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Identification of Secondary Metabolites from *Zingiber officinale* as Inhibitors for Keap 1-Nrf2 small Molecules through Molecular Docking Techniques

Nivedha Sukumar*

Abstract

Objective: *Therapeutic plants aid in treating cancer-based cell signaling pathways and preventing certain risk factors. Zingiber officinale has a variety of anti-inflammatory characteristics also contributing to enhanced immunity, reducing cancer risk and used to target the KEAP NRF2. When overexpressed in response to oxidative stress and DNA damage, elevates the risk of developing multiple cancers. NRF2 cellular sensors, which also control and coordinate their expression aid in defending healthy cells against carcinogens.* ***Methods:*** *The approach employed to accomplish this was the individual assessment of each of the 50 phytochemicals from Zingiber officinale's binding affinity for our target protein, KEAP1 NRF2. PyRx, a software programme that allows to dock the top ligands with best binding affinity and the target protein, served as the molecular docking platform for the study. In further process of the investigation, BIOVIA was used to retrieve the 3D and 2D structure of each ligand and the Protein structure retrieved from PUBsum. Along with this, the ligands bound to the target protein in PYMOL. The 8 ligands were sent for ADMET filters.* ***Results:*** *Molecular docking resulted in Gingerglycolipid A, B, C, Gingernone A,B, Cyclosalivene, viridiflourine, and Isogingernone B as the top 8 ligands with appropriate binding affinity. The ADMET lab analysis resulted in the Cyclosalivene and viridiflourine along with Gingerglycolipid A, B,C inhibiting against Keap1-Nrf2 small molecules or its pathway. Further progression of targeting this protein using ginger.* ***Conclusion:*** *These phytocompounds can help in the further suppression and the progression of the KEAP1 NRF2 protein and pathway to result in any human diseases or carcinogen activation due to Oxidative stress.*

Keyword: Keap1-NRF2 pathway, Phytocompound, ADMET lab, Molecular docking, Oxidative stress, Ginger, ARE mediated pathway, Cancer, NRF2-sMAF.

INTRODUCTION

The study and application of plants for therapeutic purposes. As an alternative to Western commercial medicine, ayurvedic medicine employs therapeutic plant studies. Around 80% of people in humanity use traditional medicine, according to estimates. Among the illnesses it aids with are Asthma, Arthritis, Digestive Problems, Eczema, High Blood Pressure, High Cholesterol Levels, and Rheumatoid Arthritis. Additionally, it teaches us to love ourselves, comprehend nature and its characteristics, and rid the body of poisons. Therefore, the use of phytocompounds of medicinal and therapeutic plants has aided in targeting specific proteins in reducing the risk of progression of cancer.

***Author for Correspondence**
Nivedha Sukumar
E-mail: Nivisukumar.7@gmail.com

Student, Department of Biotechnology, Ramaiah University of Applied Sciences, Mathikere, Bengaluru, Karnataka, India

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Zingiber officinalE also known as ginger is A flowering plant known to be utilized for both culinary purposes and traditional healing. Ginger has a wide range of medicinal properties like anti-inflammatory, antibacterial, and antiviral properties. It lowers the risk of cancer, strengthens immunity, supports cardiovascular health, and improves digestion [1]. According to a chemical investigation, ginger includes more than 400 distinct components. Carbohydrates (50-70%), lipids (3-8%), terpenes, and phenolic chemicals are the main components of ginger rhizomes. Zingiberene, bisabolene, farnesene, sesquiphellandrene, and curcumin are among the terpene components of ginger, whereas gingerol, paradols, and shogaol are phenolic substances. There are more of these gingerols (23–25%) and shogaols (18–25%) than other types [11]. Therefore, it was determined to use ginger's phytochemicals to prevent those pathways from activating and developing cancer.

Kelch-like ECH-associated protein 1, or Keap1 PDB ID 4L7B, the protein is a sensor. These protein adaptors from the BTB (Broad complex, Tram track, and Bric-a-brac)-Kelch domain family regulate the CUL3 (Cullin 3)/RBX (RING box 1) E3 ubiquitin ligase complex substrate selectivity [7], which encourages the ubiquitination and proteasome destruction of NrF2 molecules [3,]. NF-E2-related factor 2 is transported to the nucleus by Kelch-like ECH-associated protein 1 after the proteins dissociate in the cytoplasm as a result of redox-sensitive interactions. This connection leads to the expression of the catalytic subunit of gamma-glutamylcysteine synthetase. It has been found that two alternatively spliced transcript variants of this gene encode the same isoform [2, 4].

Free radicals are naturally occurring molecules that result from body metabolism. Therefore, eliminating these free radicals from the body is an incredibly important step that must be taken in order to prevent serious harm. It develops when the body is overloaded with substances such as smoking, x-ray radiation, ozone, air pollution, industrial pollutants, and even exercise. It can fight them off, but doing so harms DNA, lipids, proteins, and other biological constituents.

Nuclear factor erythroid 2-related factor 2 (Nrf2) is a regulator of cellular immunity to oxidants. Nrf2 controls both the basal and stimulated expression of a wide range of antioxidant response element-dependent genes in order to adjust the physiological and pathological effects of oxidant exposure. It activates following a variety of inputs, including growth hormones, under oxidative stress, inflammation, and other circumstances [5, 6]. Resulting in the activation of cancer cells and other health problems, such as aging. Oxidative stress stimulates a variety of chronic diseases, such as diabetes, cancer, and neurological issues [8]. To counteract these free radicals and avoid additional injury to the usual operation of the metabolism, the body produces special antioxidants [12–14].

Kelch-like ECH-associated protein-1 (Keap1) has drawn a lot of attention for its complex regulation of Nrf2, it has also become evident that this protein also interacts with other signaling pathways, including the glycogen synthase kinase-3 (GSK-3)-transducing repeat-containing protein (TrCP) axis, nuclear factor-kappa B (NF-B), Notch, AMP kinase, and E3 Due to its beneficial effects in a variety of illnesses. As a result, novel, synthetic, and targeted small molecules are being intensively researched to modulate the pathway and are currently being tried in clinical investigations [9]. Therefore, the proposed ginger derivatives' therapeutic efficacy was assessed by using the molecular docking technique and looking at their pharmacological characteristics.

