chimera as an alternative.

Way2Drug Predictive Services

Predictive tools from Way2Drug services (http://www.way2drug.com/) such as CLC-Pred for evaluating chemical potency towards tumor cell lines, ADVER-Pred for detecting adverse health effects, and PASS Target, PASS Online, and KinScreen for establishing protein targets and cellular activity was made use of. The MOL file providing each compound's structural formula has been used by CLC-Pred in which the files were evaluated individually. It was then utilized to determine cytotoxic activities in cancer cell lines, as well as a list of the compound's likelihood of being active (Pa) or inactive (Pi) in each cell line. The results were arranged according to the most inhibitory activity to the least inhibitory activity with the SDF, CSV, and PDF file formats. The SMILES format of the mentioned bioactive compounds was utilized in ADVER-Pred for the evaluation of detrimental effects such as arrhythmia, cardiac failure, hepatotoxicity, nephrotoxicity, and myocardial infarction.

Furthermore, the PASS Online and PASS Target services were also able to utilize the MOL files providing the structural formulas of the bioactive compounds. The set of identified proteins as probable targets with a direct mediated interaction was obtained using PASS Target prediction. The canonical SMILES of bioactive substances were processed to make the appropriate 2D structures for the KinScreen predictive service, which subsequently facilitated the detection of target protein kinases, novel linkages between kinases, and therapeutic implications for these molecules. The screen image was then captured and compiled into a table along with the acquisition of file formats and findings.

SwissADME

SwissADME was performed to reveal the physicochemical descriptors as well as to predict the compound's pharmacokinetic features, druglike characteristics, and medicinal chemistry responsiveness. The canonical SMILES of the compounds D-limonene, α-pinene, and caryophyllene oxide were gathered from the NCBI PubChem. These were then used to run on the website of SwissADME: http://www.swissadme.ch/index.php. The results that the system produced were then tabulated and analyzed.

Molecular Docking

The molecular docking simulations were executed using AutoDock 4 from the MGL tools 1.5.4. AutoDock simulates the binding postures and interactions of this set of inhibitors with the target. For the preparation of protein molecules, the downloaded PDB files of the receptors (3SQ9, 6DTL, 5A5J) were subjected to energy minimizations with 1000 steps and using the default options. Energy minimization was done by removing the heteroatom molecules and natural ligands on the receptors as well as adding the polar hydrogen atoms through the software BIOVIA Discovery Studio. After the completion of energy minimization for both ligands (D-limonene, α-pinene, caryophyllene oxide, temozolomide, doxorubicin) and receptors (α-7 nicotinic receptor, mitogen-activated protein kinase 6, cytochrome 2C9 P450 inhibitor), the structures were converted to the PDB format and were utilized in AutoDock 4 for molecular docking. The results were then opened through BIOVIA Discovery Studio once again and also Protein-Ligand Interaction Profiler for viewing, analysis, and interpretation of the binding between the ligand-receptor docking result, the images were then saved in PDB, and PNG or JPEG format.

Cytotoxicity Prediction of the Bioactive Compounds Against Tumor Cell Lines

The generated CLC-Pred displays the cytotoxicity prediction of the bioactive compounds caryophyllene oxide, α-pinene, and D-limonene to cancer cell lines from various tissues stored in the PASS models online (Lagunin et al., 2018). The listed results consist of the top cell line inhibited by the respective compounds from *A. elegans*

seed oil set at Pa > 0.3 significance. Table 1 shows that all compounds exhibit cytotoxic activities on various cancer cell types, including leukemia, glioma, and carcinoma. The data revealed that caryophyllene oxide would be the most cytotoxic against promyeloblast leukemia (HL-60) cell lines with a generated Pa and Pi of 0.647 and 0.012, respectively. The compound is followed by α-pinene having brain glaucoma cytotoxicity with the cancer cell line Hs 683 (Pa = 0.526, Pi = 0.048). D-limonene then succeeds the mentioned compound with thyroid gland undifferentiated carcinoma (8505C) cell line inhibition having a Pa of 0.399 and Pi of 0.015. The presented results denote that all bioactive compounds have a higher probability of activity than inactivity in the mentioned cancer cell lines.

Results

Prediction of Adverse Effects of the Bioactive Compounds

ADVER-Pred is a free web tool that predicts and estimates the compound's ability to cause adverse effects based on its structural forms (Ivanov et al., 2018). The test generated no adverse effects on two out of the three compounds, namely the α-pinene and caryophyllene oxide. D-limonene, however, generated arrhythmia side effects.

Based on Table 2, the produced Pa (0.337) and Pi (0.248) are close in range, which indicates that the side effect has a significant potential to be active and inactive. D-limonene in lower doses has been tested to cause non-significant hypotension; however, once doses are increased, intense bradycardia arrhythmia can be observed (Nascimento et al., 2019). Therefore, the results denote that the compound does not affect the heart rate by a sufficient amount. Nevertheless, an increased dosage could eventually lead to an adverse effect of arrhythmia.

Predicted Biological and Pharmacological Activities of the Bioactive Compounds

PASS Online is an online service that can predict a compound's biological and pharmacological activity based on its similarity to known physiologically active substrates (Parasuraman, 2011). Table 3 shows the predicted biological and pharmacological activity of the bioactive compounds as well as their Pa: Pi ratio at a prediction threshold of Pa > 0.7. Caryophyllene oxide and D-limonene were both predicted to have the same activities, albeit have different Pa and Pi values: (a) antineoplastic activity with Pa = 0.950, Pi = 0.004, and Pa = 0.812, Pi = 0.003, respectively; (c) transcription factor NF kappa B stimulants with Pa = 0.745, Pi = 0.004, and Pa = 0.765, Pi = 0.003, respectively. Meanwhile, α-pinene was predicted to not have any similar activity to that of caryophyllene oxide and D-limonene. α-pinene was shown to have an activity as CYP2J substrate, wherein Pa = 0.844 and Pi = 0.012. UGT1A4 substrate UGT1A4

Table 1. Tumor Cell Line Prediction Results of α-pinene, Caryophyllene Oxide, & D-limonene, Showing the Cytotoxicity Prediction of the Bioactive Compounds to Cancer Cell Lines.

Bioactive Compound	Pa	Pi	Cell-line	Cell-line full name	Tissue	Tumor type
caryophyllene oxide	0.647	0.012	HL-60	Promyeloblast leukemia	Haematopoietic and lymphoid tissue	Leukemia
α-pinene	0.526	0.048	Hs 683	Oligodendroglioma	Brain	Glioma
D-limonene	0.399	0.015	8505C	Thyroid gland undifferentiated (anaplastic) carcinoma	Thyroid	Carcinoma

