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Molecular Docking and Pharmacophore-Based Virtual Screening of Novel Inhibitors for HCV NS5B RNA-Dependent RNA Polymerase Enzyme from Crude Sesame Essential Oil.

Lukeman A.J Shittu [1, 2*], Abiodun M. Jinadu [2], Remilekun K. Shittu [3], Solomon A. Shittu[2].

[1.]Department of Anatomical Sciences, University of Abuja, Gwagwalada, Abuja, Nigeria. [2.]Research and Development Department, Jireh Laboratories International LLC, P. O. Box 1813, Alief Texas, 77411, USA. [3.]Department of Microbiology, Jireh International Laboratory, P. O. Box 882, Gwagwalada, Abuja, Nigeria.

ABSTRACT

Concern has been expressed worldwide about the rising prevalence of HCV induced acute hepatitis and chronic liver diseases with associated cirrhosis and liver cancers. However, the available synthetic drugs are ineffective for all the HCV genotypes especially in genotype-1 patients with about 40% viral response rates and numerous side effects. Besides, the availability of veritable bioinformatics tools which includes molecular docking and virtual screening studies have shown that computational generated models nowadays assists in modern drug design and development of novel and more potent inhibitors through the understanding of protein (receptors) -ligand (drugs) interaction mechanisms. Non-structural proteins especially the 5B (NS5B) is an RNA-dependent RNA polymerase implicated in the synthesis and replication of the HCV RNA; and has been a potential target for its inhibitory activities. Due to the paucity of knowledge, we aimed to determine the differential inhibitory activity of essential oils present in the crude Sesame leaves extracts on HCV-NS5B RNA dependent RNA polymerase. Using in-silico studies- a Microsoft pharmacophore-based virtual screening and molecular docking tools on the iGEMDOCK vs 2.0 software was used to dock the essential oil ligands on the generated HCV NS5B (PDB ID: 4EO6) RNA-dependent RNA polymerase protein. GC-MS of the leaves confirmed carboxylic acids and phenolic groups in the essential oils especially some potent antioxidants like alpha-linolenic acid, linoleic acid, oleic acid, etc. Moreover, Alpha-Linolenic acid/ALA (-102.2/-103.4 kcal/mol) and Linoleic acid/LA (-94.8/-109.8 kcal/mol) showed higher inhibitory impacts among the six top different docked ligands, selected based on their high differential binding affinity and pharmacological interaction energy profiles against HCV NS5B RNA polymerase activities, by forming more H-bond interactions than the NS5B co-crystallized ligand. ADMET showed that ALA is well tolerated without any apparent toxicity in the body. Hence, ALA having the highest inhibitory impacts against the HCV NS5B goes to confirm the beneficial impacts of carboxylic acids from Sesame plant in maintaining liver cellular integrity and as a natural inhibitor of HCV NS5B RNA dependent RNA Polymerase enzyme activities.

Keywords:
Sesame Leaves, GCMS, Molecular Docking, Virtual Screening, In-Silico studies, HCV NS5B RNA Polymerase, Essential Oils

***Correspondence to Author:**
Lukeman A.J Shittu Department of Anatomical Sciences, University of Abuja, Gwagwalada, Abuja, Nigeria; Research and Development Department, Jireh Laboratories International LLC

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Introduction

Primarily the crackdown of the entire human genome about a few decades ago has positively impacted on the enhanced roles of computational tools applications in modern medicine. No doubt, computer applications in the whole drug discovery and drug development pipeline system have further helped pharmaceutical companies to save millions of US dollars and time, usually lost as a result of so many failed clinical drug trials in bringing drugs to reality. Besides, Computer-Aided-Drug-Discovery (CADD) approaches are now extensively been used in the drug investigation to enhance the efficiency of the entire drug discovery process [1].

The liver is considered the largest organ in the human body, weighing on the average, about 1.44–1.66 kg (3.2–3.7 lb) for both sexes [2]; as a result, it is tasked with enormous responsibilities which include metabolism and detoxifications of ingested substances in the body. Which further predisposed the liver to suffer reversible or irreversible damages due to several mechanisms caused by toxins, infectious agents; although, these are usually insidious in several conditions like hepatitis. One of such implicated agents is viral hepatitis caused by different type of viruses, such as hepatitis A, B, C, D, and E. In addition, jaundice is usually one of the most apparent characteristic features of viral hepatitis. As such, early detection of viral hepatitis and its treatment will indeed have a significant impact on the disease progression and prognosis. Hence, there is the need for a rapid and proper diagnosis of viral hepatitis through serological testing of the patient's blood for antiviral antibodies detection [3] with characteristic features of liver disease. Since, over 60% of the acute sufferers progress to the chronic stage of the infection [4].

Moreover, in recent times, hepatitis C virus (HCV) liver infection has become a major health concerns with significant economic and medical impacts, affecting humans with about 170 million chronic sufferers estimated worldwide [5,6]. Also, it is considered as one of the primary causes of cirrhosis and hepatocellular carcinoma (HCC); and a leading cause for liver transplantation worldwide [5-7]. In addition, HCV belongs to the family-Flaviviridae and genus- hepacivirus [8]. The HCV genome contains about 9.6 kilobase pairs in length that encode a polyprotein with over 3000 amino acids, which is cleaved by HCV genome proteases to 10 structural (S) and non-structural (NS) proteins [8,9]. The HCV non-structural (NS) protein especially, the NS5B polymerase, is an RNA-directed/dependent RNA polymerase and is also responsible for the complete replicate of the HCV RNA genome that is, it can catalyze the synthesis of both the positive (genomic) and negative (template) strand HCV RNA. However, it has no functional equivalent in mammalian cells. In addition, clinical evidence has shown that NS5B is essential for HCV replication [10] and can be detected with biochemical testing [8].

Recently, a dual therapy is said to be more effective than the usual single vaccine treatment approach, for example, the combination of pegylated interferon and ribavirin vaccines yielded a higher response rate against the HCV than a separate individual treatment [9,11]. However, both vaccines are still characterized with severe side effects such as hemolytic anemia, renal failure, etc. [11]. In addition, studies have shown that there have been no effective vaccines or therapy without any significant side effects [6,9,11].

Moreover, the past two decades have witnessed several strategies used to inhibit HCV NS - viral proteins with NS5B RNA-dependent RNA polymerase emerging as one of the most attractive target choices for most anti-HCV drugs development for obvious reasons as already stated [8,10]. Furthermore, a growing number of small molecules have been reported as allosteric inhibitors of NS5B with only a very few of them still active in clinical trials in recent times [5-7]. However,

limited studies are available on medicinal small molecules, as HCV NS5B RNA-dependent, RNA polymerase inhibitors from other plants like *Acacia concinna* [9], *Phyllanthus niruri* [10].

Hence, recently expressed is the searching for ideal natural medicinal antimicrobial agents with effective antiviral activity against the HCV NS5B RNA-directed RNA polymerase with minimal or no side effects as compared to the available synthetic agents in the markets [12-16]. Interestingly, phytoestrogens are one of such natural plant-based estrogenic agents that have recently attracted so much attention during the last decade because of their essential health benefits to humans. They include four broad classes of phytochemicals namely the lignans (sesame seed and flaxseed), isoflavonoids (soybeans), stilbenes (peanuts and grape) and coumestans (mung beans and clover) [17-20]. Moreover, phytoestrogens tend to mimic endogenous estrogens. Depending on their concentrations in the body, they act either as agonists or antagonists by displaying the endogenous estrogens from their estrogens receptors (Era and Erb) binding sites among their other known mechanisms of action [15,18,19,21].

Besides, sesame plant is one of the most abundant food sources of phytoestrogenic lignans, a valuable phytochemical celebrated by man since the dawn of civilization [15,18,19,22]. They are also now increasingly being incorporated into human diet worldwide because of their reported health benefits [15,18,19]. Moreover, sesame plant is inherently rich in essential oils, trace elements/ minerals [such as calcium, iron, magnesium, zinc, manganese, selenium, molybdenum, copper and phosphorus]. It also rich in amino acids, multi-vitamins, primary and secondary metabolites [12,14,18,19,22-28].

Moreover, all parts of the sesame plant such as the seed, oil, leaves and roots are of great folkloric medicinal values locally. Also, subsistence farmers in some areas of Nigeria (Northern/NN, South-west/SWN and Middle-belt/MBN) consumed sesame as staple food sources. In addition, sesame bears different local names based on the areas of cultivations. Like ekuku–gogoro (SWN- for black-seed), yanmoti (SWN-for white-seed), ridi (NN); and Beni (MBN/English) or gingelly (English) in Asia and Africa [12,14,15,18,19,29]. Also, sesame bioavailability in the body after its oral ingestion in man revealed that the lignans usually undergo extensive metabolism in the intestine depending on the characteristics of the individuals' intestinal microflora to produce mammalian enterolignans, enterodiol and enterolactone [14,22].