METHODS

Ligand Retrieval

The first step to the methods we used to dock the target protein was the retrieval of 50 phytochemicals looking at the pharmacological and medicinal properties of ginger from the software called IMPPACT (https://cb.imsc.res.in/imppat/) [16]. We collected the canonical smiles, 2d structure, and 3d structure along with the CID number of all 50 ligands. (In SDF format).

Protein Retrieval and Purification

The target protein Keap1, PDB ID 4L7B, was retrieved from the PDB website (https://www.rcsb.org/). Purification of the target protein is necessary to characterize its relationships,

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structure, and function. Before isolating the target protein from all other proteins, the purification procedure may first separate the mixture's protein and non-protein components [10]. Therefore, the undesirable b chains, ligand groups, and hetatom were removed to purify the protein. This procedure was carried out on a BIOVIA Discovery Studio software platform. The protein structure was then neutralized by the insertion of polar ends. Then it was archived in pdf format.

Protein Structure Validation

With a better understanding of how a protein functions due to its structure, we may develop theories about how to influence, regulate, or modify it. Comprehending a protein's 3D structure is essential for controlling or altering a protein's function, predicting which chemicals will bind to a protein and understanding numerous biological interactions, assisting in the discovery of new drugs, or even creating our own [10]. Using PDB-derived 3D structures of the protein we used PDBSUM (http://www.ebi.ac.uk/thornton-srv/databases/pdbsum/Generate.html) generate to get a Ramachandran plot of KEAP 1. PROSA was used to retrieve the Z-score as well (https://prosa.services.came.sbg.ac.at/prosa.php). Hydropathy plots were collected from BIOVIA. Now that we have collected all the supporting information for the validation of the target protein. Molecular docking was conducted next.

Molecular Docking

A technique to analyse how two or more different molecules interact, they could be drugs or ligands, this is called molecular docking. It makes predictions about the compatibility of a ligand with a target protein or small molecule, or about the optimum kind of ligand to use in a certain situation. The 50 ligands that were retrieved were loaded and docked with the purified target protein Keap1 after converting it into a macromolecule the ligands were run and docked against Keap1 one by one until we got the results in CSD format. Molecular docking was done in a software database called PyRx. Blind docking was done instead of site-specific docking. The top 8 ligands which had the least binding affinity were docked together using Pymol. The dimensions of the grid during molecular docking were X:9.5958; Y:16.416; Z: -11.9187 [Centre], X:49.9081; Y:47.1491; Z:51.9520.

Visualisation

Visual mapping intuitively illustrates the whole knowledge structure, research framework, and development trends of a subject, visual mapping is a very important tool for researchers to swiftly identify the general research state and hotspots. many applications of computer graphics, scientific visualization, and information visualization in the biological sciences. downloading both the 2D and 3D structures of each of the top 8 ligands' docked complexes. The docked structures' hydrophobicity was also collected. Additionally, measurements of the non-bonds were gathered. Visualization was done in BIOVIA. The flowchart from Figure 1 shows the complete step-by-step protocol used for the analysis in precision.

ADME Screening

ADME allows one to evaluate the different aspects of a drug in discovery. It analysis the adsorption, distributional, medicinal, physiochemical, and toxicity properties of a particular drug. This was done from a software called ADMET lab 2.0 (https://admetmesh.scbdd.com/). The results retrieved from molecular docking and the ligands with the best binding affinity were sent for ADMET lab analysis using the canonical smiles of the top 8 ligands.

RESULTS

Protein Structure Analysis

In light of the findings of the research, we can say that we were successful in extracting 50 ligands (phytochemicals) from ginger, a medicinal plant. The target protein, KEAP 1 (PDB NO: 4L7B) was downloaded from the PDB website. Later, this protein was purified using BIOVIA (https://discover.3ds.com/discovery-studio-visualizer-download), a bioinformatics application. The next phase involved verifying the protein’s secondary structures and the Ramachandran plot as well as

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the hydropathy plot using PUBSUM (http://www.ebi.ac.uk/thornton-srv/databases/pdbsum/Generate.html). The 3D structure of the protein KEAP Nrf2 protein is given in the result part Figure 2.