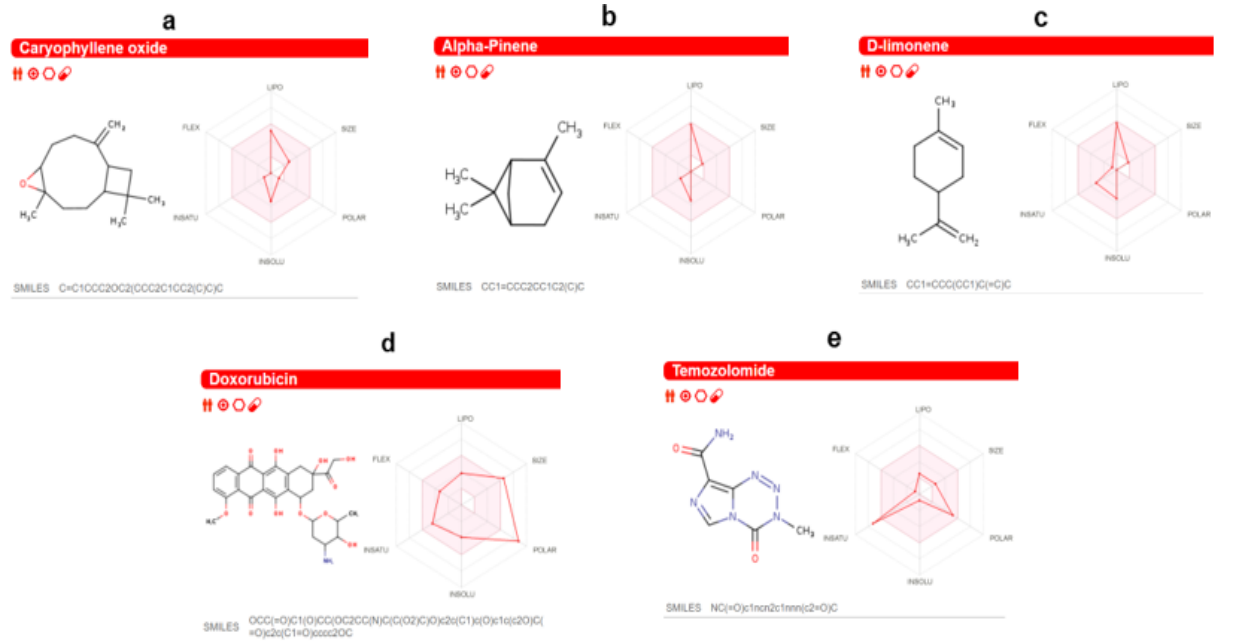

Figure 1. The Bioavailability Radar and Canonical SMILES of Bioactive Compounds Using the SwissADME web Tool. (a) caryophyllene oxide, (b) α-pinene, (c) D-limonene, (d) Doxorubicin, and (e) Temozolomide.

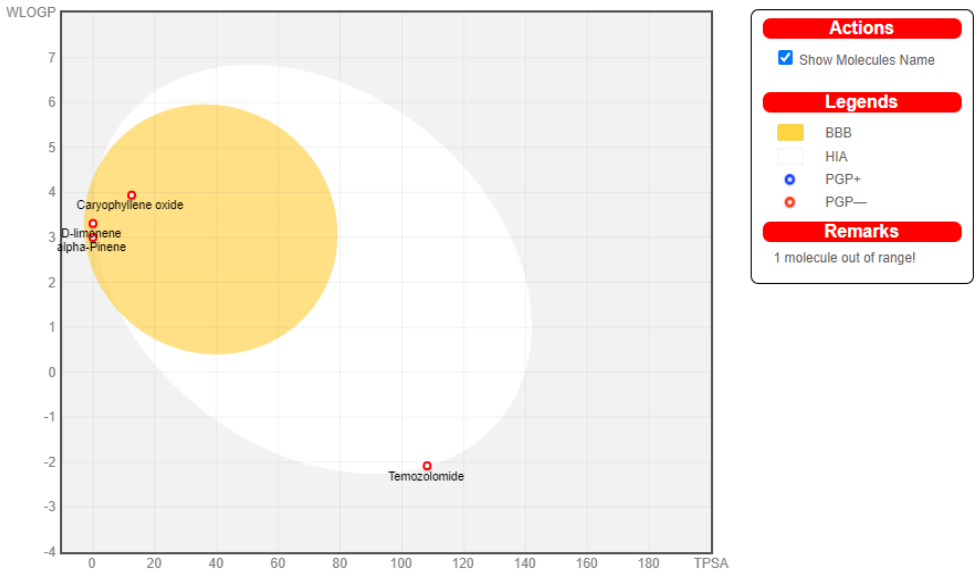

Figure 2. BOILED-Egg Model of Gastrointestinal (GI) Absorption, Blood-Brain Barrier (BBB) Penetration, and P-Glycoprotein (PGP) Activity of the Target and Standard Molecules. The standard doxorubicin is out of the model's range.

Table 2. Tabulated Results of the ADVER-Pred Test on Caryophyllene Oxide, α-Pinene, & D-Limonene.

Bioactive Compound	ADVER-Pred Result
caryophyllene oxide	Adverse effects are not predicted
α-pinene	Adverse effects are not predicted
D-limonene	Side effect: Arrhythmia Pa = 0.337 Pi = 0.248

substrate was also predicted with Pa = 0.708 and Pi = 0.005. Lastly, α-pinene also displayed CYP2J2 substrate activity with Pa = 0.724 and Pi = 0.028. The generated high Pa indicates that all compounds possess a high active probability of cancer-related activities. Table 3 shows the predicted biological and pharmacological activity of the bioactive compounds as well as their Pa: Pi ratio at a prediction threshold of Pa > 0.7.

Table 3. PASS Online Results of Caryophyllene Oxide, α-Pinene, & D-Limonene Predicting the Possible Biological and Pharmacological Activities of the Compounds.

Bioactive Compound	Pa	Pi	Activity
	0.95	0.004	Antineoplastic
	0.836	0.006	Apoptosis agent
caryophyllene oxide	0.745	0.004	Transcription factor NF kappa B stimulant
	0.844	0.012	CYP2J substrate
	0.708	0.005	UGT1A4 substrate
α-pinene	0.724	0.028	CYP2J2 substrate
	0.816	0.007	Apoptosis agent
	0.812	0.01	Antineoplastic
D-limonene	0.765	0.003	Transcription factor NF kappa B stimulant

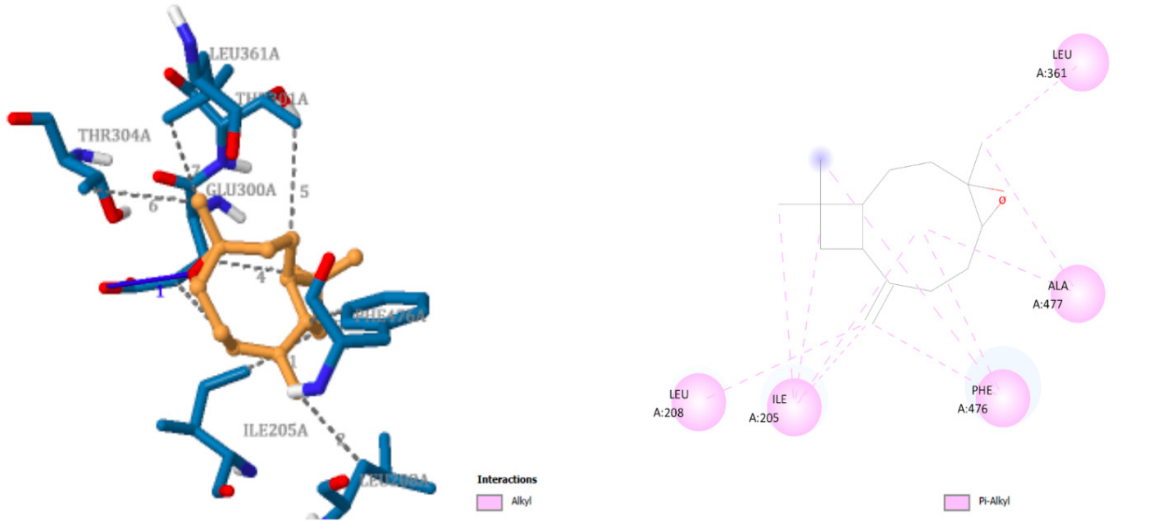

Figure 3. 3D (Left) Diagram from Protein-Ligand Interaction Profiler & 2D (Right) Diagram from BIOVIA Discovery Studio of Caryophyllene Oxide and Cytochrome C29 P450.