More so, molecular docking is a software technique that fits a small molecule into a target's binding sites and is used to study the binding affinity modes of protein (large molecule) with ligands and inhibitors (usually small molecules) [30,31]. Thus, docking tools can be used to predict the structure of the intermolecular complex formed between two or more molecules including matching the molecules with overall minimum energy. In addition, it gives the prediction of absolute ligand binding affinities, as well as the binding orientation of ligands [31,32]. Hence, its application in drug discovery and drug design process, since drugs-receptor interactions are also highly specific and complimentary. Similarly, studies have shown that the predicted binding affinities of the ligands from the dry laboratory are usually in excellent agreement with the wet laboratory/ experimental values with an average deviation of 0.61 +/- 0.4 kcal/mol [32].

Hence, we aim to study the inhibitory impact of the different natural active essential oil (small molecules) extracted from sesame plants (Sesamum radiatum-black) against the HCV NS5B RNA polymerase enzyme (belonging to transferase/transferase inhibitor enzyme class) using *in-silico* studies (virtual screening and molecular docking) on the iGEMDOCK computational suite [31]. In addition, to confirm

the folkloric claim of sesame leaves effectiveness as an anti-viral agent.

Material and Methods

Plant-source and preparation

The leaves from the sesame plant species (Sesamum radiatum, Schum and Thonn, Pedaliacaea family) purchased from a local vendor in SW Nigeria were processed accordingly. The extraction was allowed to proceed for five days (120 h) in the Refrigerator at 4°C for the stability of the essential oils. The crude extracts solution obtained was regarded as the full concentration with the crude extract later reconstituted for further (in-vitro) studies as stated in previous studies [12,13,15,16,18,19].

Phytochemical screening using gas chromatography-mass spectral (GC/MS)

Crude methanolic extracts of Sesame leaves were analyzed by GC/MS. The GC analyses were performed using a Hewlett Packard gas chromatograph (model 6890) equipped with a flame ionization detector and injector MS transfer line temperature of 230°C respectively. Also, a fused silica capillary column HP-InnoWax (30 in x 0.25 mm, film thickness 0.25 µm) was used.

The oven temperature was set at 50 °C for 5 minutes holding time and then gradually raised from 50-230°C at a rate of 2 °C /min. Helium is the carrier gas used at a flow rate of 22cm/sec. One milliliter of extract mixed with methanol (80%), at a split ratio of 1:30 was later injected into the system [12,13,15,16,33].

GC/MS analyses were carried out on an Agilent Technologies Network mass spectrometer (model 5973) coupled to H.P gas chromatograph (model 6890) equipped with NBS 75K Library Software data.

The capillary column and GC conditions were as stated above and helium was used as the carrier gas with a flow rate of 22cm/s. The mass spectra were recorded at 70 eV/200°C at a scanning rate of 1scan/sec with a run time of 90 minutes as reflected in previous studies [12,13,15,16]. Compound identification carried out by comparing the obtained GC relative retention times and mass spectra to those of authentic substances analyzed under the same conditions; using their retention indices (RI) and by comparison to reference compound [12,13,15,16].

Molecular docking studies

The iGEMDOCK v2.1 software suite [http://gemdock.life.nctu.edu.tw/dock] is a complete standalone docking and virtual screening tool that was used to dock the NS5B protein of the HCV with the extracted essential oil molecules from sesame plant.

Preparation of the protein structure

Protein structure retrieval- The Fasta sequence obtained from the HCV NS5B protein was subjected to sequence similarity search by using the BLASTP program [34] and the protein molecule having 100% sequence identity structure was then retrieved from the Brookhaven RCSB Protein Data Bank (https://www.rscb.org) [35]. Thus, the three-dimensional (3D) structure for the target HCV NS5B protein, an RNA-dependent RNA polymerase protein, obtained at a resolution of 1.79Å root mean square deviations (RMSD); and corresponding to the PDB ID- 4EO6 was downloaded in pdf format. The HCV NS5B protein was later uploaded into the iGEMDOCK software [31] for further analysis and docking studies.

Protein structure visualization

The three-3-dimension (3D), PDB ID- 4EO6 protein structure that was retrieved from RCSB PDB was visualized using the iGEMDOCK in-built RasMol v 2.7.5 software, flexible, robust molecular visualization software (http://rasmol.org/) [36]. Also, the bound ligand in the protein structure was visualized and removed before in-silico analysis.

Active binding site preparation

Previous studies have shown that the HCV NS5B protein has catalytic and allosteric active

binding sites for its enzymatic activity [9] as also predicted in this present study using the CASTp webserver [37]. This tool helps identify ligand binding sites, using energy interaction scheme between the protein and a simple Van der Waal's probe to locate energetically favorable binding sites of a ligand. Here, the CASTP showed the binding site-amino acid residues, participating in the active site of the macromolecule (protein), including calculation of the binding active site's area and volume, by using the probe with a radius of 4Å. The obtained active site residues were then mapped on to the HCV NS5B structure to check exactly where the residues are present.

Moreover, the iGEMDOCK software [31] has its own in-built protein binding site prediction, which centered on the protein's ligand (0S2) in itself at any given radius (3-30Å) for docking reference. The binding site of the ligand-0S2 in the HCV NS5B protein was set at a radius of 4.0 A for the present study=docking reference. The platform also showed a RasMol displayed of the referenced, centered ligand (0S2) in its active binding sites on the protein as reflected in figure 1. In addition, the binding site for the target protein –NS5B was prepared at a probe radius of 4 Å for each platform and the top 6 compounds with higher binding energy and scores were selected in the present study. Moreover, the PDBsum analysis of the HCV NS5B 4EO6 protein-ligand (0S2) binding sites was carried out to validate information on the binding site of the protein in the present study (http://www.ebi.ac.uk/thornton-srv/databases/pdbsum/) [38].

Ligands/Compounds preparation

The ligand molecules used for the present docking process were the natural essential oil compounds extracted and analyzed from the leaves of Sesame plants using Gas Chromatography-Mass Spectroscopy [[12,13,15,16]. The extracted compounds were then subjected to extensive database/library survey, especially, with the PubChem library (https://pubchem.ncbi.nlm.nih.gov) [39] to identify their structures, properties and uses further.

In addition, the 3D structure of each essential oil compound downloaded from the PubChem database in SDF format was then loaded into the Chimera 1.11.2 software (http://www.rbvi.ucsf.edu/chimera) [40]. Which was further built through a geometric energy minimization process (assigning bond, hydrogen atoms, charges and flexible torsion) to be converted into pdf format and saved as a MOL file for further study.

The Molecular docking process

This was performed by docking each ligand separately into all the potential active sites detected on NS5B-RNA-dependent RNA-polymerase enzyme. At first, the molecules were prepared and bonds, with bond orders, explicit hydrogen's atoms, charges, flexible torsions assigned to both the protein and ligands.

Furthermore, all the prepared volatile essential oils compounds saved in pdf format (MOL files) were uploaded into the iGEMDOCK software [31] using the following set parameters by default: Population size: 200, Generations: 70, Number of solutions: 3. The output path was set for data retrieval with the 'Start docking' option clicked; and, when docking was completed, a post-screening/analysis of the docked ligands was done.

The iGEMDOCK is unique because it measures the ligand docked fitness as the total energy (Etot) of a predicted pose in the binding site

as dissected into the following terms below:

Etot = Ebind + Epharma + Eligpre, where Ebind is the empirical binding energy used during the molecular docking; Epharma is the energy of binding-site pharmacophores; Eligpre is a penalty value if the ligand unsatisfied the ligand preferences.

In addition, the Epharma and Eligpre were used to improve the number of true positives by discriminating active compounds from hundreds of thousands of non-active compounds.