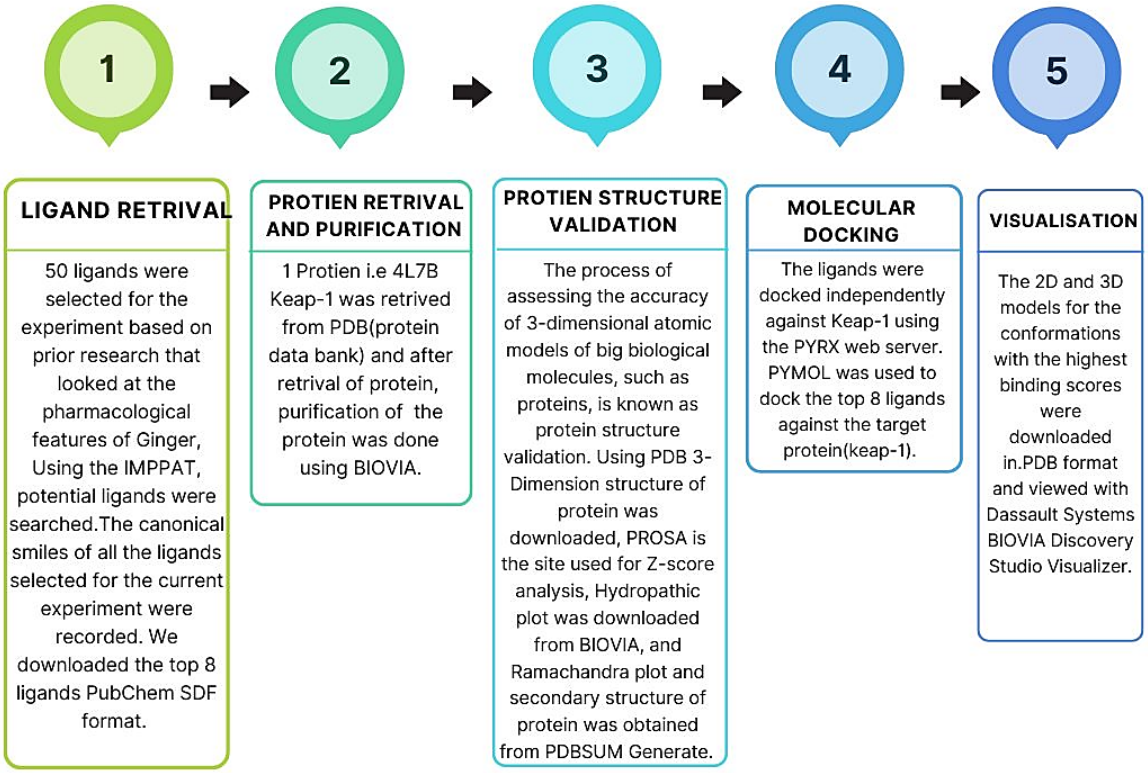
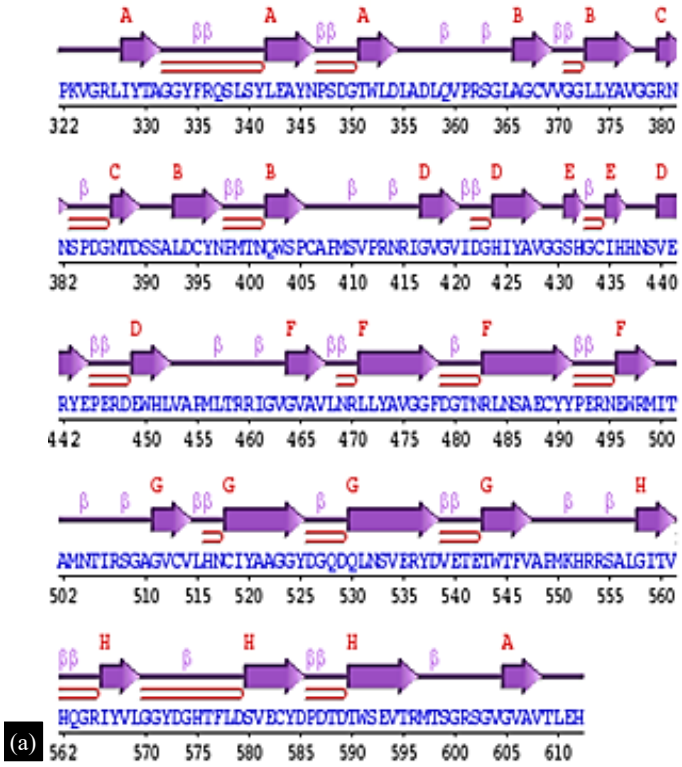


Figure 1. Process of methodology. Depicts the methods used to pursue this investigation. 1. Ligand retrieval, 2. Protein retrieval and purification, 3. Protein structure and validation, 4. Molecular Docking, 5. Visualization.



(a)

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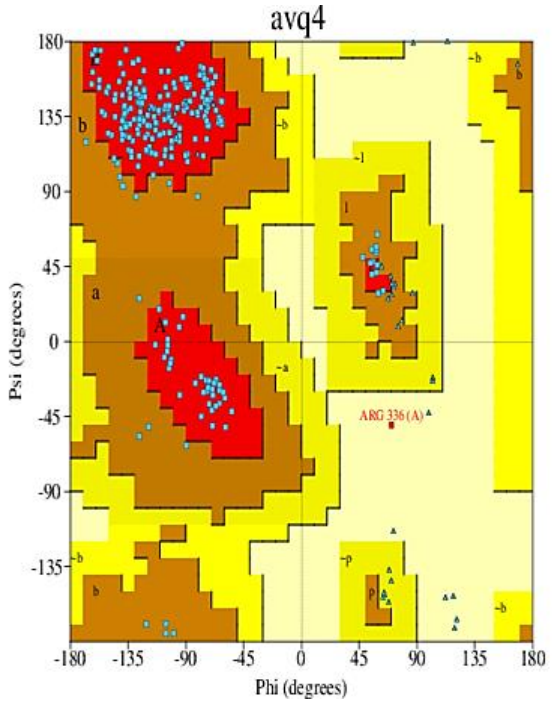
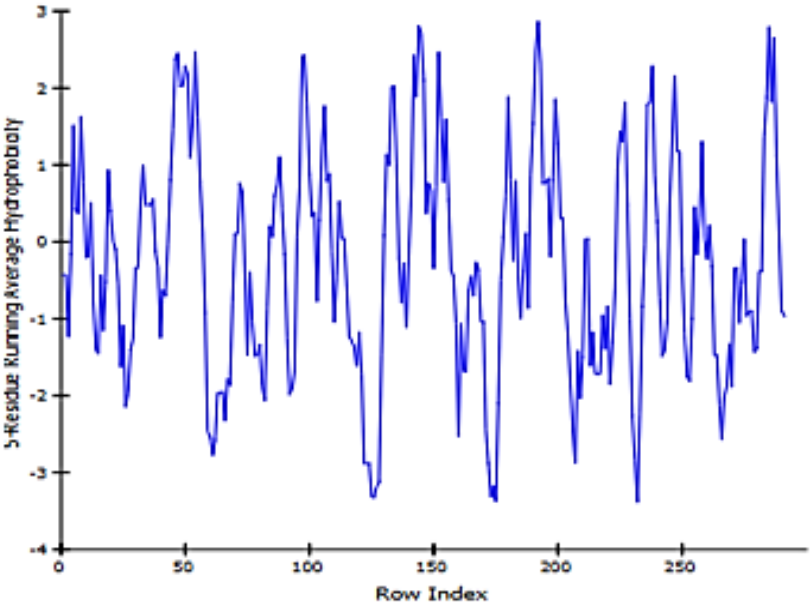
(b)



(c)



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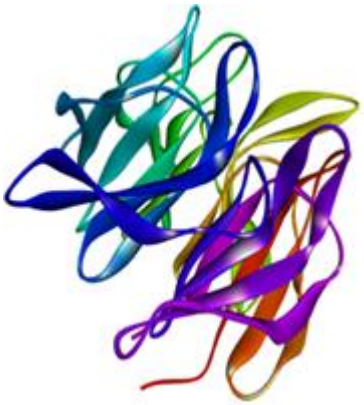