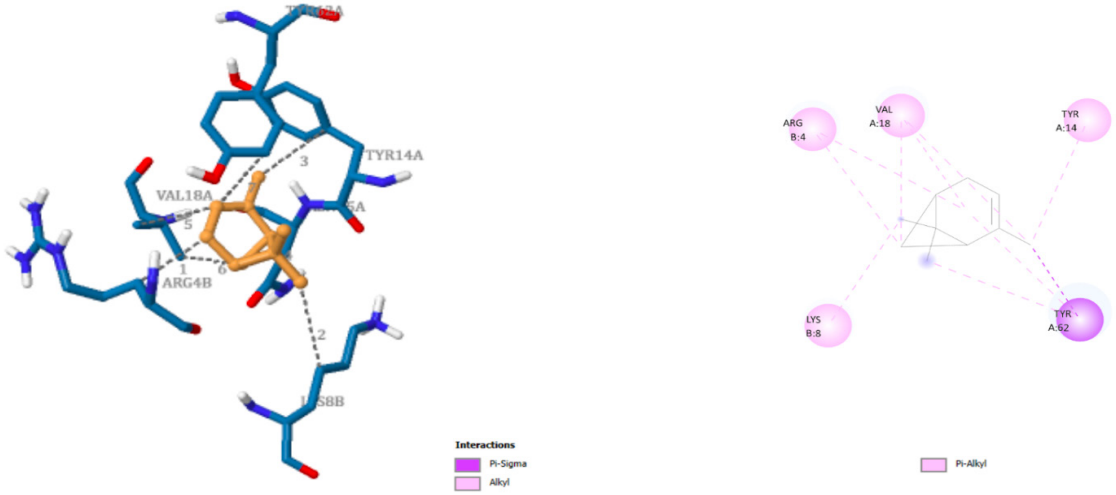

Figure 4. 3D (Left) Diagram from Protein-Ligand Interaction Profiler & 2D (Right) Diagram from BIOVIA Discovery Studio of α-Pinene and α-7 Nicotinic Receptor

Table 4. PASS Target Results of Caryophyllene Oxide, α-Pinene, & D-Limonene Predicting the Interactions of Human Protein Targets from Different Organisms with Drug-Like Compounds.

Bioactive Compound	Target name	Confidence	ChEMBL ID
caryophyllene oxide	Cytochrome P450 2C9	0.6805	CHEMBL3397
α-pinene	Neuronal acetylcholine receptor protein α-7 subunit	0.6233	CHEMBL2492
D-limonene	Mitogen-activated protein kinase 6	0.3988	CHEMBL4309

Predicted Human Target Proteins Interacting with the Bioactive Compounds

PASS Online is an online service that can predict a compound's biological and pharmacological activity based on its similarity to known physiologically active substrates (Parasuraman, 2011) (Table 4).

Predicted Kinase Targets of the Bioactive Compounds

The KinScreen results (Table 5) revealed that all three bioactive compounds had high confidence in the

Table 5. Kin Screen Results of Caryophyllene Oxide, α-Pinene, and D-Limonene

Bioactive Compound	Confidence	Name	UniProt ID	ChEMBL ID	Prediction accuracy (AUC, LOO CV)
caryophyllene oxide	0.74	Serine/threonine-protein kinase NEK6	Q9HC98	CHEMBL4309	0.7
α-pinene	0.69	Serine/threonine-protein kinase NEK6	Q9HC98	CHEMBL4309	0.7
D-limonene	0.69	Serine/threonine-protein kinase NEK6	Q9HC98	CHEMBL4309	0.7

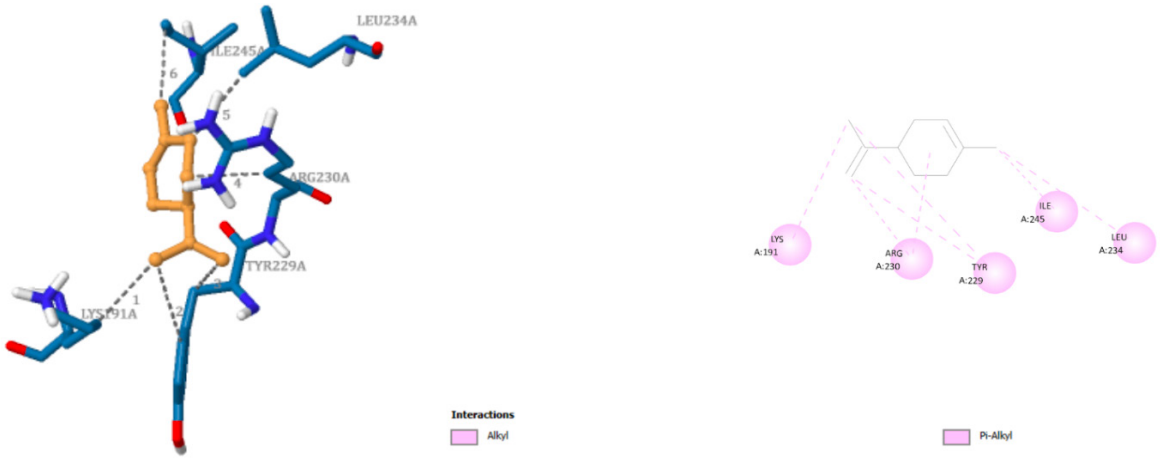

Figure 5. 3D (Left) Diagram from Protein-Ligand Interaction Profiler & 2D (Right) Diagram from BIOVIA Discovery Studio of D-limonene and Mitogen-Activated Protein Kinase 6.

Table 6. Lipinski's Rule of Five for ADME and Drug-Likeness Parameters for the Target Compounds and Anticancer Standards

Target Compounds	Lipinski's Rule of Five					Drug-likeness		
	MW (g/mol) <500	H-bond acceptors <10	H-bond donors <5	MLog P<5	Lipinski violations <2	GI absorption	BBB permeant	Bioavailability
Caryophyllene oxide	220.35	1	0	3.67	0	High	Yes	0.55
α-pinene	136.23	0	0	4.29	1	Low	Yes	0.55
D-limonene	136.23	0	0	3.27	0	Low	Yes	0.55
Anticancer Standards	MW (g/mol) <500	H-bond acceptors <10	H-bond donors <5	MLogP <5	Lipinski violations <2	GI absorption	BBB permeant	Bioavailability
Doxorubicin	543.53	12	6	-2.1	3	Low	No	0.17
Temozolomide	194.15	5	1	-0.98	0	High	No	0.55