Table 1. GCMS OF SESAME ESSENTIAL OIL COMPOUNDS

S/N	Natural Essential Oil Compounds. IUPAC NAMES	PubChem CID
1.	Alpha Linolenic acid/ZZZ-9,12,15 octadecatrienoic acid	5280934
2.	Linoleic acid/ZZ-9,12 octadecadienoic acid	5280450
3.	Oleic acid/Z-9 octadecenoic acid	445639
4.	Stearic acid/octadecanoic acid	5281
5.	Palmitoleic acid/Z-9 hexadecenoic acid	445638
6.	Palmitic acid/n-hexadecanoic acid	985
7.	Arachidic acid (Eicosanoic acid)	10467
8.	Myristic acid/tetradecanoic acid	11005
9.	Capric acid/decanoic acid	2969

Table 2. DIFFERENT DOCKED LIGANDS WITH DIFFERENTIAL TOTAL BINDING ENERGY PROFILES

S/N	Compounds-natural essential oil	Total Binding Energy-(ΔG)	VDW	HBond	Elec
1	cav4eo6_0S2-5280934-1.pdb	-102.182	-75.9332	-25.3281	-0.92051
2	cav4eo6_0S2-4EO6_0S2-0.pdb	-94.783	-83.981	-10.5	-0.30203
3	cav4eo6_0S2-5280450-2.pdb	-90.4195	-76.5509	-11.9463	-1.92235
4	cav4eo6_0S2-445639-2.pdb	-86.6814	-71.6403	-12.6542	-2.3869
5	cav4eo6_0S2-5281-0.pdb	-85.7358	-70.1779	-13.1378	-2.42015
6	cav4eo6_0S2-445638-2.pdb	-81.9587	-62.4851	-16.3349	-3.13872
7	cav4eo6_0S2-985-2.pdb	-79.7233	-69.0078	-10.3451	-0.3704
8	cav4eo6_0S2-10467-1.pdb	-79.5048	-71.1209	-8.38389	0
9	cav4eo6_0S2-11005-0.pdb	-78.6797	-65.7108	-10.8687	-2.10016
10	cav4eo6_0S2-2969-2.pdb	-71.1036	-52.01	-15.9798	-3.11378

Table 3. SHOWING DIFFERENT ESSENTIAL OIL COMPOUNDS WITH TOTAL BINDING ENERGY AND PHARMACOLOGICAL INTERACTION ENERGY PROFILES.

Compounds-natural essential oil	Total Binding Energy (ΔG)	Energy (pharmacological interaction)
cav4eo6_0S2-5280934-1.pdb	-102.2	-103.69
cav4eo6_0S2-4EO6_0S2-0.pdb	-94.8	-109.78
cav4eo6_0S2-5280450-2.pdb	-90.4	-109.42
cav4eo6_0S2-445639-2.pdb	-86.7	-105.63
cav4eo6_0S2-5281-0.pdb	-85.7	-99.78
cav4eo6_0S2-445638-2.pdb	-82.0	-126.1
cav4eo6_0S2-985-2.pdb	-79.7	-96.68
cav4eo6_0S2-10467-1.pdb	-79.5	-100.01
cav4eo6_0S2-11005-0.pdb	-78.7	-97.63
cav4eo6_0S2-2969-2.pdb	-71.1	-130.72

Table 4. TOTAL BINDING ENERGY AND PHARMACOLOGICAL ENERGY INTERACTION OF DOCKED ESSENTIAL OIL COMPOUNDS WITH HCV NS5B POLYMERASE ENZYME

Compounds-natural essential oil	Total Binding Energy (ΔG)	E(pharma)	E-S-ARG -422	H-S-ARG -422	H-M-SER -476	V-S-LEU -419	V-S-TYR -477	V-M-TRP-528
		Z-score	-1.00	1.76	2.59	3.78	3.75	4.33
		W(pharma)	0.00	0.68	1.00	0.87	0.86	1.00
cav4eo6_0S2-5280934-1.pdb	-102.2	-103.69	-0.30268	-5.69153	-3.5	-10.6845	-8.79276	-20.3821
cav4eo6_0S2-4EO6_0S 2-0.pdb	-94.8	-109.78	0	0	-7	-12.3625	-10.3967	-12.7606
cav4eo6_0S2-5280450-2.pdb	-90.4	-109.42	0	0	-3.5	-4.89336	-4.28332	-16.0269
cav4eo6_0S2-445639-2.pdb	-86.7	-105.63	0	0	-3.5	-4.55577	-5.11734	-15.4994
cav4eo6_0S2-5281-0.pdb	-85.7	-99.78	-2.42015	-6.2154	0	-4.09811	-4.95913	-17.3809
cav4eo6_0S2-445638-2.pdb	-82	-126.1	-3.13872	-13.8349	0	-3.61437	-10.0076	-13.1083
cav4eo6_0S2-985-2.pdb	-79.7	-96.68	0	0	-7	-6.26139	-7.83893	-10.9176
cav4eo6_0S2-10467-1.pdb	-79.5	-100.01	0	0	-3.25368	-4.28418	-7.37566	-12.9762
cav4eo6_0S2-11005-0.pdb	-78.7	-97.63	-2.10016	-5.04046	0	-4.8591	-9.00146	-10.0388
cav4eo6_0S2-2969-2.pdb	-71.1	-130.72	-3.11377	-13.4798	0	-8.18988	-2.78033	-10.7763

Z score ≥ 0.5 is considered significant

S-SIDE CHAIN OF NS5B ARG -422 =ARGININE, 422

M-MAIN CHAIN OF NS5B SER- 476 = SERINE, 476

H-HYDROGEN BOND FORMATION LEU-419 = LEUCINE, 419

E- ELECTROSTATIC BOND FORMATION TYR-477 = TYROSINE, 477

VDW- VANDER-WAAL BOND FORMATION TRP-528 = TRYPTOPHAN, 528

Table 5. PREDICTED PROPERTIES OF ALPHA-LINOLENIC ACID

PROPERTY	VALUE	SOURCE
Water Solubility	0.000266 mg/mL	ALOGPS
logP	6.62	ALOGPS
logP	6.06	ChemAxon
logS	-6	ALOGPS
pKa (Strongest Acidic)	4.99	ChemAxon
Physiological Charge	-1	ChemAxon
Hydrogen Acceptor Count	2	ChemAxon
Hydrogen Donor Count	1	ChemAxon
Polar Surface Area	37.3 $Å^2$	ChemAxon
Rotatable Bond Count	13	ChemAxon
Refractivity	89.64 $m^3 \cdot mol^{-1}$	ChemAxon
Polarizability	34.98 $Å^3$	ChemAxon
Number of Rings	0	ChemAxon

Table 6. PREDICTED ADMET FEATURES OF ALPHA-LINOLENIC ACID

PROPERETY	VALUE	PROBABILITY
Human Intestinal Absorption	+	0.9896
Blood Brain Barrier	+	0.9314
Caco-2 permeable	+	0.7735
P-glycoprotein substrate	Non-substrate	0.6766
P-glycoprotein inhibitor I	Non-inhibitor	0.9499
P-glycoprotein inhibitor II	Non-inhibitor	0.9025
Renal organic cation transporter	Non-inhibitor	0.9311
CYP450 2C9 substrate	Non-substrate	0.7735
CYP450 2D6 substrate	Non-substrate	0.9081
CYP450 3A4 substrate	Non-substrate	0.6884
CYP450 1A2 substrate	Inhibitor	0.6915
CYP450 2C9 inhibitor	Non-inhibitor	0.8798
CYP450 2D6 inhibitor	Non-inhibitor	0.9631
CYP450 2C19 inhibitor	Non-inhibitor	0.9638
CYP450 3A4 inhibitor	Non-inhibitor	0.9465
CYP450 inhibitory promiscuity	Low CYP Inhibitory Promiscuity	0.9426
Ames test	Non AMES toxic	0.9132
Carcinogenicity	Non-carcinogens	0.6502
Biodegradation	Ready biodegradable	0.7808
Rat acute toxicity	1.4499 LD50, mol/kg	Not applicable
hERG inhibition (predictor I)	Weak inhibitor	0.8818
hERG inhibition (predictor II)	Non-inhibitor	0.9315

Table 7. SEQUENCE ALIGNMENT RESULT OF BLASTPOF 4EO6 PROTEIN

Sequences producing significant alignments:							
Select Protein Sequence	Description	Max score	Total score	Query cover	E value	identity	Accession
Select seq pdb|4EO6|A	Chain A, HCV NS5B Polymerase Inhibitors: Tri-Substituted Acylhydrazines As Tertiary Amide Bioisosteres	1205	1205	100%	0,0	100%	4EO6_A

Table 8. SHOWING THE AMINO ACID RESIDUES INTERACTIONS OF CO-CRYSALLIZED LIGAND OS2 FOR THE 4EO6-CHAIN A.