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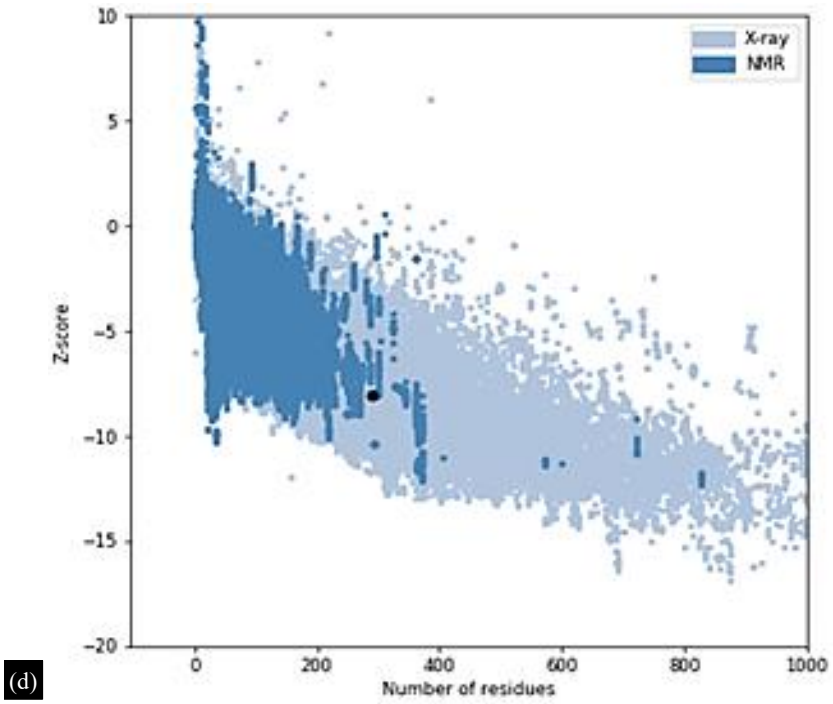

(d)

Figure 2. Represents Structural analysis (a) 2D structure of KEAP1-NRF2; (b) Ramachandran plot of KEAP1-NRF2; (c) Hydropathy plot; (d) 3D structure of KEAP1-NRF2; (e) Z-score value of KEAP1 NRF2.

Ramachandran Plot

We have got the results of the Ramachandran plot that was derived and described the motifs and pro-motifs as described. The pro motifs of the protein consist of 8 sheets, 17 beta hairpins, 10 beta bulges, 28 strands, 40 beta turns, and 1 gamma turn. Thus, this was what resulted from the Ramachandran plot and the 3D structure derivation from the PROSA website.

Selection of Phytocompounds

Table 1 lists the top 8 phytochemicals or ligands and their binding affinity, that is compatible with KEAP 1 Nrf2 small molecules and their mechanism for reducing the health risks that this protein creates. Table 2 displays the results of the docked ligand structures along with their canonical smiles and CID numbers ligands, that are most compatible with the proteins.

Table 1. Binding affinity of the top 8 ligand with the KEAP1-NRF2 Protein.

Ligand	Binding Affinity
pu4l7b_10910653_uff_E=44366466906.71	-8.6
pu4l7b_5318568_uff_E=400.21	-8.3
pu4l7b_5317592_uff_E=1218.67	-8.2
pu4l7b_101635263_uff_E=910.58	-7.9
pu4l7b_10349562_uff_E=993.96	-7.9
pu4l7b_5281775_uff_E=2426.26	-7.9
pu4l7b_10009754_uff_E=1112.15	-7.7
pu4l7b_519960_uff_E=4464.29	-7.2

SMILES, also known as the Simplified Molecular Input Line Entry System, is a tool used to translate the three-dimensional structure of a molecule into a set of symbols that computer software programs can easily comprehend. It also describes the bonding and chirality of molecules. The canonical smiles format of these top 8 ligands or phytochemicals is therefore presented below, according to Table 2.

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Table 2. Canonical smiles of top 8 ligands suitable for Keap1 Nrf2 along with PubChem CID no.

Compound	Canonical smiles	PubChem CID No.
Gingerglycolipid B	CCCCCC=CCC=CCCCCCCCC(=O)OCC(COC1C(C(C(C(O1)COC2C(C(C(C(O2)CO)O)O)O)O)O)O)O	10009754
Gingerglycolipid A	CCC=CCC=CCC=CCCCCCCCC(=O)OCC(COC1C(C(C(C(O1)COC2C(C(C(C(O2)CO)O)O)O)O)O)O)O	10349562
Isogingereone B	COC1=CC(=CC(=C1O)OC)CCC(=O)C=CCCC2=CC(=C(C=C2)O)OC	5318568
Gingerenone B	COC1=CC(=CC(=C1O)OC)CCC=CC(=O)CCC2=CC(=C(C=C2)O)OC	5317592
Gingerenone A	COC1=C(C=CC(=C1)CCC=CC(=O)CCC2=CC(=C(C=C2)O)OC)O	5281775
Gingerglycolipid C	CCCCCCCCC=CCCCCCCCC(=O)OCC(COC1C(C(C(C(O1)COC2C(C(C(C(O2)CO)O)O)O)O)O)O)O	10635263
Viridiflorene	CC1CCC2=C(CCC3C(C12)C3(C)C)C	10910653
Cyclosativene	CC(C)C1CCC2(C3C1C4C2(C4C3)C)C	519960

Molecular Docking and Visualization

PyRx (https://pyrx.sourceforge.io/) was used to automatically dock 50 ligands at the molecular level, and its secondary structure was obtained. We selected the top 8 ligands with the lowest binding affinities. The complex structure was obtained by separately docking each of the eight ligands with the KEAP 1 protein in Pymol.

To further visualize these structures, BIOVIA was used to acquire the 3D and 2D structures of the docked structures, non-bonds, and hydrophobicity of the structure. The suitability of the top 8 phytochemicals or ligands for human consumption as a form of medication was evaluated using ADME.

The protein is colored orange, while the ligand color varies for identification purposes. It also shows the amino acid present on which ends of the bond. Along with this non-bond measurements of all eight ligands are given below the structures. Figure 3 shows the 3D and 2d structures of interactions of Gingerglycolipid A and B with KEAP1-NRF2. Figure 4 shows interactions of Gingernone B and Isogingernone B with KEAP1-NRF2. Figure 5 and Figure 6 show interactions of Gingerglycolipid C and Gingernone A, Viridiflourene and Cyclosalivene with KEAP1-NRF2 respectively.