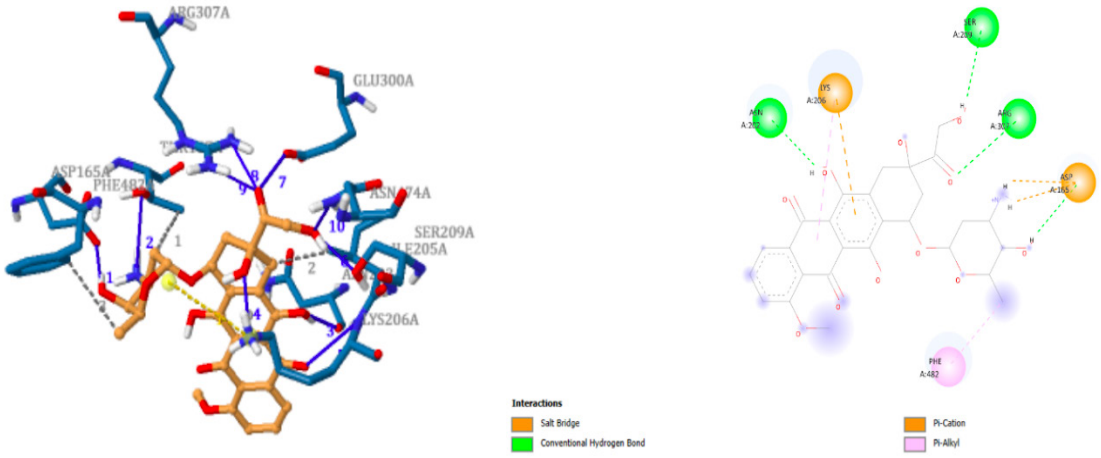

Figure 6. 3D (Left) Diagram from Protein-Ligand Interaction Profiler & 2D (Right) Diagram from BIOVIA Discovery Studio of D-limonene and Mitogen-Activated Protein Kinase 6.

protein target serine/threonine-protein kinase NEK6. Because these compounds have been found to exhibit serine/threonine-protein kinase activity, several studies on the kinase family have revealed that they are involved in both apoptotic activity and cell proliferation via cell cycle regulation (Cross et al., 2009; Jin et al., 2015). Few reported studies specifically focus on bioactive compounds, including serine/threonine kinases, other than the fact that these bioactive chemicals have different apoptotic properties than other protein kinases.

Table 7. Summary of the Bioactive Compounds with Their Respective Receptors

No.	Protein	Compound	Binding Energy (Kcal/Mol)	Inhibition constant (Ki) (mM)	No. of H-bonds	Amino Acid involved in interaction
1	Cytochrome 2C9 P450 inhibitor (5A5J)	Caryophyllene oxide	-6.82	10.05	1	Ile205(A), Leu208(A), Glu300(A), Thr304(A), Leu361(A), Phe476(A)
2	α-7 nicotinic receptor (3SQ9)	α-pinene	-5.48	96.32	0	Arg4(B), Lys8(B), Tyr14(A), Asn15(A), Val18(A), Tyr62(A)
3	Mitogen-activated protein kinase 6 (6DTL)	D-limonene	-5.59	79.87	0	Lys191(A), Tyr229(A), Arg230(A), Leu234(A), Ile245(A)

Table 8. Summary of Caryophyllene Oxide, Doxorubicin, & Temozolomide Docked with Cytochrome 2C9 P450 Inhibitor

No.	Protein	Compound	Binding Energy (Kcal/Mol)	Inhibition constant (Ki) (mM)	No. of H-bonds	Amino Acid involved in interaction
1	Cytochrome 2C9 P450 inhibitor (5A5J)	Caryophyllene oxide	-6.82	10.05	1	Ile205(A), Leu208(A), Glu300(A), Thr304(A), Leu361(A), Phe476(A)
2	Cytochrome 2C9 P450 inhibitor	Doxorubicin	-5.99	40.59	10	Asp165(A), Thr167(A), Ile205(A), Phe482(A), Asn202(A), Lys206(A), Ser209(A), Glu300(A), Arg307(A), Asn474(A)
3	Cytochrome 2C9 P450 inhibitor	Temozolomide	-3.65	2.11	2	Ile207(A), Tyr225(A)

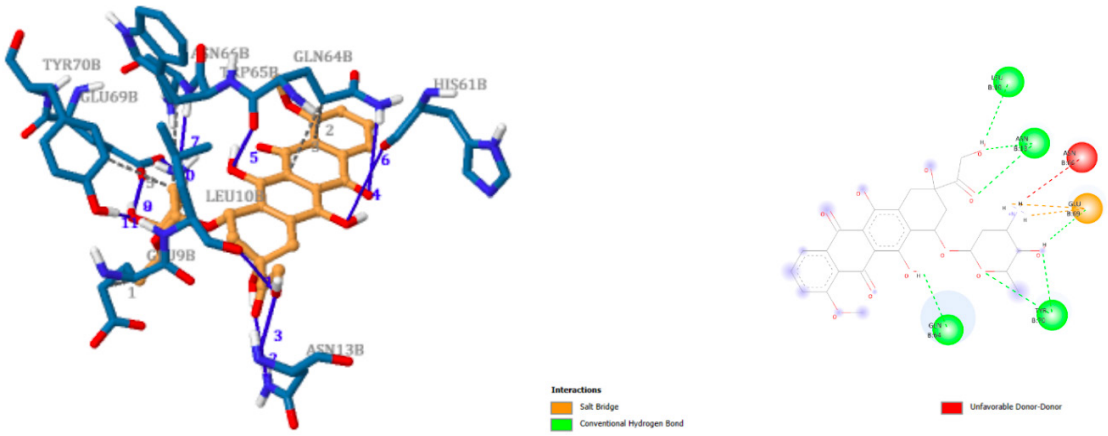

Figure 7. 3D (Left) Diagram from Protein-Ligand Interaction Profiler & 2D (Right) Diagram from BIOVIA Discovery Studio of Doxorubicin and α-7 Nicotinic Receptor

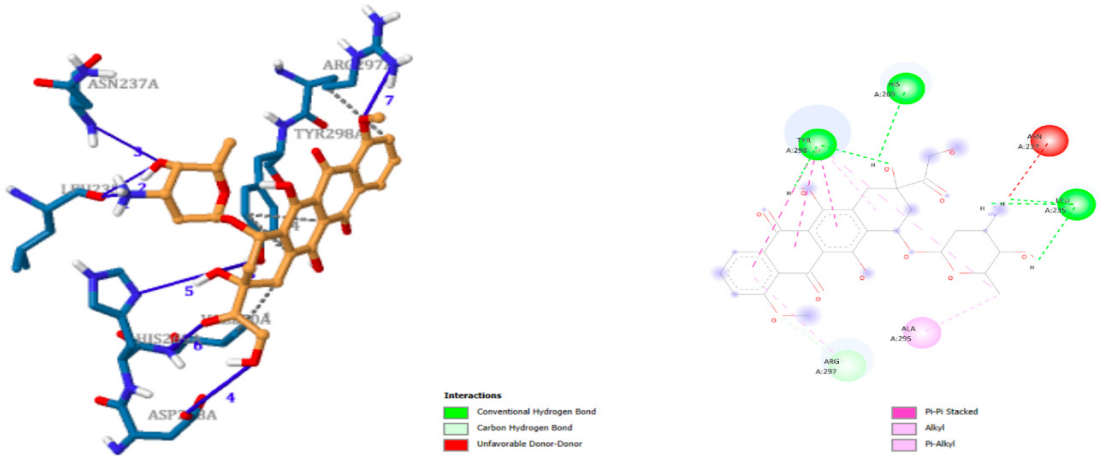

Figure 8. 3D (Left) Diagram from Protein-Ligand Interaction Profiler & 2D (Right) Diagram from BIOVIA Discovery Studio of doxorubicin and Mitogen-Activated Protein Kinase 6

SwissADME ADME and Drug-Likeness Profiling

For one or more compounds, the SwissADME Webserver allows the computation of essential physicochemical, pharmacokinetic, drug-likeness, and

Table 9. Summary of α-Pinene, Doxorubicin, & Temozolomide docked with α-7 Nicotinic Receptor (3SQ9).