Hydrogen bonds

	<----- A T O M 1 ----->				<----- A T O M 2 ----->				
S/N no.	Atom name	Atom name	Res no.	Res Chain	Atom no.	Atom name	Atom name	Res no.	Res Chain	Distance
1.	3661	N	SER	476 A	8718	O10	0S2	600	A	2.81
2.	3667	N	TYR	477 A	8717	O09	0S2	600	A	2.87

Non-bonded contacts

	<----- A T O M 1 ----->				<----- A T O M 2 ----->				
no.	Atom name	Atom name	Res no.	Res Chain	Atom no.	Atom name	Atom name	Res no.	Res Chain	Distance
1.	3205	CD1	LEU	419 A	---	8705	C18	0S2	600 A	3.71
2.	3206	CD2	LEU	419 A	---	8712	S04	0S2	600 A	3.88
3.	3230	CB	ARG	422 A	---	8720	C22	0S2	600 A	3.57
4.	3235	NH1	ARG	422 A	---	8707	C20	0S2	600 A	3.72
5.	3237	N	MET	423 A	---	8720	C22	0S2	600 A	3.89
6.	3646	O	LEU	474 A	---	8707	C20	0S2	600 A	3.79
7.	3652	CA	HIS	475 A	---	8718	O10	0S2	600 A	3.54
8.	3653	C	HIS	475 A	---	8718	O10	0S2	600 A	3.62
9.	3655	CB	HIS	475 A	---	8718	O10	0S2	600 A	3.62
10.	3661	N	SER	476 A	---	8716	C08	0S2	600 A	3.40
11.	3661	N	SER	476 A	---	8717	O09	0S2	600 A	3.20
12.	3661	N	SER	476 A	---	8718	O10	0S2	600 A	2.81
13.	3662	CA	SER	476 A	---	8717	O09	0S2	600 A	3.32
14.	3662	CA	SER	476 A	---	8718	O10	0S2	600 A	3.73
15.	3663	C	SER	476 A	---	8717	O09	0S2	600 A	3.56
16.	3667	N	TYR	477 A	---	8717	O09	0S2	600 A	2.87
17.	3668	CA	TYR	477 A	---	8717	O09	0S2	600 A	3.85
18.	3671	CB	TYR	477 A	---	8717	O09	0S2	600 A	3.79
19.	3673	CD1	TYR	477 A	---	8717	O09	0S2	600 A	3.68
20.	3710	CG1	ILE	482 A	---	8712	S04	0S2	600 A	3.76
21.	3712	CD1	ILE	482 A	---	8712	S04	0S2	600 A	3.70
22.	3712	CD1	ILE	482 A	---	8717	O09	0S2	600 A	3.76
23.	3737	CG1	VAL	485 A	---	8725	C27	0S2	600 A	3.57
24.	3739	N	ALA	486 A	---	8725	C27	0S2	600 A	3.88
25.	3824	CD2	LEU	497 A	---	8710	C02	0S2	600 A	3.61
26.	4074	CB	TRP	528 A	---	8706	C19	0S2	600 A	3.88
27.	4075	CG	TRP	528 A	---	8706	C19	0S2	600 A	3.79
28.	4075	CG	TRP	528 A	---	8720	C22	0S2	600 A	3.83
29.	4077	CD2	TRP	528 A	---	8706	C19	0S2	600 A	3.65
30.	4077	CD2	TRP	528 A	---	8720	C22	0S2	600 A	3.70
31.	4080	CE3	TRP	528 A	---	8705	C18	0S2	600 A	3.79
32.	4080	CE3	TRP	528 A	---	8706	C19	0S2	600 A	3.61

Number of hydrogen bonds: 2

Number of non-bonded contacts: 32.

The empirical binding energy (Ebind) is given as:

Ebind = Einter + Eintra + Epenal. Where Einter and Eintra are the intermolecular and intramolecular energy, respectively, Epenal is a large penalty value if the ligand is out of range of the search box. In this paper, Epenal is set to 10000 (http://gemdock.life.nctu.edu.tw/dock) [41].

Moreover, iGEMDOCK has an empirical scoring function for each ligand posed obtained during docking, which is estimated as:

Fitness = vdW + Hbond + Elec. Where vdW represents van der Waal energy, while, Hbond and Elect stood for hydrogen bonding energy and the electro statistic energy respectively.

Thus, the docking process resulted in binding affinities in kcal/mol and docking run time due to energy-related conformational changes involving the receptor-protein and ligands with the surrounding solution. Moreover, the virtual drug screening scores for the top five phytochemicals-natural essential oil exhibiting higher binding affinity and pharmacological interaction are obtained from iGEMDOCK [31]. The highly negative energy score represents the stronger binding affinity and positive score represents poor or no effective binding between the HCV NS5B -4EO6 protein receptor and the natural essential oil small molecule. However, the molecule with the lowest binding energy is considered as the best inhibitor for the present study.

ADME and Drug Likeness Analysis

Molecular properties like membrane permeability and bioavailability of leading compounds are always associated with some fundamental molecular descriptor such as logP (partition coefficient), molecular weight (MWt), or the number of hydrogen bond acceptors and donors in a molecule, logP (partition coefficient) [42]. These molecular properties were useful in formulating “rule of five” [43]. Lipinski’s rule states that molecule with good membrane permeability must have molecular weight value ≤ 500, Hydrogen Bond Donor ≤ 5, Hydrogen Bond Acceptor ≤ 10 and partition coefficient (LogP) value ≤ 5. Therefore, Lipinski’s Rule of Five [43] was used to test the bioavailability characteristics such as Adsorption, Distribution, Metabolism and Elimination (ADME) of the ligands used in this study.

Prediction of Compound Toxicity

The AdmetSAR database was also used to predict the adverse effects and other properties such as Absorption, Distribution, Metabolism, Excretion and the Toxicity of the natural essential oil compounds -ADMET profiles (http://www.admetexp.org) [43] in the present study.

Results

The CASTP of HCV NS5B – 4EO6 protein showed that there are 15 binding pockets with pocket # -13 having the largest area and volume of 962.8 Å^2 and 1345.1 Å^3 respectively; and, calculated at a radius of 4.0Å. In addition, the iGEMDOCK’s platform showed a RasMol display of centered ligand (0S2) in its active binding sites on the protein as reflected in figure 1a.

The HCV NS5B structure has double chain such as chain-A represented in blue color, chain-B represented in white with different atoms constituting various colors; its N-terminal indicating the starting point of the structure and C-terminal indicating the end point of the structure. Also, RasMol image of the whole HCV NS5B protein showing both double chains A and B with its centered ligands as prepared from the iGEMDOCK suite [31] is shown in figure 2a-b.

The GC-MS finding of the extracted natural essential oil (small molecules) from the Sesame plant with their respective chemical ID numbers as retrieved from the PubChem library are shown in table 1. Therefore, the following natural essential oil extracted from the GC-MS analysis of Sesame leaves includes: (ZZZ)-9,12,15 octadecatrienoic acid, (ZZ)-9,12 octadecadienoic acid, (Z)-9 octadecenoic acid,

FIGURE 1. SHOWING STICK DISPLAY OF 4EO6 LIGAND-OS2 IN ITS ACTIVE SITE OF HCV NS5B PROTEIN (AT 4 Å).