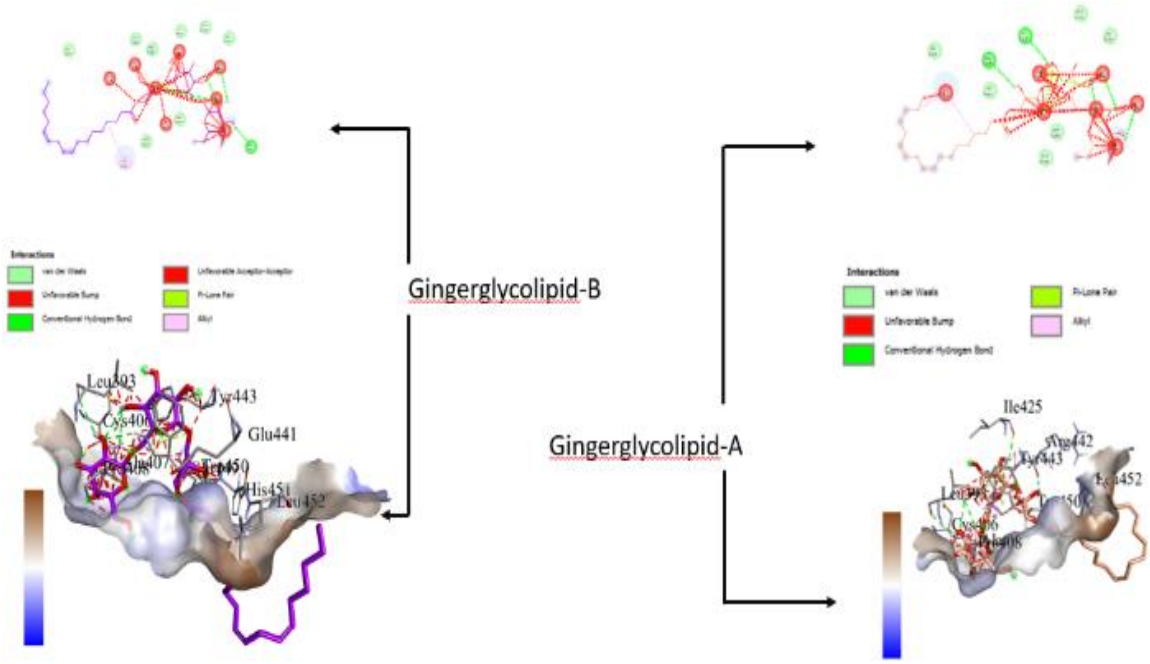

Figure 3. 3D and 2D structures interactions of Gingerglycolipid A and B with KEAP1-NRF2.

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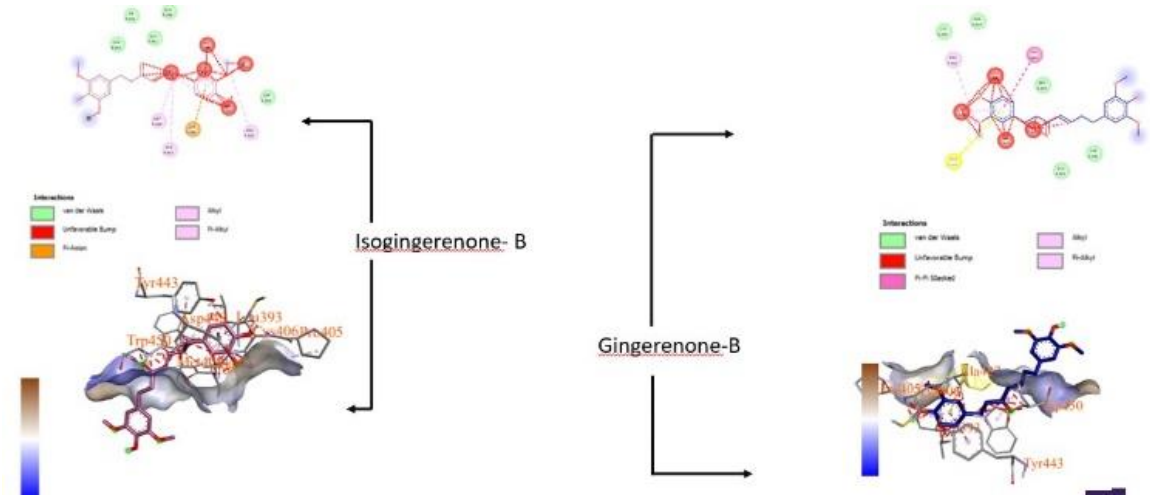

Figure 4. 3D and 2D structures interactions of Gingernone B and Isogingernone B with KEAP1-NRF2.

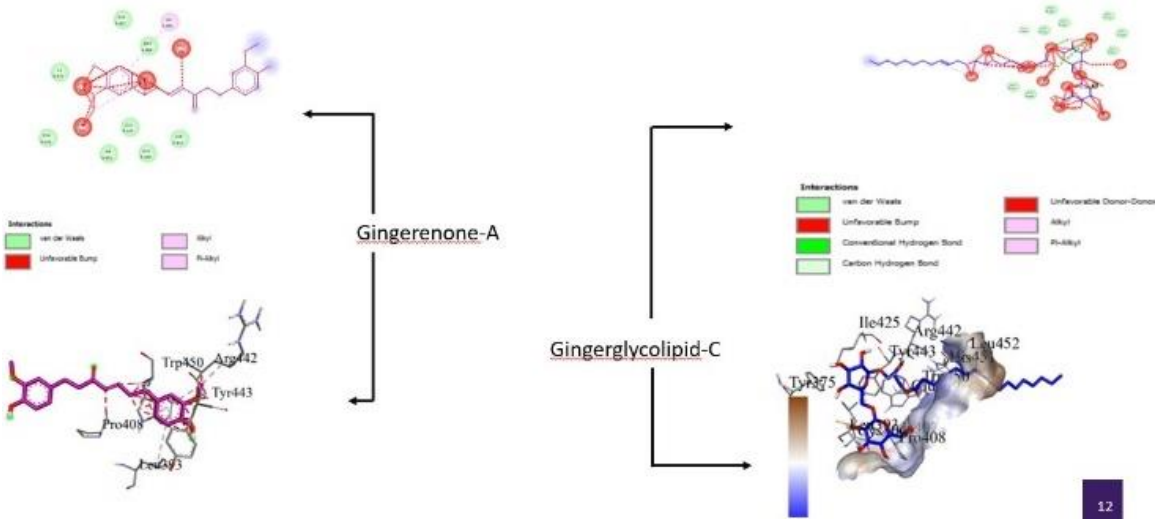

Figure 5. 3D and 2D structures interactions of Gingerglycolipid C and Gingernone A with KEAP1-NRF2.

