In-silico screening of lung cancer inhibiting potential of the chemical Constituents of n-hexane extract of *Elaisiguineenses*

Abstract

Palm oil is an edible vegetable oil that is rich in saturated fat, it is derived from the mesocarp of the oil palm fruit especially the African oil palm fruit (*Elaisiguineenses),* this study investigates the possible inhibition of lung cancer cells using the bioactive constituents of *Elaisiguineenses.* The chemical constituents of the oil were extracted in n-hexane, analyzed with Fourier transform infrared spectrometer (FTIR) and gas chromatography-mass spectrometry (GC–MS) studies. Absorption, distribution, metabolism and elimination and toxicity (ADMET) properties were predicted using ADMETSAR online softwarewhile the molecular docking was achieved using the AutodockVina found in PYRX software. The ADMET properties of the constituents showed that theyare good drug leads for lung cancer treatment. The docking result revealed that1,4,10,13-tetraoxa-7,16- dithiacyclooctadecane gave a good binding affinity (-5.8 kcal mol^{-1})that is close to that of the control (Osimertinib) (-7.8 k cal mol^{-1}). The obtained results showed that palm oilmay be a promising lead candidate for the treatment of lung cancer.

Keywords: lung cancer, *Elaisiguineenses,* osimertinib, molecular docking, PYRX,

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1. Introduction

“Cancer is one of the most deadly diseases known in developing African countries especially Nigeria” [1-2]. “Cancer has limited chances for curing it, especially when it goes beyond the early stage. It occurs as a result of biochemical alteration in the genetic and epigenetic mechanisms while targeting many tracts in growth and malignant progression towards incurable lethal disease” [3]. Most cancer treatment methods especially chemotherapeutic agents offer some level of toxicity to the patient showing severe side effects. Natural anticancer products have proven to be reliable when it comes to the management of the disease [4-6]. Lung cancer is one of the major life threatening diseases in Nigeria today; this may be attributed to the careless life style of people living in this area. Majority of the young men

smoke tobacco and hard drugs. Molecular docking is a method used to model the interaction between natural compounds and target protein [7-9], the binding site area around the amino acid residues plays an important role in binding with drugs or ligands [10]. "Molecular docking correlates the computational and experimental approaches and has been of great assistance in the identification and development of promising and novel compounds" [11-12]. West AfricaPalm oil (*Elaisiguineenses*)is one of the most beneficial food products in the world today. It is made from the fruit of the African oil palm.It is used for preparing meals and also as an ingredient in ice cream, margarine, and many other food products. It is also used traditionally for the control of rheumatism headaches, pains, arterial thrombosis, cancer, cardiovascular diseases and an atherosclerosis because of the very rich phytonutrients it contains [13-14]. "The biomass extracted from palm oil industries which include oil palm frond, oil palm leaves, oil palm trunks, mesocarp fibers,empty fruit bunches, palm kernel shells, and palm oil mill are poorly utilized" [15-16].This work tries to identify some natural lung cancer inhibitors from palm oil through molecular docking method.

2.0 Materials and Methods

2.1 **Collection of plant Materials**

Fresh palm fruits were harvested from a local village in Imo State Nigeria and were identified by Prof. Martins Mbagwu of the Plant Science and Biotechnology department Imo State University, Owerri. After proper identification and authentication, the fruits were ground with care so that the hardnut does not crack. The nut was separated from the fiber and the fiber was subsequently dipped in hexane and allowed to stand for 24 hours after which the oil was filtered and stored in an air tight container for further use [17].

2.2 **FTIR Analysis**

Fourier transform infrared spectroscopy (FTIR) analysis was conducted on the extract to identify the functional groups therein. The analysis was carried out at a frequency range of 4000-400 cm^{-1}. The extracted oil was encapsulated in 200 mg of KBr salt pellet, using a mortar and pestle, compressed into a thin pellet, the sample scans was set at 30 to calibrate it. Resolution was set to 8.2 and the Background Scans was set at [18].

2.3 Gas-Mass Chromatography Analysis

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The extracted oil sample was subjected to GC-MS experiment usingan Agilent technology gas-mass chromatography instrument (model: 19091S-433UI) USA. The conditions for the gas chromatography part were set with the parameter as follows: A HP-5ms capillary standard nonpolar column Ultra Inert 0 °C—325 °C (350 °C): 30 m x 250 µm x 0.25 µm was used. The temperature range was 50 – 325℃, held at 50 ℃ for 2 min, the rate was 5 ℃/min and at 180 ℃ for 0 minutes increased to 20 ℃/min at 270 ℃ for 5 min, the carrier gas used was helium at a rate of 1 mL per minute. The results obtained were analyzed by comparing with the spectra program imbibed in the National Institute of Standards and Technology (NIST) mass spectral library [19].