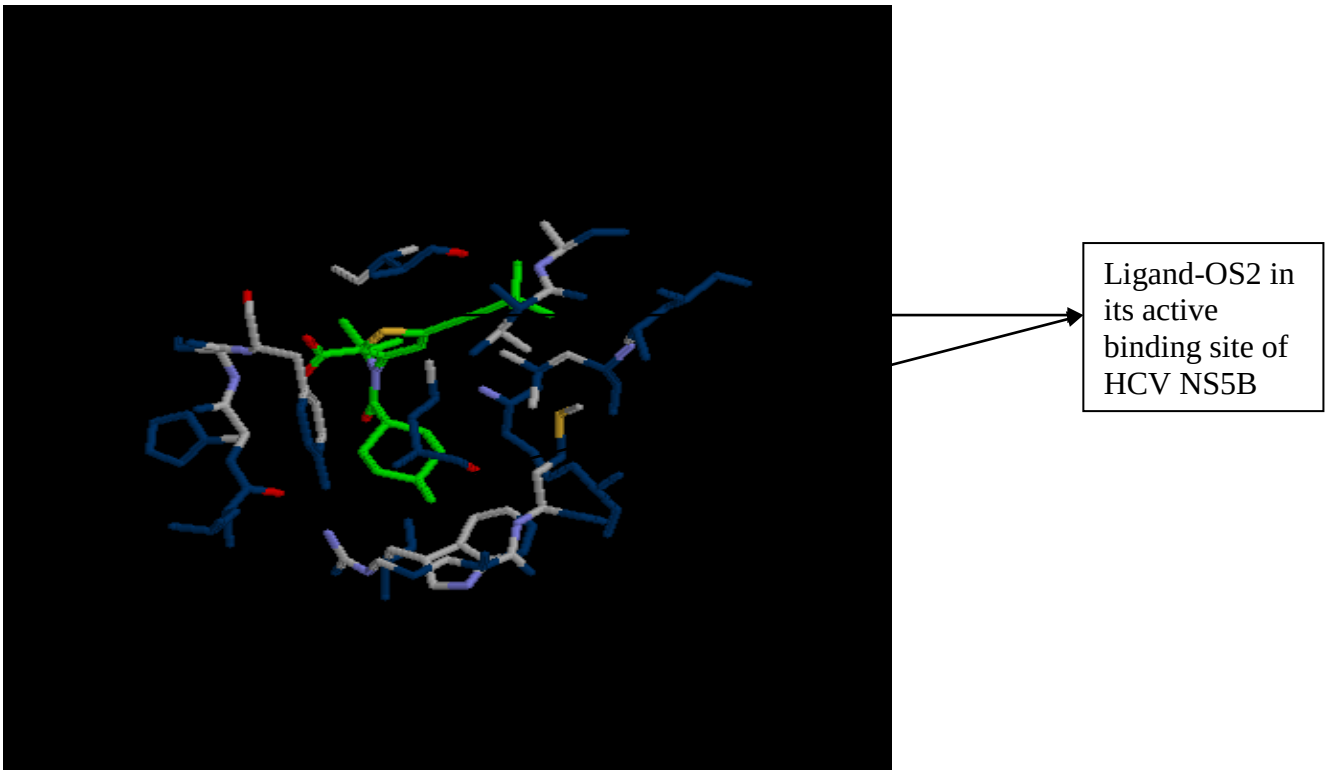
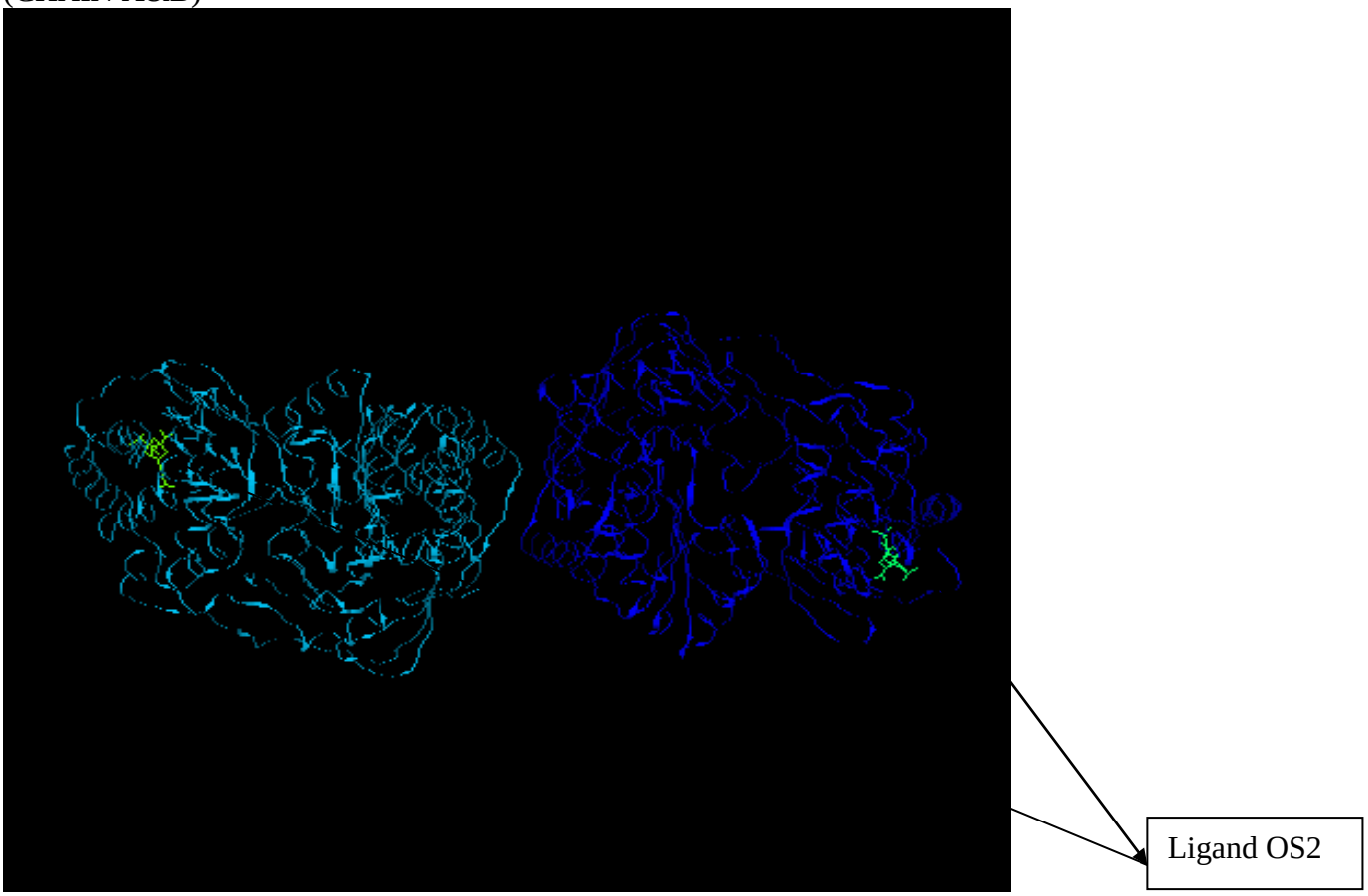
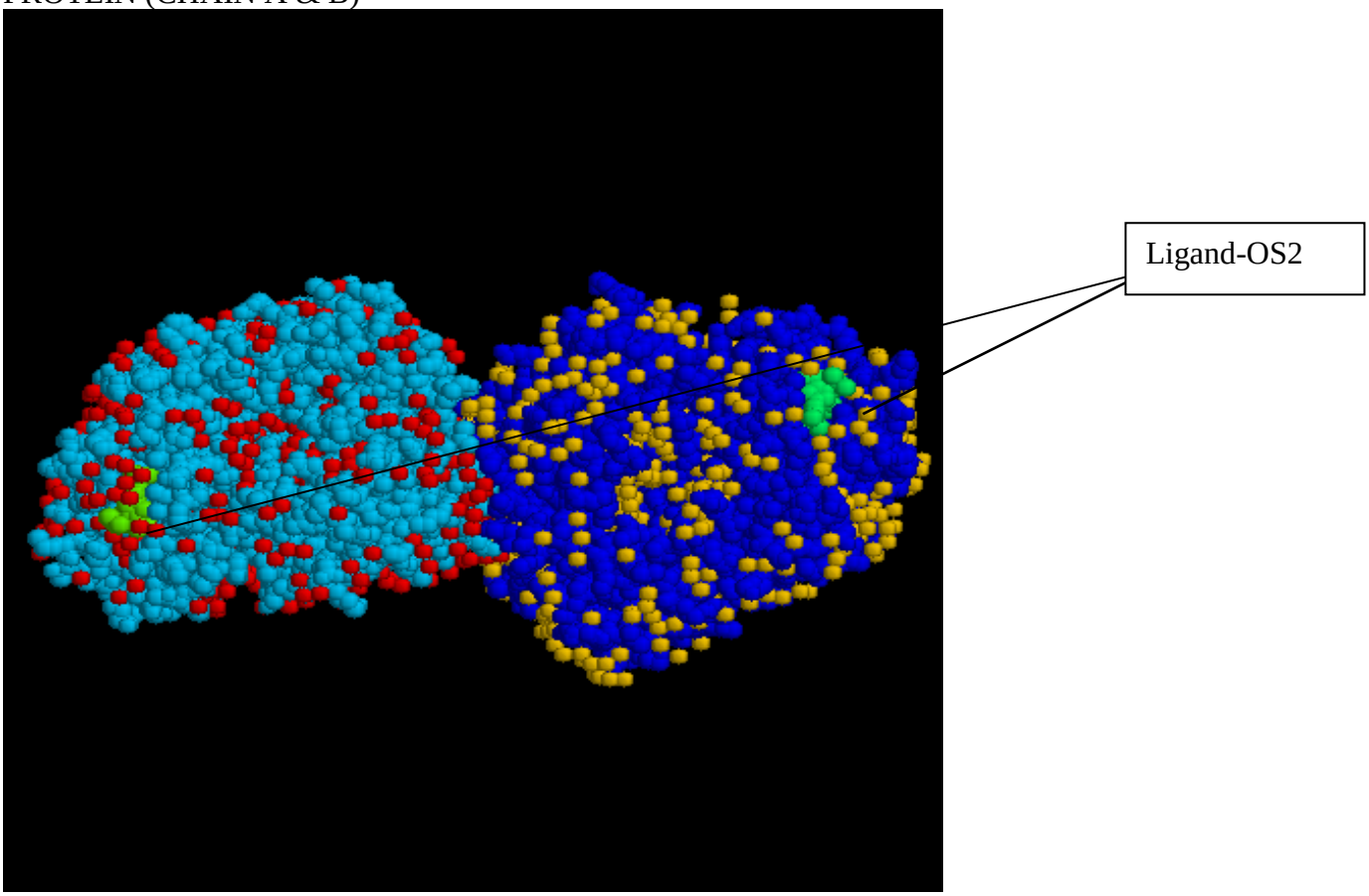


Figure 2a. SHOWING THE RIBBON-DISPLAY OF THE DOUBLE CHAINS- HCV NS5B PROTEIN (CHAIN A&B)



CHAIN A-CONTAINS SKYE GREEN COLORED ATOMS, OS2 LIGAND- YELLOW GREEN, CHAIN B- CONTAINS DEEP BLUE COLORED ATOMS, OS2 LIGAND- YELLOW GREEN.