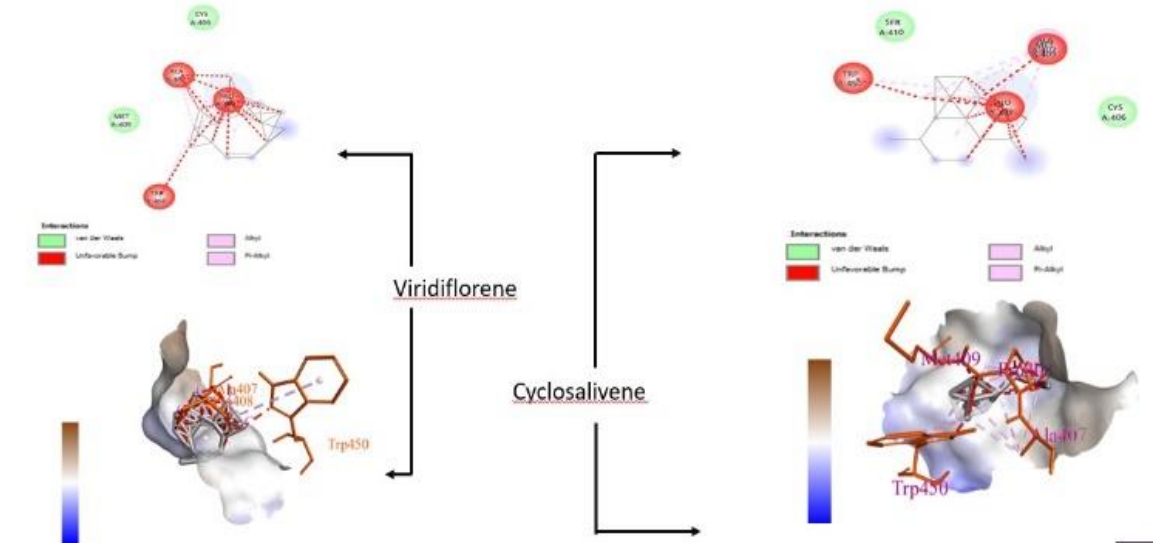

Figure 6. 3D and 2D structures interactions of Viridiflourene and Cyclosalivene with KEAP1-NRF2.

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ADMET LAB ANALYSIS

Utilizing ADMET (https://admetmesh.scbdd.com/) profiling during clinical development aids in minimizing risks. We are aware that drug development failures may result from efficacy or safety issues, which is why ADMET profiling is necessary to determine whether or not a molecule is fit to advance to the clinical stage. Analyses of chemical adsorption, distribution, metabolism, excretion, and toxicity are included in the ADMET lab analysis.

Table 3. Adsorption properties of top 8 ligands

ID	Caco-2	MDCK	Pgp-inh	Pgp-sub	HIA	F(20%)	F(30%)
10910653	-4.592	1.64E-05	0.035	0	0.003	0.249	0.303
5318569	-4.719	1.71E-05	0.114	0.028	0.012	0.052	0.068
5317592	-4.83	1.77E-05	0.942	0.472	0.007	0.128	0.056
101635263	-5.595	4.00E-05	0.679	0.28	0.988	0.851	0.994
10349562	-5.415	9.45E-05	0	0.845	0.964	0.174	0.997
5281775	-4.758	2.07E-05	0.303	0.008	0.012	0.033	0.04
10009754	-5.482	6.59E-05	0.006	0.501	0.979	0.264	0.997
519960	-4.63	6.35E-05	0.001	0	0.003	0.012	0.817

Cyclosalivene seems to have the best adsorption properties but lacks in MDCK factor and is very poor. Isogingerenone B has reasonable adsorption properties among every other phytochemical as seen from the Table 3.

Table 4. Distribution properties of top 8 ligands.

ID	BBB	PPB	VDss	Fu
10910653	0.747	98.04%	5.308	1.96%
5318568	0.083	93.86%	0.397	5.35%
5317592	0.019	95.57%	0.375	1.84%
101635263	0.157	92.76%	0.368	6.36%
10349562	0.165	53.18%	0.221	13.02%
5281775	0.085	97.97%	0.584	1.74%
10009754	0.163	82.57%	0.319	7.92%
519960	0.73	95.94%	1.683	3.41%

From the Table 4 Gingerglycolips A and B have very low plasma protein binding, therefore it being a drug its binding rate would be very weak or bad. Gingernone B and Gingerglycolipd C have good PPB and VD rate. Therefore, they appear to have the best distributional properties.

Table 5. Toxicity properties of ligand

ID	hERG	DILI	Ames	FDAMDD	Carcinogenicity	IGC50	LC50
10910653	0.016	0.325	0.006	0.534	0.047	4.475	5.142
5318568	0.03	0.094	0.026	0.71	0.267	4.016	3.992
5317592	0.097	0.071	0.027	0.928	0.198	4.946	5.423
101635263	0.104	0.008	0.145	0.001	0.132	5.263	5.378
10349562	0.008	0.005	0.285	0.001	0.146	4.219	4.366
5281775	0.037	0.125	0.084	0.477	0.754	4.391	4.293
10009754	0.044	0.007	0.236	0.001	0.199	4.898	4.951
519960	0.051	0.047	0.022	0.823	0.041	4.376	4.635

In Table 5 Gingerglycolipid A (10349562) has been seen to have the least toxicity compared to other ligands keeping in mind the hERG, DILI, Ames, though it has a poor carcinogenicity rate. The least carcinogenic is cyclosalivine and viridiflourine.

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Table 6. Medicinal properties of top 8 ligands.

ID	QED	PAINS	Lipinski	Fsp3	Synth
10910653	0.513	0	Accepted	0.867	4.148
5318568	0.604	0	Accepted	0.318	2.545
5317592	0.604	0	Accepted	0.318	2.436
101635263	0.04	0	Rejected	0.909	4.802
10349562	0.043	0	Rejected	0.788	5.044
5281775	0.668	0	Accepted	0.286	2.457
10009754	0.041	0	Rejected	0.848	4.915
519960	0.606	0	Accepted	1	5.834

Table 6 interprets that Isogingerenone B and Gingernone B seem to be the fittest and medicinally accepted phytochemicals to be used as drugs for clinical use.