No.	Protein	Compound	Binding Energy (Kcal/Mol)	Inhibition constant (Ki) (mM)	No. of H-bonds	Amino Acid involved in interaction
1	α-7 nicotinic receptor (3SQ9)	α-pinene	-5.48	96.32	0	Arg4(B), Lys8(B), Tyr14(A), Asn15(A), Val18(A), Tyr62(A)
2	α-7 nicotinic receptor	Doxorubicin	-8.62	479.38	11	Glu9(B), Gln64(B), Trp65(B), Tyr70(B), Leu10(B), Asn13(B), His61(B), Glu69(B)
3	α-7 nicotinic receptor	Temozolomide	-6.02	38.37	6	Gln3(C), Arg4(C), Asn15(B), Val18(B), Tyr62(B)

Table 10. Summary of D-limonene, Doxorubicin, & Temozolomide docked with Mitogen-Activated Protein Kinase 6 .

No.	Protein	Compound	Binding Energy (Kcal/Mol)	Inhibition constant (Ki) (mM)	No. of H-bonds	Amino Acid involved in interaction
1	Mitogen-activated protein kinase 6 (6DTL)	D-limonene	-5.59	79.87	0	Lys191(A), Tyr229(A), Arg230(A), Leu234(A), Ile245(A)
2	Mitogen-activated protein kinase 6	Doxorubicin	-5.58	81.68	8	Val270(A), Arg297(A). Tyr298(A), Leu235(A), Asn237(A), Asp268(A), His269(A)
3	Mitogen-activated protein kinase 6	Temozolomide	-4.84	282.85	8	Ser238(A), Thr242(A), Pro333(A), Arg334(A)

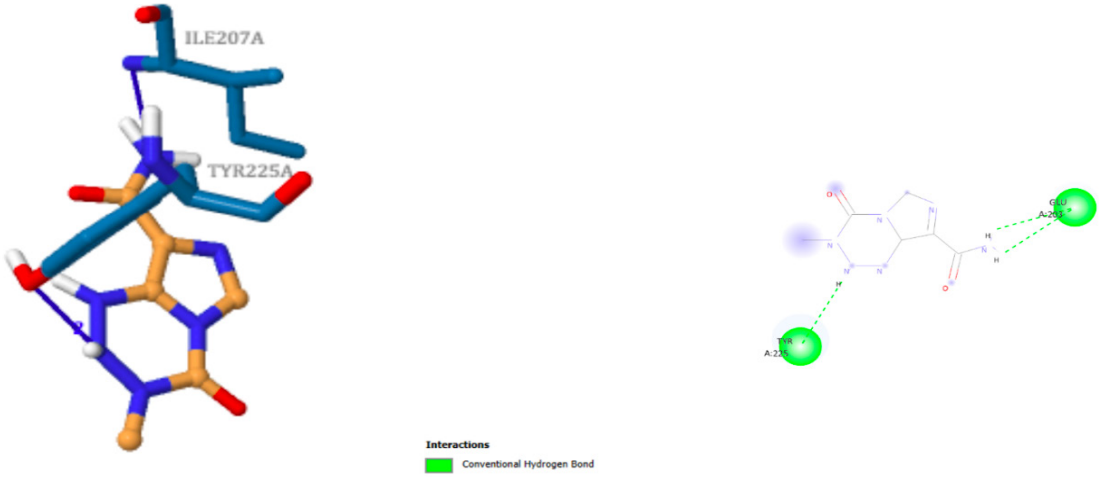

Figure 9. 3D (Left) Diagram from Protein-Ligand Interaction Profiler & 2D (Right) Diagram from BIOVIA Discovery Studio of Temozolomide and Cytochrome 2C9 P450 Inhibitor.

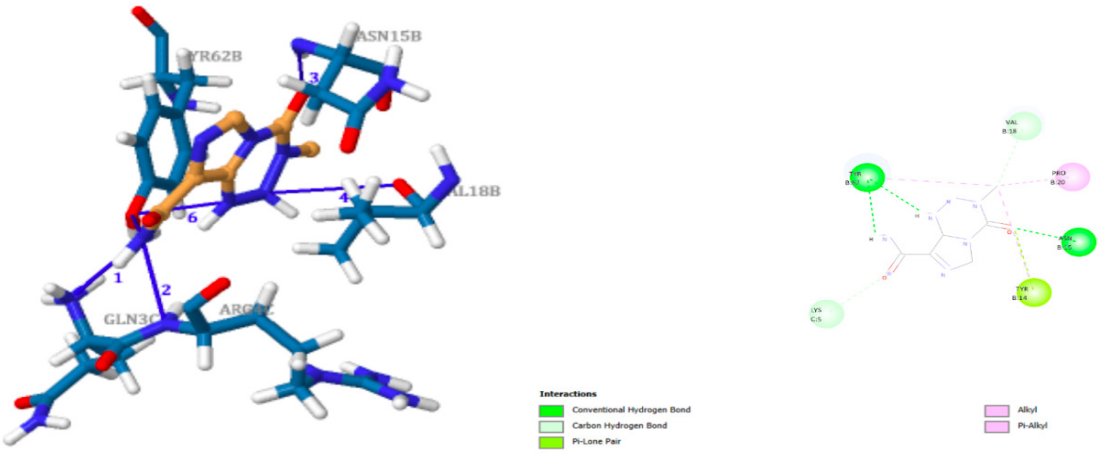

Figure 10. 3D (Left) Diagram from Protein-Ligand Interaction Profiler & 2D (Right) Diagram from BIOVIA Discovery Studio of Temozolomide and α-7 Nicotinic Receptor.

associated characteristics. The target bioactive compounds D-limonene, α-pinene, and caryophyllene oxide and their corresponding established anticancer standards namely doxorubicin and temozolomide were evaluated using the website, SwissADME. According to a study, the pharmacological interactions and structural features of compounds can influence the molecule's behavior in humans (Ndombera et al., 2019).

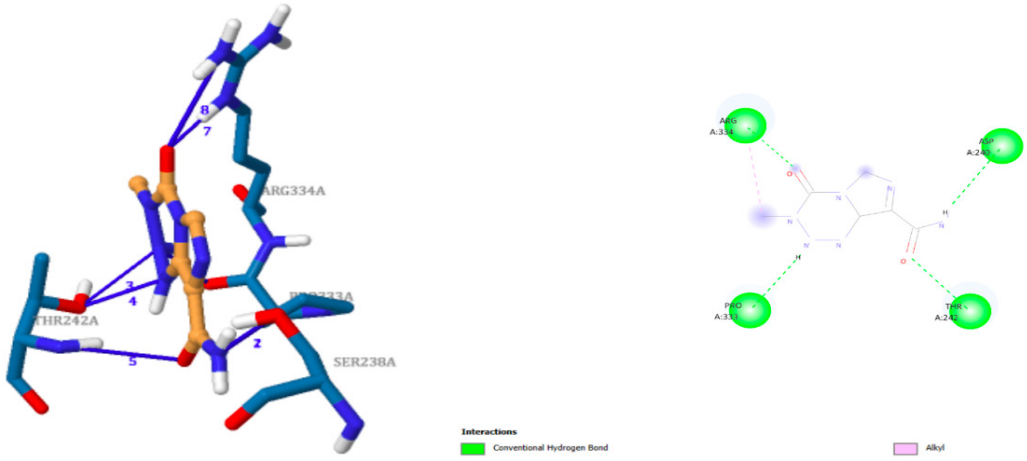

Figure 11. 3D (Left) Diagram from Protein-Ligand Interaction Profiler & 2D (Right) Diagram from BIOVIA Discovery Studio of Temozolomide and Mitogen-activated Protein Kinase 6.