Figure 2b. SHOWING THE BALL AND SOCKET DISPLAY OF THE DOUBLE CHAIN HCV NS5B PROTEIN (CHAIN A & B)



CHAIN A-CONTAINS SKYE BLUE AND RED COLORED ATOMS, OS2 LIGAND- YELLOW GREEN,CHAIN B-CONTAINS DEEP BLUE AND YELLOW COLORED ATOMS, OS2 LIGAND- SEAFOAM GREEN.

FIGURE 3a. SHOWING STICK DISPLAY OF CAV4EO6_0S2-5280934-1.PDB

(Z)-9 hexadecenoic acid, octadecanoic acid, eicosanoic acid, tetradecanoic acid, decanoic acid, dodecanoic acid, methyl salicylate acid, etc.

The essential oil molecules extracted from the Sesame plant were docked using iGEMDOCK software [31] with their respective docked scores, total binding energy, Van-der Waal energy, electrostatic and hydrogen bonding profiles are reflected in table 2.

The iGEMDOCK analysis for the different molecules revealed their docking scores and energies, which included the ligands such as Alpha-Linolenic acid/ALA (-102.2), Linoleic acid/LA (-94.8), Oleic acid/OA (-86.7), Stearic acid/SA (-85.7), Palmitoleic acid/POA (-82.0), Palmitic acid/PA (-79.7) among other essential oils analyzed. Thus, alpha-linolenic/ALA acid appeared to have the highest binding affinity score and ranked top among the different docked ligands as reflected in table 2 and figure 4. In addition, the RasMol image visualization for each of the top 6 ranked docked poses for each essential oil molecule is shown in figure 3a-f.

The iGEMDOCK –total binding energy score is compared to the pharmacological energy (E-pharma) for each extracted essential oil molecule as analyzed and reflected in table 3 and figure 5. Which include ligands viz: Alpha-Linolenic acid/ALA (-102.2/-103.4), Linoleic acid/LA (-94.8/-109.8), Oleic acid/OA (-86.7/-109.4), Stearic acid/SA (-85.7/-99.8), Palmitoleic acid/POA (-82.0/-126.1), Palmitic acid/PA (-79.7/-96.7).

Table 4 showed the result of the different amino acid residues involved in the various pharmacological bonding interactions between the different carboxylic acids ligands and the HCV NS5B RNA dependent RNA polymerase enzyme.

The bioactivity and druggableness of ALA as described and calculated using specific vital molecular descriptors. Such as molecular weight, polar surface area volume, hydrogen bond donor, hydrogen bond acceptor, LogP, polarizability, lipophilicity and lipophobicity are all summed up in the Lipinski rule of five as shown in table 5. The LogP is an estimate of the overall lipophilicity of a molecule, a factor that influences the behavior of such molecule in a wide range of biological membranes, hepatic clearance, selectivity and non-specific toxicity [42]. As such, based on the rule of five [43], ALA has a Lipinski's score of 2.

ADMET profile was evaluated using the admetSAR for predicted classification and regression values were calculated for a-linolenic acid/ALA (with the highest binding energy); by using different types of models such as blood-brain-barrier, human intestinal absorption, Caco2 permeability. They all showed positive values indicating that the ALA molecule passes all the tested models with no significant side effects on absorption as shown in table 6. In addition, various Cytochrome P450 (CYP) substrate and inhibitor models were calculated in the case of metabolism and the result showed that they are non-substrate and non-inhibitor except CYP450 1A2 Inhibitor. Moreover, for toxicity study, ALA was found to be Ames non-toxic and non-carcinogenic as reflected in table 6.

The finding of the sequence alignment of the NS5B protein with 100% identity is displayed in table 7. In addition, the PDBsum findings of the HCV NS5B 4EO6 protein-co-crystallized ligand (0S2) binding sites as analyzed are shown in table 8.

Discussion

Undoubtedly, humanity is increasingly exposed to evolving viral strains, bacterial multidrug resistance, undruggable targets, epidemics and accelerated lifestyle-associated risks, since the dawn of civilization. However, the demand for smart and robust technologies that are capable of handling the generated biological big data as par the 5 Vs': volume, velocity, variety, veracity and value, cannot be overemphasized [45].