2.4 Ligands preparation and identification

"The three dimension (3D) structures of the compounds identified in the GC-MS experiment were downloaded from the pubchem online database. The obtained compounds were minimized at PyRx virtual screening tool with the aid of universal force field with 200 steps and changed to AutoDock ligands and used for the molecular docking procedures" [20].

2.5. Absorption, distribution, metabolism and elimination and toxicity (ADMET) screening

The identified compounds were submitted to ADMETsar2 server for drug-like properties, pharmacokinetics, and pharmacodynamics parameter examination [21].

2.6. Identification and preparation of molecular targets

The three dimension (3D) structure of epidermal growth factor receptor protein targetwith PDB ID: 4zau was downloaded from protein data bank (PDB database). The protein was prepared using discovery studio where the interfering crystallographic water molecules and co-crystallized ligand were removed; the active site was discovered using pymol software. Further preparation was done by addition of polar hydrogen. The prepared protein was saved as protein data bank file and used for the molecular docking analysis.

2.7 Docking Analysis

Docking studies of the compounds on the prepared protein was achieved with the Autodockvina imbibed in PYRX software. Depending on several scoring functions, the PYRX software allowsone to virtually screen a library of compounds and detects the strongest binders. The ligand binding site of Osimertinib was chosen to be the active site of the 4zau protein. The results from this study showed that all the docked compounds bind either at the active site or very close to the site

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[22].The amino acid residues involved in covalent interaction around the active site of the chosen protein are Leu718, Val 726, Ala 743, Rhu 723, Thr 790, Pro 794 and Cys 797. The docked results were subsequently visualized in discovery studies for further analysis.

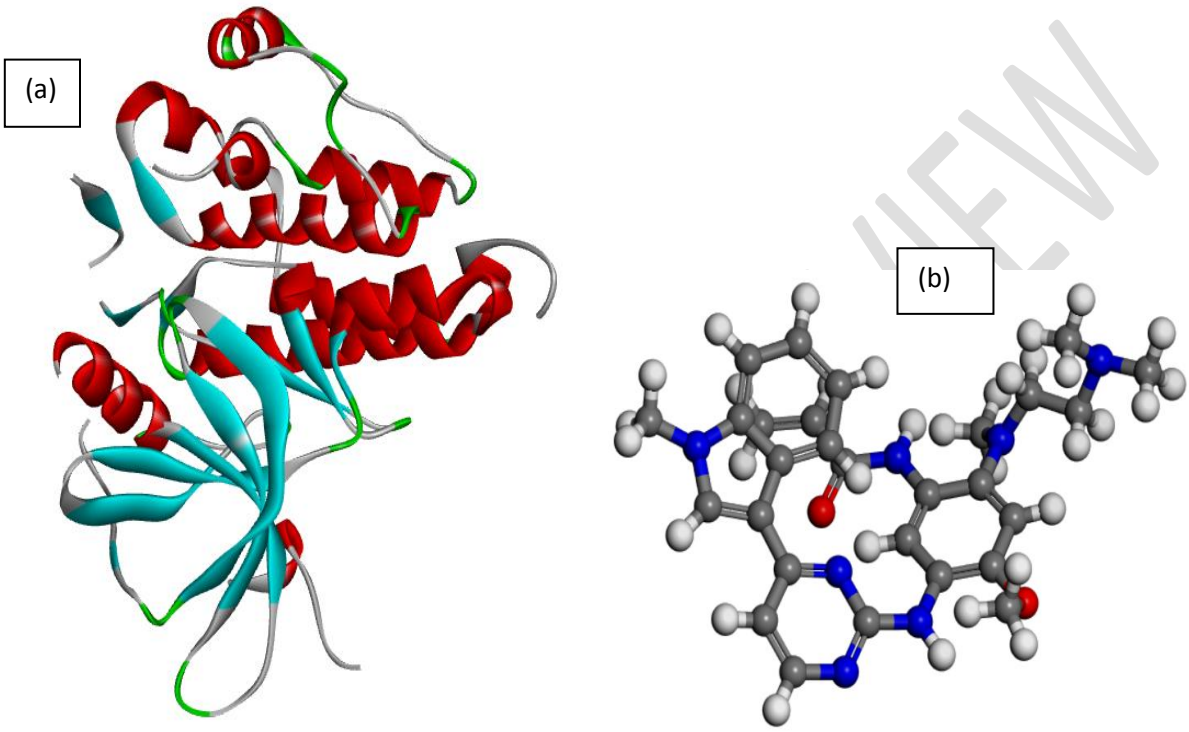

Figure 1: Three dimension (3D) view of (a) 4zau protein and (b) Osimertinib(Atom legend: white = H; gray = C; red = O; blue = N.)