Autodock 4: Molecular Docking Analysis

After finishing the molecular docking in Autodock 4, the visualization of results was done in BIOVIA Discovery Studio and Protein-Ligand Interaction Profiler (PLIP) to visualize in 3D and 2D conformations the ligand-protein complex showcasing the interacting amino acids.

Discussion

The compound α-pinene displays neuroprotective effects and tumor suppression of various cancer cell types, which aligns with the generated CLC result where the compound shows cytotoxicity in brain tissue glioma (Salehi et al., 2019). By inhibiting mitogen-activated protein kinases (MAPKs) and the nuclear factor-kappa B (NF-B) pathway, α-pinene has demonstrated to have anti-inflammatory, antioxidant, and neuroprotective properties both in vitro and in vivo as observed by the decrease of pro-inflammatory markers such as interleukin-6 (IL-6), tumour necrosis factor (TNF), and nitric oxide (NO). (Weston-Green et al., 2021; Khoshnazar et al., 2019).

Caryophyllene oxide, on the other hand, demonstrated anticancer activities in human osteosarcoma cells, human leukemia cancer cells, human gastric cancer cells, and lung cancer cell lines which are observed to be the top cell line in which the bioactive compound was shown to have cytotoxicity (Ahmed et al., 2022; Pan et al., 2016). This can be linked to the upregulation of p21CIP1 and p27KIP1 and the establishment of G1 phase cell cycle arrest by downregulating cyclin D1 and other cyclin-dependent kinases (Ahmed et al., 2022).

Lastly, D-limonene, with other terpenoids, was reported to have induced cellular apoptosis in human thyroid carcinoma but exhibited no cytotoxic effect in healthy fibroblasts in a study (Catalani et al., 2017). Also, D-limonene inhibits the growth of breast cancer cells in MCF 7 cells by causing G2/M phase arrest through dysregulation of Cyclin B1/CDK1 (Mandal et al., 2022).

On the other hand, it was shown in Table 5 that caryophyllene oxide and D-limonene pose cytotoxic activity on normal human cell lines. It is an important obligatory step to determine as well because, through this normal cell line determination, researchers may be able to assess and specify which cell line to focus on for evaluation of the compounds, which in turn may lead to the discovery of metabolite development, which can become an ultimate source of toxicity study for possible cancer treatment (Shah et al., 2019; Calhelha et al., 2014). Through the studies mentioned, α-pinene, caryophyllene oxide, and D-limonene all have expressed anticancer, among other pharmacological effects, which aligns with the generated results of them inhibiting cell lines from the CLC-Pred. Accordingly, the following studies can establish the cytotoxicity prediction results to be accurate.

Various antineoplastic drugs have already been established, such as busulfan, doxorubicin, methotrexate, and vandetanib, however, different effects can occur in mid-to-high doses (National Institute of Diabetes and Digestive Kidney Diseases, 2021). Caryophyllene oxide is predicted to have no diverse effects, as seen in Table 2, giving it an advantage against the previously mentioned established drugs. Caryophyllene oxide is a terpene, specifically sesquiterpenes, with anti-metastatic and anti-inflammatory effects. As such, the predicted categorization of caryophyllene oxide validates the compound's ability to have anticancer properties (Ahmed et al., 2022). D-limonene is another compound that was predicted to have antineoplastic activity. However, unlike caryophyllene oxide, D-limonene was predicted to have an adverse effect on arrhythmia (Table 6) in higher doses. A comment in study regarding the effects of D-limonene on skin tumor development remarked that with the data presented in the study, they were able to show that D-limonene reduces the proliferation of tumor growth (Kapoor, 2013; Chaudhary et al., 2012). The study also stated other studies regarding the inhibitory effect of D-limonene against the phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) pathway. Furthermore, it was also revealed that D-limonene acts as an enhancer of the antineoplastic effect of other chemotherapeutic agents (Zhou et al., 2021; Kapoor, 2013). Thus, showing that D-limonene is a potential antineoplastic agent.

Apoptosis is efficient in removing abnormal cells arising due to DNA damage during the developmental process. Regulated by several genes involved in several signaling pathways, deregulation of apoptosis leads to cancer via uncontrolled cell proliferation. Therefore, targeting apoptosis by natural compounds may restore the disrupted apoptotic pathway in cancer cells that might be a promising approach in the treatment of various cancers. Caryophyllene oxide was shown to have an apoptotic effect against PC-3 androgen-independent prostate cancer cells via depolarization of the mitochondrial membrane (Mandal et al., 2022; Delgado et al., 2021). Caryophyllene oxide in the study was also shown to activate caspase-7 for apoptosis. However, studies regarding the apoptotic effect of caryophyllene oxide against HL-60 tumor cells (predicted tumor cell line, see Table 1) have yet to be investigated. S Studies have shown that caryophyllene oxide enhances DNA fragmentation and the expression of p21 and p53. These occurrences result in cell cycle arrest, which is accompanied by a considerable activation of apoptosis via downregulation of Bcl-2, upregulation of Bax and caspases (-3,-7, and 9) (Shahabana et al., 2023; Delgado C et al., 2021).

D-Limonene was also predicted to be an apoptosis agent. Similar to caryophyllene oxide, D-limonene has also been investigated for its pharmacological properties. A study investigated the effect of D-limonene against A549 and H1299 lung cancer cell lines (Mandal et al., 2022; Yu et al., 2018). Their findings showed that D-limonene inhibits the proliferation of lung cancer cell lines and induces apoptosis via autophagy. The pharmacological properties of both caryophyllene oxide and D-limonene, a great Pa: Pi ratio, as well as previous studies investigating their anticancer properties, suggest that both bioactive compounds are potential apoptosis agents.

Nuclear factor-kappa B (NF-kB), being a transcription factor, is engaged in regulating several genes that are involved in cell survival, proliferation, differentiation, as well as inflammatory and immunological responses.

Moreover, it is involved in the formation and activation of lymphocytes; it is required for both innate and adaptive immune responses (Shabana et al., 2023; Giuliani et al., 2018). Transcription factor NF-kB acts as a promoter of apoptosis, though at times it also acts as an anti-apoptotic factor, interpretation of whether NF-kB is pro-apoptosis or anti-apoptotic depends on the context it is being used (Lin et al., 1999). A link between the NF-kB pathway and apoptosis validates the results shown in Table 3 concerning the antiproliferative effect of the caryophyllene oxide and D-limonene. For instance, D-limonene reduces dopaminergic neurodegeneration caused by rotenone by regulating neuroinflammation, hippo signaling, and apoptosis (Eddin et al., 2021 and 2023). Our results *in silico* imply that limonene and plants or plant products containing limonene may be used to justify their traditional applications or to find alternate treatments for currently available pharmaceuticals with minimal to no side effects. Moreso, caryophyllene oxides have a complex polypharmacological profile with properties that include blocking, suppressing, chemosensitizing, and cytoprotection. This suggests that they may be useful as chemopreventive agents that can be used for both adjuvant and preventive purposes (Di Sotto et al., 2020). Moreso, through the down-modulation of NF-B-regulated gene products, it is hypothesized that the chemopreventive action of caryophyllene oxide potentiates TNF-induced apoptosis and inhibits invasion. (Kim et al., 2014; Di Sotto et al., 2020)

Cytochromes P450 (CYPs) enzymes are vital in the development and treatment of cancer. They are involved in the activation of chemotherapeutic drugs, as well as the metabolic activation of several precarcinogens. CYPs can either activate or inactivate the cytotoxic effect of anticancer drugs. However, their involvement in the activation and inactivation of carcinogens makes them a crucial target for cancer therapy (Rodriguez-Antona et al., 2006). α-pinene was predicted to be both a substrate of CYP2J and CYP2J2. CYP2J belongs to family 2 subfamily J of CYP, while CYP 2J2 belongs to family 2 subfamily J member 2 of CYP. The CYP 2 family is known to have a role in the activation and inactivation of precarcinogens. CYP2J2 has been identified to have a tumor-specific expression (Alzahrani et al., 2020). CYP2J2 was found to be overexpressed in human cancer cell lines and promotes the neoplastic phenotype of carcinoma cells. The mRNA and/or protein levels of CYP2J2 are known to be involved in the formation of all four epoxyeicosatrienoic acids (EETs). Studies have shown that the presence of CYP2J2 can also be found in the pituitary gland (Jiang et al., 2005). CYP2J2's presence in the brain (due to the pituitary gland) may validate earlier prediction of α-pinene being potent to the Hs 683 glioma tumor cell line (see Table 1).