FIGURE 3b. SHOWING STICK DISPLAY OF CAV4EO6_0S2-5280450-1.PDB

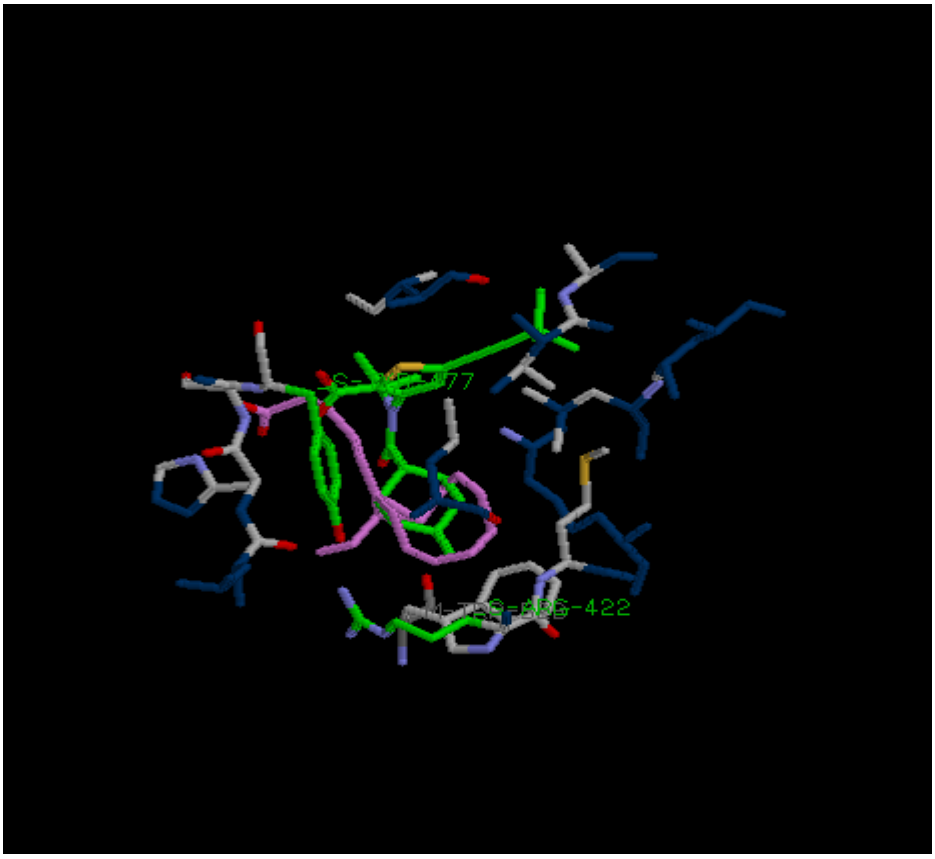


FIGURE 3c. SHOWING STICK DISPLAY OF CAV4EO6_0S2-445639-1.PDB

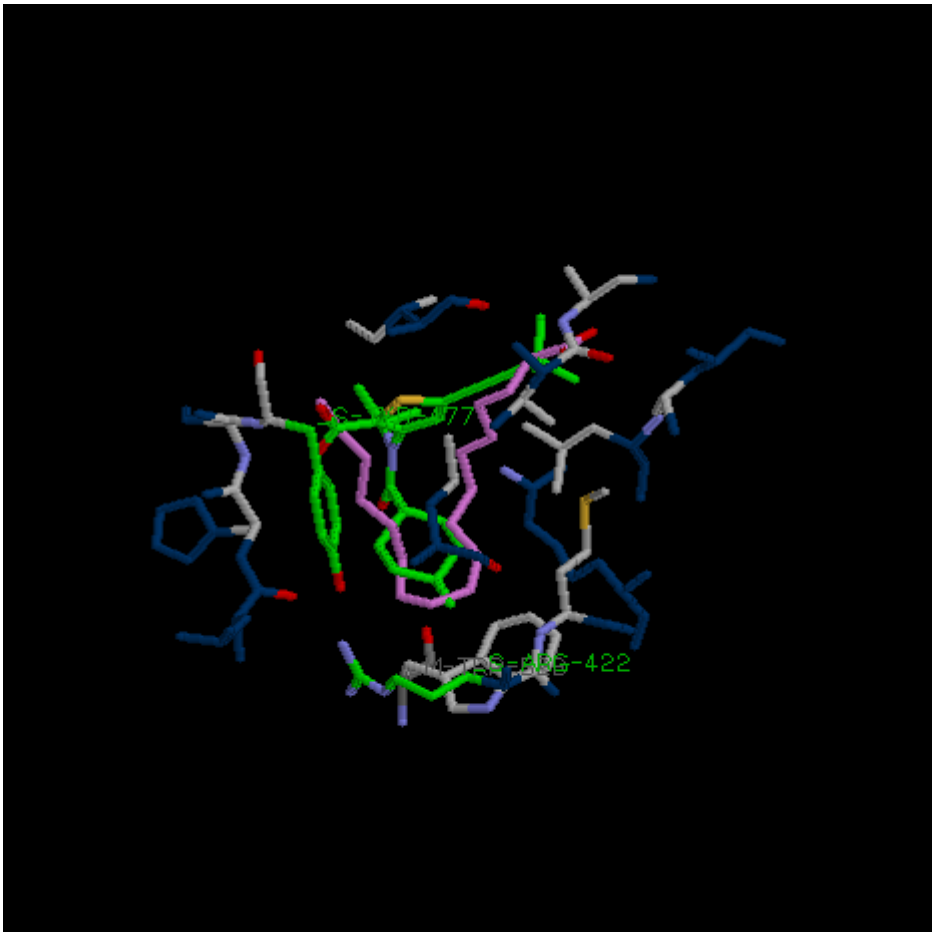


FIGURE 3d. SHOWING STICK DISPLAY OF CAV4EO6_0S2-5281-1.PDB

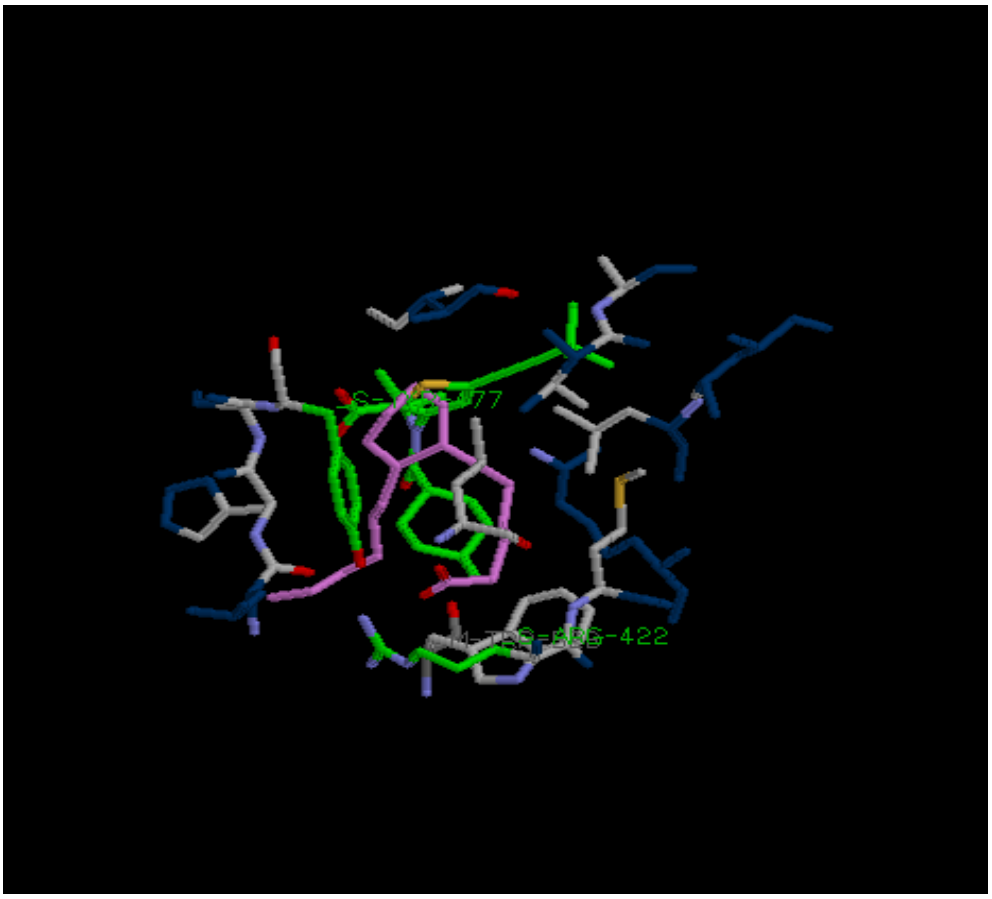


FIGURE 3e. SHOWING STICK DISPLAY OF CAV4EO6_0S2-445638-1.PDB

Thus, unlike the traditional wet laboratory system, molecular docking technique and other bioinformatics tools (important techniques in Computer-Aided Drug Design -CADD) operate in the dry laboratory system. CADD is an evolving area in structure-based drug design that has the computational power to screen million of chemical libraries in a matter of minutes; as such they are less labor-intensive, very convenient and efficient time-saving processes. In addition, molecular docking has served as one of the most critical objectives in structure-based drug design and molecular biology, which modeled and comprehend molecular interactions between protein and ligands [45]. Hence, having a CADD platform that is standalone, fast/sharp, effective, flexible, accurate, reproducible and multi-tasking are some of the critical factors that matter for high validity and reliability of obtained predictive values.

Molecular dynamic (MD) stimulation is combined with linear interaction energy approximation (LIE) in the present study. This is based on the assumption that free energy binding (ΔG) is linearly/directly proportional to changes in the van der Waals and electrostatic energy of the system, as previously proposed [32,46]. Which, has hitherto provided a good model for further prediction of absolute binding affinities and binding orientation of ligands as well [32].

Moreover, the iGEMDOCK v2.1 suite [31] was found to execute flexible docking for each ligand by generating protein-ligand interaction profiles of electrostatic (E), hydrogen-bonding (H), and Van der Waal's (V) interactions based on the LIE model. This is very interactive and has automatically integrated virtual screening (VS) environment with different phases from preparation through molecular docking and post-docking/screening analysis with pharmacological interactions studies between the different docked ligands and the protein. It also provides the interactive interfaces to prepare both the target protein (using the specified coordinates obtained from the PDB) and the screening ligands/compounds database carried out using the atom coordinates gotten from the compound library to infer the ligand binding site area, atom formal charge and atom types for each ligand studied [31, 46]. Furthermore, iGEMDOCK [31,47] infers the pharmacological interactions and clusters the screening compounds for the post-screening analysis based on these profiles and the screening compound structures. Finally, it re-ranks and visualizes the screening compounds by combining the pharmacological interactions and energy-based scoring function of GEMDOCK [31,46]. Besides, other studies have shown that iGEMDOCK has a better overall performance in docking simulations compared to other docking software [31,47,48]. Thus, the iGEMDOCK v2.1 suite [31,46] was adopted for the present in-silico studies.

The result of the GC-MS analysis done on sesame plant (black Sesame) were similar to what obtained in previous studies on sesame leaves [12,13,15,16]. These findings were also similar to that of a recent study that showed Sesame is very rich in carboxylic acids in a relatively balanced content, which includes saturated (SFA)-23.05%, monounsaturated (MUFA)-33.65% and polyunsaturated fatty acids (PUFA)-39.7% in the seed-oil [28]. Moreover, other study has shown that the PUFA content mainly the Linoleic acid/LA (n-6) and ALA (n-3) are relatively similar depending on the varieties of sesame being analyzed [23]. Henceforth, virtual screening and molecular docking were done by taking the 4EO6 as a target for HCV NS5B RNA-dependent RNA polymerase enzyme inhibitory activity against the various extracted natural essential oil compounds using the iGEMDOCK v2.1 suite [31,46].

Based on the result, Alpha- Linolenic acid (ALA) was found to be the top natural essential oil compounds with the highest binding energy affinity (ΔG score -most negative dock score) of -102.2 kcal/mol against NS5B RNA-directed

FIGURE 3f. SHOWING STICK DISPLAY OF CAV4EO6_0S2-985-1.PDB.