Table 7. Physicochemical properties of top 8 Ligands. hERG: The human ether-a-go-go related gene; DILI: Drug-induced liver injury; AMES: The Ames test for mutagenicity; FDAMDD: The maximum recommended daily dose, carcinogenicity, and 96-hour fathead minnow LC50 were examined.

ID	MW	nHA	nHD	nRot	nRing	MaxRing	nHet	nRig	Flex	TPSA	LogS	LogD	LogP
10910653	204.19	0	0	0	3	11	0	13	0	0	-5.87	4.623	4.148
5318568	386.17	6	2	10	2	6	6	14	0.714	85.22	-3.668	2.74	2.545
5317592	386.17	6	2	10	2	6	6	14	0.714	85.22	-4.722	3.207	2.436
101635263	680.4	14	8	25	2	6	14	14	1.786	225.06	-2.922	3.089	4.802
10349562	676.37	14	8	23	2	6	14	16	1.438	225.06	-2.446	2.462	5.044
5281775	356.16	5	2	9	2	6	5	14	0.643	75.99	-3.45	2.941	2.457
10009754	678.38	14	8	24	2	6	14	15	1.6	225.06	-2.726	2.833	4.915
519960	204.19	0	0	1	5	0	0	13	0.077	0	-5.283	4.508	5.834

Table 7 gives us the interpreted data that Gingerglycolipids A, B, and C are a little more than the optimal molecular weight, however, nHD, nRing, and nHA are way above the optimal range along with TPSA. Cyclosalivene and Viridiflourene have the least molecular weight and are in the range as well. While Viridiflourene has more MaxRing value than Cyclosalivene. Although these two have higher LogS values and LogP. Therefore, from the above interpretation, we can say that. Cyclosalivene and Viridiflourene seem to be the most suitable fit compared to the physiochemical properties of other ligands.

Emperical Decision: 0–0.3: excellent (green); 0.3–0.7: medium (yellow); 0.7–1.0(++): poor (red)

PPB- >90%- excellent, below -poor

MDCK > -2*10(-6)

Caco-2 0.42> **Molecular weight-** 100–600 optimal

DISCUSSION

The emergence of molecular oxygen caused a substantial alteration in the Earth's environment and posed a brand-new barrier to the development of sophisticated life. Although the increase in ambient oxygen made it possible to utilize organic substrates more efficiently, it also added a burden of ROS that put cellular homeostasis at risk.

Secondary Structure of KEAP Nrf2

The 3D structure of the protein KEAP Nrf2 protein is given in the result part Figure 2. After the retrieval of protein and ligands on viewing the secondary structure analysis and its validation of the protein we have got the results of the Ramachandran plot that was derived described the motifs and pro-

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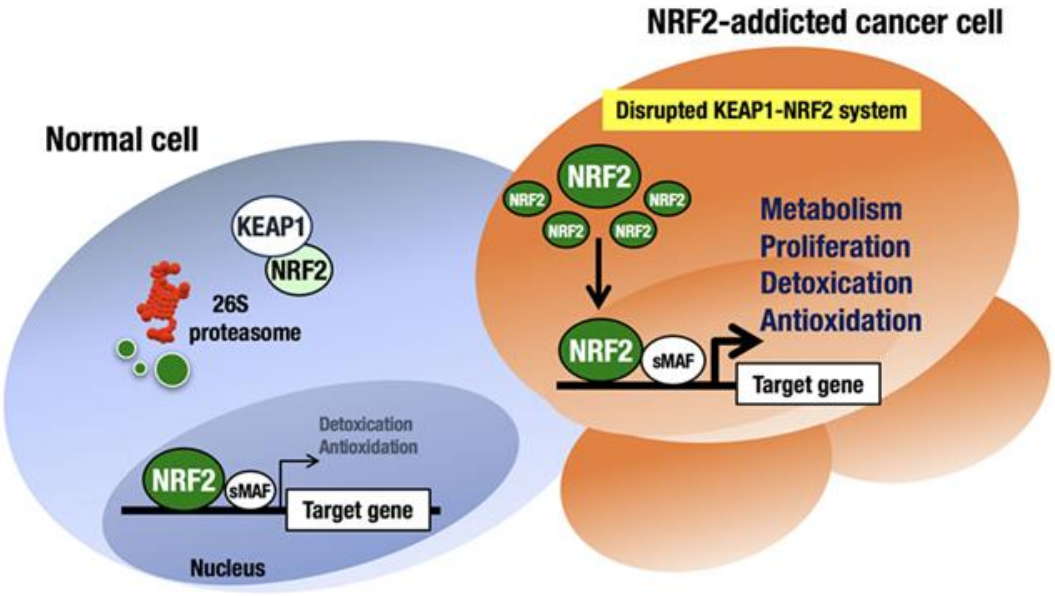
motifs as described. The pro motifs of the protein consist of 8 sheets, 17 beta hairpins, 10 beta bulges, 28 strands, 40 beta turns, and 1 gamma turn. Thus, this was what resulted from the Ramachandran plot and the 3D structure derivation from the PROSA website.

KEAP1-NRF2 Pathway

It has been proposed that the KEAP1/NRF2 pathway, a crucial cellular antioxidant defense system present in nearly all metazoans, evolved in part in response to increased global oxygenation. To resist or avoid the uncommon pathophysiological state of broad redox and nutritional stress that transformation appears to consistently produce, cancer cells must evolve defense mechanisms [15]. We concluded from what we observed that there is a phytocompound that is appropriate for use in medicine to inhibit Keap1 Nrf2 molecules without endangering any clinical trials.

The Keap1 NRF2 pathway is ARE mediated (AU-rich elements) which causes the rapid degradation of mRNA which is present in the 3′ untranslated regions (3′UTRs). The keap1-nrf2 pathway is responsible for several cell signalling mechanisms where exactly is in tissues subjected to long-term oxidative stress, activation of the NRF2/KEAP1 pathway has been identified as a cancer suppressor. When a cell is healthy or not under stress, Keap1 connects to the Nrf2 molecule to create the 26s proteasome, which quickly breaks down the Nrfs. Later, the Nrf2 molecule binds to the sMAF molecule and targets the target protein, leading to detoxification and antioxidation. Stabilized NRF2 enters the nucleus and forms a heterodimer with a small MAF (sMAF) transcription factor. When the NRF2-sMAF heterodimer binds to an antioxidant-responsive element (ARE) or an electrophile-responsive element, several cytoprotective genes are made to begin transcription. Figure 7 shows the mechanism of the KEAP 1-Nrf2 addicted cancer cell with and without anti-cancer drug radiation.