UDPglucuronosyltransferases (UGTs) catalyze the conversion of a variety of hydrophobic endogenous and exogenous substrates to inactive, more hydrophilic molecules using UDP-glucuronic acid (Greer et al., 2014). In another study by Romero-Lorca et al., (2015), they investigated the effect of various glucuronidase genotypes such as UGT1A4 on the metabolism of tamoxifen in breast cancer patients (Romero-Lorca et al., 2015). Tamoxifen is an established anti-cancer drug for breast cancer. They were able to determine that UGT1A4 is a substrate-dependent glucuronidation enzyme that produces glucuronidated Tamoxifen metabolites as well as several carcinogenic chemicals, androgens, progestins, and plant steroids. CYPs and UGT1A4 play a vital role in the metabolism of bioactive compounds. α-pinene being a substrate of both CYPs and UGT1A4 could have an underlying mechanism regarding its metabolism in the body. As such, the results suggest that each bioactive compound has a cancer-related activity, revealing a link between apoptosis induction and cell proliferation regulation.

Caryophyllene oxide was predicted to have a cytotoxic effect against promyeloblast leukemia, as seen in Table 1. The amino acids implicated in CYP2C9-substrate/inhibitor binding in the active site of a Cytochrome P450 family 2 subfamily C member 9 (CYP2C9) homology model correlated well with the most relative points of contact, according to the binding affinity of CYP2C9 (National Center for Biotechnology Information, 2021). As a result, there is a high level of confidence in the relationship between caryophyllene oxide and the CYP2C9 target. According to Alzahrani and Rajendran (2020), the overexpression of P450 contributes to the proliferation of various cancer types. The upregulation of specific P450 family members in cancer makes them prospective targets for cancer therapy. Moreover, P450-mediated metabolism that occurs at the tumor site or the site of anticancer drug action can represent a novel way of targeting tumor cells. Hence, caryophyllene oxide's ability to target Cytochrome P450 2C9 makes it a potential drug candidate capable of producing a novel approach to treating promyeloblast leukemia. Nevertheless, the available data are insufficient to draw any clinical conclusion for the recommendation of caryophyllene oxide in the management of tumor diseases. Thus, more research are needed to determine the most effective doses for the beneficial roles of these compounds in the cancer chemotheraphy and its potential benefits in targeting mitochondria of cancer cells using both experimental and human studies.

α-Pinene is a monoterpene that is, known to have antimicrobial, apoptotic, anti-metastatic, and antibiotic properties (Salehi et al., 2019; Pandey et al., 2022). It is also known to suppress MAPKs and the NF-B pathway, making it a promising agent for the treatment of various inflammatory diseases. Through the nuclear factor-kappa B-cyclooxygenase-2 pathway, α-Pinene suppresses inflammatory response and provides protection against brain ischemia. (Shabani et al., 2023; Salehi et al., 2019). The compound has a direct interaction with the protein target, neuronal acetylcholine receptor protein α-7 subunit (NACHRA7), which is consistent with the findings in Table 1, which reveal that α-pinene is cytotoxic to brain glaucoma cells. NACHRA7 has been linked to cognitive impairments as a ligand-gated ion channel. It binds to positive allosteric modulator ligands that avoid direct contact with the acetylcholine binding site, allowing induced agonists to respond more quickly (Balsera et al., 2014). As previously stated, α-pinene has been shown to have neuroprotective and tumor-suppressing

properties in a variety of cancer cell types. Hence, a thorough understanding of the molecular mechanisms behind the anti-inflammatory characteristics of α-Pinene and its derivatives to help in the development of targeted disease-specific therapeutics because different pathways may contribute differently to inflammation in distinct cancer cell types.

Finally, D-limonene has direct contact with the Mitogen-activated protein kinase 6 targets. D-limonene activity is predicted to be identified due to the target's interactions with numerous assay components provided by the software, resulting in activity variations. Furthermore, it was found that the target can influence NFkB activity, which has played a crucial role in 4-HPR-induced apoptosis in prostate cancer cells (Shimada et al., 2002).

It was discovered that β-caryophyllene oxide not only inhibited the constitutive activation of the PI3K/AKT/mTOR/S6K1 signaling cascade in tumor cells but also activated numerous protein kinases, including ERK, JNK, and p38 MAPK, and increased the production of reactive oxygen species from mitochondria, linking it to apoptosis (Ahmed et al., 2022; Park et al., 2011). Meanwhile, α-pinene reduced the invasiveness of highly metastatic human breast cancer cells by inhibiting induced tumor necrosis factor (TNF); TNF is a primary cytokine involved in regulating systemic inflammation and is produced by a variety of cells, including macrophages, lymphocytes TNF is one of the primary cytokines involved in regulating systemic inflammation and is produced by a variety of cells, including macrophages, lymphocytes, and others (Kang et al., 2016). Lastly, D-limonene increased nitric oxide levels by triggering apoptosis through two pathways, which are low-concentration H2O2 generation and ERK pathway activation, where high-concentration protein farnesylation inhibition and O2 production takes place (Manuele et al., 2010).

Since serine/threonine kinases are enzymes that catalyze the transfer of a phosphate from ATP to a protein substrate, such as a serine or threonine amino acid residue (Diallo et al., 2011), and the following KinScreen results show that the bioactive compounds exhibit this property. A study also discovered that inhibition of serine/threonine kinases (STK) was successful in a considerable percentage of human cancers (Capra et al., 2006). As such, further research into the cell cycle progression of the following protein kinases of the compounds will undoubtedly benefit cancer research.

As observed in Table 5, Lipinski's rule of five for ADME and drug-likeness parameters for the target bioactive compounds in comparison to the established anticancer standards. The target compounds and the standard temozolomide passed Lipinski's rules and are all absorbable in the gastrointestinal (GI) tract, except for doxorubicin. The bioavailability score of 0.55 indicates that the target compounds were orally bioavailable based on the rapid assessment of their drug-likeness and their Furthermore, descriptors specified a physicochemical range on each axis, with the pink area depicting the radar plot of the molecule that varies wholly into the drug-like category (Daina et al., 2017). It can be seen that the compounds α-pinene and D-limonene exhibited good bioavailability radar as displayed in Figure 1, indicating that the mentioned compounds fall within optimal ranges in terms of properties including lipophilicity, size, polarity, solubility, saturation, and flexibility. Hence, are good drug candidates. The software SwissADME was also able to generate a BOILED-Egg prediction model of the compounds. Figure 3 portrays the BOILED-Egg model of the target compounds, which shows the gastrointestinal (GI) absorption, blood-brain barrier (BBB) penetration, and P-glycoprotein (PGP) activity of the target and standard molecules. It can also be observed that the standard temozolomide showed high GI absorption. It is also far from the yolk part, suggesting that the molecule doesn't cross the blood-brain barrier (BBB) and has few central adverse effects. On the other hand, the standard doxorubicin showed low GI absorption and is out of range, as depicted in the BOILED-Egg model.