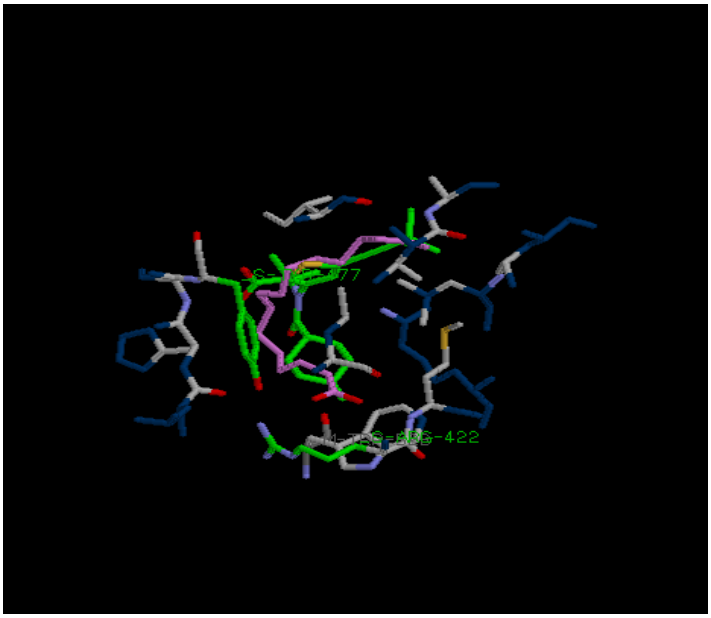
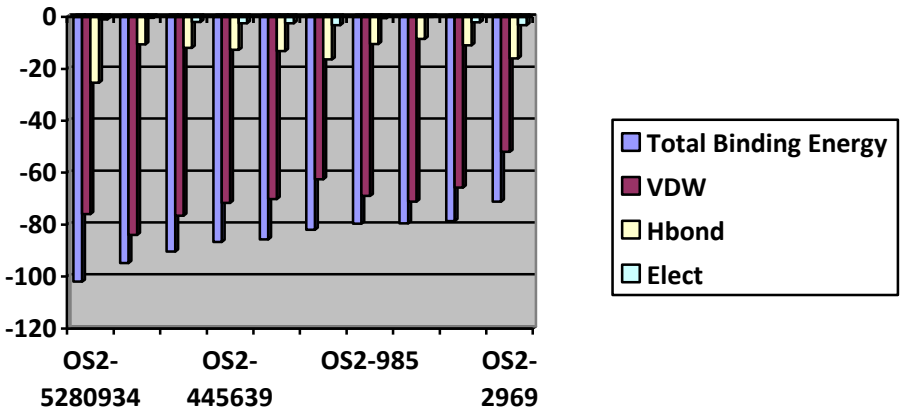
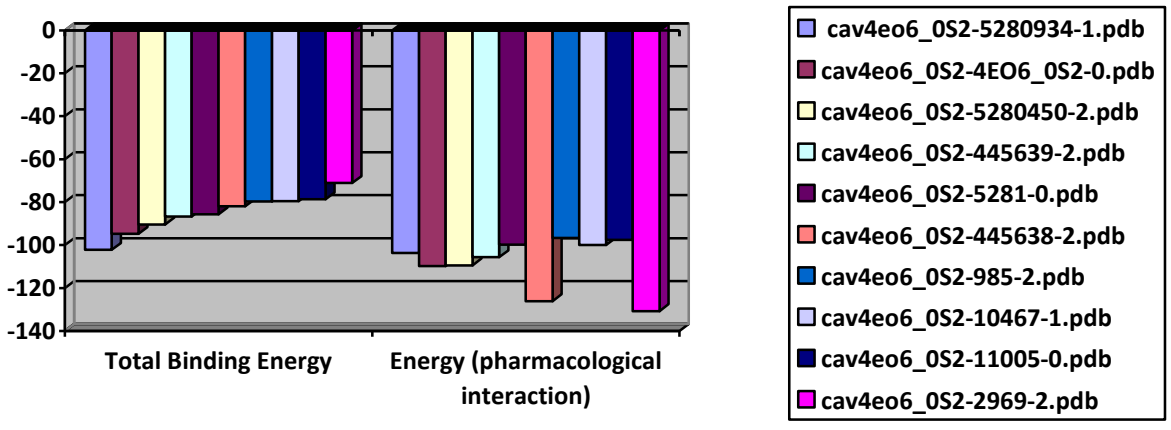


Figure 4. DIFFERENTIAL TOTAL ENERGY BINDING PROFILES



1 cav4eo6_0S2-5280934-1.pdb 2cav4eo6_0S2-4EO6_0S2-0.pdb 3 cav4eo6_0S2-5280450-2.pdb
4 cav4eo6_0S2-445639-2.pdb 5 cav4eo6_0S2-5281-0.pdb 6 cav4eo6_0S2-445638-2.pdb
7 cav4eo6_0S2-985-2.pdb 8 cav4eo6_0S2-10467-1.pdb 9 cav4eo6_0S2-11005-0.pdb
10 cav4eo6_0S2-2969-2.pdb

Figure 5. SHOWING THE TOTAL BINDING ENERGY COMPARED TO E-PHARMA



RNA polymerase. Which, implied that ALA as compared to other compounds fits precisely well into the active binding cavity/site of the NS5B receptor molecule by forming an energetically most stable drug-receptor complex with more number of H-bond interactions than the original co-crystallized ligand (0S2) in NS5B protein as reflected in table 2.

The total binding energy (∆G) and pharmacological interaction energy (E-pharma) of the protein-ligand (drug) interactions were considered in the present study as shown in table 3. This is based on the fact that hierarchical clustering and the profiling derived from the pharmacological scoring function (E-pharma) are different significantly from those derived from the energy-based scoring function (∆G) alone [47]. Hence, compounds with high pharmacological scores tend to be structurally and chemically similar to the active compounds, in describing how fit the natural essential oil binds into the binding pockets of HCV NS5B RNA polymerase. In addition, the E-pharma for each docked ligand was found to be higher than the ∆G, indicating that pharmacological interactions are more useful for identifying active compounds, which showed active biological interaction and binding of the HCV NS5B protein molecule. Thus, palmitoleic acid/POA was found to have the highest E-pharma of 126.1 kcal/mol of all the docked ligands, despite being ranked 5th based on the free binding energy profile as reflected in table 3.

Conversely, in another similar NS5B RNA-directed RNA polymerase study; Palmitic acid/PA (SFA) was found as the essential oil ligand with the highest ∆G score among the other docked ligands and may be attributed to its relatively higher percentage (about 23% PA) in the Acacia concinna pod [9,49]. Thus, in comparison, Alpha-Linolenic acid/ALA was found to have the highest ∆G score among the other docked essential oil ligands. This may be due to PUFA (including Linoleic acid/LA) found to have a relatively higher percentage (of about 37.4%) among the fatty acid contents in Sesame [28]. Also, ALA content in the Sesame leaves tends to be more than that in the seeds [Shittu, unpublished work]. More so, PUFA contents in sesame plant are relatively similar depending on the cultivated varieties [23].

In addition, the major fatty acids in sesame seeds such as Linoleic acid methyl ester/LA (37.40%)-PUFA and oleic acid methyl ester/ OA-MUFA (29.09%) [28], were found to have similar major impact on the HCV NS5B RNA dependent RNA polymerase enzyme inhibitory activity, ranked 2nd and 3rd molecules respectively as seen in table 2. Moreover, a recent study has revealed that intake of unsaturated fats like oleic acid (richly available in Sesame plant) is more beneficial to human health, in that it allows for better flexibility and fluidity of the cell membrane functional structures, especially the endoplasmic reticulum (ER) components; thereby, prompting their proper functioning such as easy transport of substances in and out of the ER compared to the stiff, solid-like patches obtained in the palmitic acid/PA (SFA) treated-ER membrane [50, 51]. In addition, Oleate/OA supplementation was found to reverse palmitate/PA-induced ER stress and lipotoxicity in the ER of primary hepatocytes and the H4IIEC3 hepatoma cell line [51]. Although, palmitate is reported to be the most abundant fatty acid found in the circulation [52] that helps to promotes phospholipid synthesis and its accumulation in the ER [51,53].

Based on pharmacological interactions, the amino acid residues Arg-422 (1.0), Met-233 (0.52) and Ser-476(0.22) with high hydrogen-bonding preferences (≥ 0.5) interact with the structural water to form the hydrogen bonding network. This is essential for the NS5B receptor inhibitory activities induced by natural essential oil inhibitors as shown in table 4. In addition, hydrophobic interacting residues include Leu-491 (0.48), Arg-422(0.64), His-475(0.39), His-

475(0.48), Try-477(0.45), Trp-528(0.35), Trp-528(1.0), with high vdW interaction preferences (≥ 0.5) making contact with the side or main carbon chain scaffolds of the active compounds. These residues also contributed to the major vdW interactions for the ligand binding of NS5B RNA polymerase receptor. Thus, ALA formed H-bonding with Arg-422 and hydrophobic bonding with residues Arg-422, His-475, Try-477, Trp-528, and Trp-528.

Based on the results, the ADMET properties predicted showed that ALA is a readily bio-degradable, druggable molecule with no significant side effects on absorption, metabolism and toxicity in the body and thus, fit for human consumption. This further confirms the beneficial roles of carboxylic acids especially the omega-6 and omega-3 played in maintaining the integrity of the liver cells in general.

To the best of our knowledge, after extensive and thorough literature search, this is the first in-silico studies done on the natural essential oil compounds of Sesame species against HCV NS5B polymerase-protein.

Conclusion

No doubt, protein-ligand interaction plays a significant role in structure-based drug designing. As such, the use of iGEMDOCK tool has demonstrated its efficacy in the present study in successfully elucidating the novel inhibitory anti-viral (hepatitis) agents from Sesame plant.

Based on the present study, Apha-Linolenic acid/ALA (-102.2) is considered the molecule with the highest binding energy/score and to be a very effective natural potential inhibitor of HCV NS5B RNA dependent RNA polymerase with no significant side effects. Moreover, the study reflects the importance of volatile natural essential oil compounds extracted from Sesame plant and further confirms their folkloric uses as natural anti-viral agent as demonstrated.

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