This similarity led to the discovery of the NRF2-sMAF-mediated control of cytoprotective gene expression via ARE/EpRE. Globally, it seems that NRF2-sMAF regulates networks involved in metabolism and cytoprotection A subset of important NRF2-sMAF target genes encode detoxifying and antioxidative enzymes. The epigenetic silencing of the KEAP1 gene has been associated with both KEAP1 downregulation and NRF2 overexpression. NRF2 inducers shield healthy cells from carcinogens whereas NRF2 inhibitors seek to block the progression of cancer. However, free radicals are not detoxified when there is an excess of only Nrf2 produced due to oxidative stress [6]. Since these alterations are so numerous and diverse, KEAP1/NRF2 signaling can respond to additional cellular physiologic stressors. The main antioxidant transcription factor NFE2L2 (nuclear factor erythroid 2-like 2), often known as NRF2, is primarily controlled negatively by KEAP1 [2, 6].



(a)

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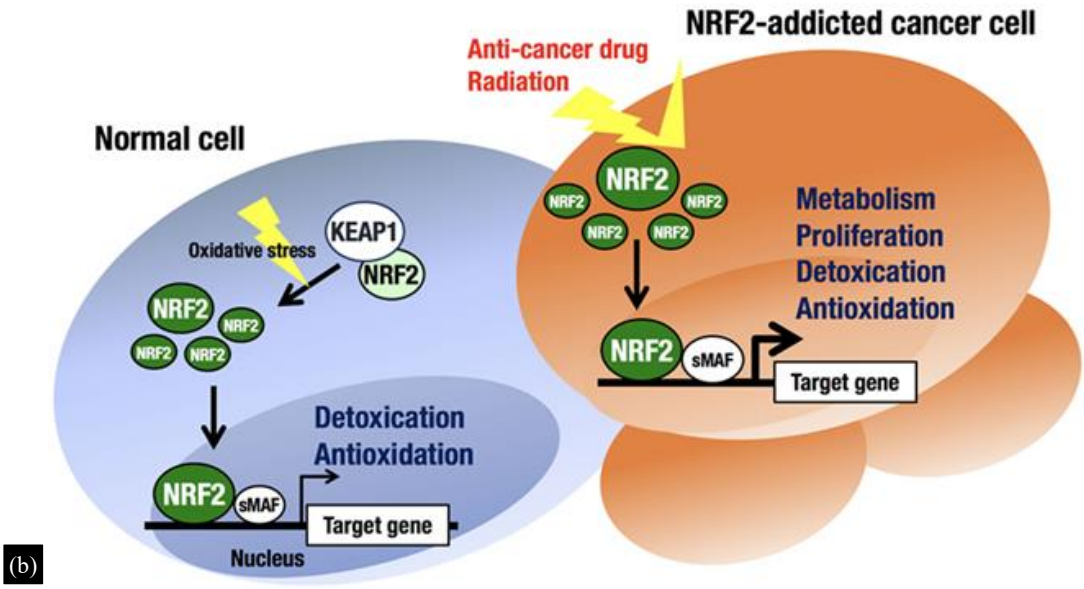

Figure 7. KEAP 1 NRF2 pathway in normal and under the influence of anti-cancer drug radiation condition in an NRF2- addicted cancer cell, (Keiko Taguchi,2007).

Table 8. Hydrophobic interactions and hydropathy interpretation.

Index	Residue	AA	Distance	Ligand Atom	Protein Atom
1.	407A	ALA	3.76	2774	795
2.	408A	PRO	1.66	2781	802
3.	408A	PRO	1.31	2770	799
4.	450A	TRP	3.29	2775	1199

The hydropathy interactions of the protein are described in the aforementioned Table 8. Graphs of z-scores, hydrophobicity, and hydropathy plots were examined. The top eight appropriate ligands are included in Table 9 along with their binding affinities, which will aid in interpreting the strength or compatibility of the protein and ligand binding.

Table 9. Top 8 ligands and their binding affinities

S.N.	Compound	Binding Affinity
1.	Gingerglycolipid B	-8.6
2.	Gingerglycolipid A	-8.3
3.	Isogingerenone B	-8.2
4.	Gingerenone B	-7.9
5.	Gingerenone A	-7.9
6.	Gingerglycolipid C	-7.9
7.	Viridiflorene	-7.7
8.	Cyclosalivene.	-7.2

The visualization and ADMET lab analysis were completed later in the final phase, which is a critical part of the overall research, and the data was interpreted. This phase is essential to the entire process since visualizing the docked complexes is just as significant as conducting a clinical analysis to determine whether the drug will be accepted.

The best ligands or phytocompounds that can be utilized without fearing compromising the human body's metabolism are believed to be Cyclosalivene and viridiflourine. They may work best when used individually or in combination with other components and interpreted to have the best physiochemical

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properties and toxicity properties and are accepted by the Lipinski rule of drug development and design though they are falling short in the binding affinity towards the protein.

Gingerglycolipid A, B, and C have also proved to be clinically acceptable to be used as an inhibitor against Keap1-Nrf2 small molecules or its pathway. Although they lack in the field of physiochemical properties of the ligands.

CONCLUSION

Therefore, based on the results of this investigation, we have determined that the ligands cyclosalivene and viridiflourine can be used for future clinical research. We may also state that gingerglycolipid A binds to the KEAP1-NRF2 protein with the highest binding affinity. However, gingerglycolipds A, B, and C all have the most effective components to prevent cancer from progressing as a result of the target proteins (KEAP1-NRF2). For these compounds, additional in-vitro research can be conducted.

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Abbreviations

KEAP- Kelch-like ECH-associated protein 1

Nrf2- nuclear factor erythroid 2-related factor 2

ARE - AU-rich elements

MDCK - Madin-Darby canine kidney

hERG- human Ether-a-go-go Related Gene) assay

DILI- Drug-induced liver injury

PPB- Plasma protein binding

VD- volume of distribution

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