As observed in Table 5, Lipinski's rule of five for ADME and drug-likeness parameters for the target bioactive compounds in comparison to the established anticancer standards. The target compounds and the standard temozolomide passed Lipinski's rules and are all absorbable in the gastrointestinal (GI) tract, except for doxorubicin. The bioavailability score of 0.55 indicates that the target compounds were orally bioavailable based on the rapid assessment of their drug-likeness and their Furthermore, descriptors specified a physicochemical range on each axis, with the pink area depicting the radar plot of the molecule that varies wholly into the drug-like category (Daina et al., 2017). It can be seen that the compounds α-pinene and D-limonene exhibited good bioavailability radar as displayed in Figure 2, indicating that the mentioned compounds fall within optimal ranges in terms of properties including lipophilicity, size, polarity, solubility, saturation, and flexibility. Hence, are good drug candidates.

The software SwissADME was also able to generate a BOILED-Egg prediction model of the compounds. Figure 2 portrays the BOILED-Egg model of the target compounds, which shows the gastrointestinal (GI) absorption, blood-brain barrier (BBB) penetration, and P-glycoprotein (PGP) activity of the target and standard molecules. It can also be observed that the standard temozolomide showed high GI absorption. It is also far from the yolk part, suggesting that the molecule doesn't cross the blood-brain barrier (BBB) and has few central adverse effects. On the other hand, the standard doxorubicin showed low GI absorption and is out of range, as depicted in the BOILED-Egg model.

The following interactions between the ligand caryophyllene oxide and the receptor cytochrome 2C9 P450 inhibitor may be shown in Figure 3, with seven amino acids engaged in the protein-ligand binding interaction. All interacting chains A are isoleucine 205, leucine 208, glutamic acid 300, threonine 304, leucine 361, and phenylalanine 476. Chain A glutamic acid 300 has only one hydrogen bond, while the rest have pi-alkyl (in pink) hydrophobic interactions. The interaction of the α-7 nicotinic receptor and α-pinene (Figure 4) was discovered to involve several amino acids. Chain B arginine 4 and B lysine 8, as well as the rest of chain A

tyrosine 14, asparagine 15, valine 18, and tyrosine 62, are the following. As seen in the 2D picture, all amino acids have hydrophobic contacts, with chain A tyrosine 62 (in purple) revealing that the interaction is pi-sigma and the remainder (in pink) being pi-alkyl. D-limonene exhibits the same pi-alkyl chain A hydrophobic interactions with various amino acids as caryophyllene oxide. Figure 5 shows lysine 191, tyrosine 229, arginine 230, leucine 234, and isoleucine 245 as amino acids.

Comparing Table 7 to Table 6, it is clear that the SwissADME results for the amount of hydrogen bonds match the molecular docking results, as there are no hydrogen bonds with α-pinene and D-limonene, only with caryophyllene oxide. The binding energy and inhibition constant of caryophyllene oxide are the lowest of the three chemicals investigated. The bioactive compound is the most stable of the three and the most effective as an inhibitor with 10.05 mM as the inhibition constant which correlates to IC_{50}.

As caryophyllene oxide as compared to the anticancer drugs demonstrated in Table 8, it was determined that the bioactive compound had the highest binding energy, even though temozolomide has the lowest inhibition constant of the three, indicating that temozolomide is the preferred choice for its inhibitory effects whilst caryophyllene oxide for its potency against cytochrome 2C9 P450 inhibitor. However, in Table 9, In comparison to α-pinene and the standards, Doxorubicin has the lowest binding energy and the highest inhibition constant. While Doxorubicin is the most stable out of the three, its drug potency to inhibit α-7 nicotinic receptor is the lowest, with Temozolomide having 38.37 millimolar. α-pinene has the highest binding energy of the two standards, therefore further confirming that its complexity is the least stable. Lastly, in Table 10, D-limonene has the lowest binding energy with -5.59 Kcal/Mol and an inhibition constant of 79.87 millimolar than the two standards. When observed further, it is clear that the results of D-limonene are not that far from Doxorubicin having its values closer than the bioactive compound with Temozolomide. The compound Temozolomide is the least desirable drug to inhibit or influence mitogen-activated protein kinase 6. Figures 6-11 depict the 3D and 2D conformations of the standards.

In conclusion, evidence of the pharmaceutical potential of the seed oil of *Alpinia elegans* is determined *in silico* manner and is, therefore, all theoretical. It is concluded that the compounds D-limonene, α-pinene, and caryophyllene oxide can induce apoptosis and inhibit cellular proliferation in several cancer cells since the resulting binding energies suggest that the compounds are highly stable with strong complexes between receptors, making them a promising chemotherapeutic agent. Though these compounds are also found in other natural products such as the Citrus aurantium (bitter orange), Cannabis sativa (marijuana), Humulus lupulus (common hop), Salvia officinalis (sage), and Salvia rosmarinus (rosemary) in various concentrations, this study as an active area of research has shown significant results with the specific combination of these compounds and their concentration on *Alpinia elegans*. The specific complexes that they form with the receptors present how they can complement conventional experimental methods. Moreover, the target compounds were predicted to be orally bioavailable, have good absorption, and showed better results compared to one of the standard anticancer drugs. Of the three bioactive compounds, caryophyllene oxide obtained the best results based on the generated *in silico* data, thus the best compound from the plant's seed oil.

in silico study enables us to predict possible reaction/s based on a given structure/s of compounds and its bioavailability - before synthesizing them for high throughput screening of pharmaceutical targets against cancer. The modifications in the functional group of the compound may elicit enhanced bioactivity, ADMETox, molecular dynamics, etc. Given that *A. elegans* is an endemic medicinal plant in the Philippines and meager studies are available on the plant's bioactivity with chemopreventive action against certain types of cancer. This finding presents a unique perspective on how these bioactive compounds could potentially lead to cancer treatments with fewer adverse effects than current treatments highlighting the potential of *in silico* methods for drug discovery. To validate the outcomes of future investigations, comprehensive in vitro and in vivo pharmacokinetic and pharmacodynamic profiles need to be done. With the help of *in silico* prediction, it is possible to identify and concentrate on those bioactive compounds and/or their metabolites that show promise for overcoming challenges in the search for alternative cancer chemotherapeutic leads.

Author Contribution Statement

JMNC, RIDGB, MSCB, MAFO, and MRSB conceptualized the *in silico* experimental design and computational framework, and analysis and interpretation of data. JMNC, RIDGB, MSCB, and MAFO carried out the data collection and wrote the manuscript with input and guidance from LAS and MRSB. MRSB was in charge of the paper's overall concept, development, and approval of the final manuscript version.

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Approval

This study is part of an approved undergraduate thesis that has been approved and accepted as partial fulfillment of the requirements for the degree of Bachelor of Science in Biochemistry at the University of Santo Tomas, Manila,

Philippines.

Data Availability

The model simulations based on this study are too extensive to archive. Instead, we provide all the information needed to replicate the simulations. Due to confidentiality agreements, supporting data can only be made available to bona fide researchers subject to a non-disclosure agreement. Details of the data and how to request access are available from the corresponding author at the University of Santo Tomas, Department of Biochemistry.

Study Registration

The study is not registered in any registering dataset.

Conflict of Interest

The authors declare that they have no competing interests.

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