3.0 Results

3.1 FTIR Result

Fourier transform infrared spectroscopy (FTIR) analysis was used to identify the functional groups present in the Hexane extract *Elaisiguineenses.*The presence of some functional groups was observed. The major functional groups are summarized in Table 1 while the spectrum is shown in Figure 2. The functional groups observed is similar to those reported elsewhere to enhance drug activity [23].

Table 1 Major functional groups found in n-Hexane extract of *Elaisiguineenses*

Absorption peak (cm^{-1})	Functional group	Appearance
3008.0	O-H of carboxylic acid	Weak Broad
2922.2	Asymmetric sp^3 C-H	Strong sharp
2855.1	Symmetric Sp3 C-H	H Strong sharp
1744.4, 1710.8	$C = O$	Strong sharp
1481.1	$C - H$	Sharp

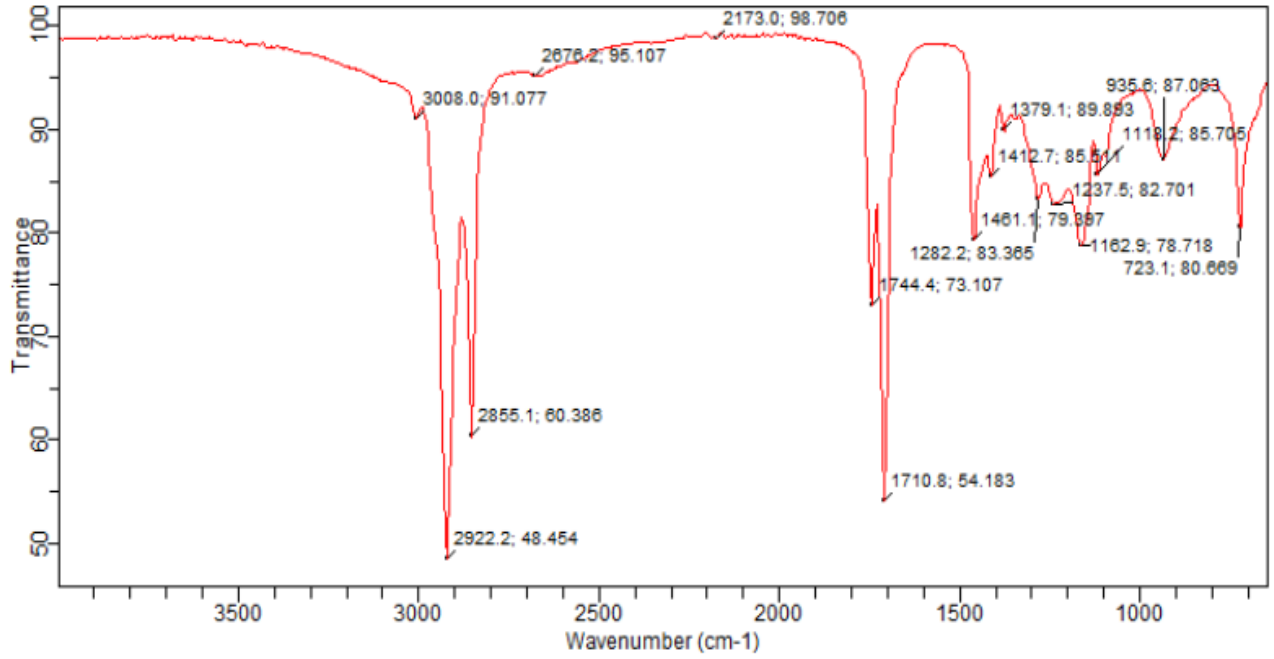

Figure 2. FTIR spectra of the n-hexane extract of *Elaisiguineenses*

3.2 GC-MS Result

The Hexane extracted oil was injected into an AgilantGas chromatography-mass spectrophotometer (model 19091S-433UI) for examination and analysis. The chromatogram is presented in Figure 3 while Table 2gives the compounds identified from the GC-MS analysis.**N-(2-{[2-**(dimethylamino)ethyl](methyl)amino}-4-methoxy-5-{[4-(1-methyl-1H-indol-3-yl)pyrimidin-2-yl]amino}phenyl)prop-2-enamide (**Osimertinib)**with Pubchem Id

71496458, molecular formula C_{28} H_{33} N_7 O_2 and weight 499.6 g/mol was used ascontrol drug toidentify compounds with good binding affinities for the studied cancer protein.

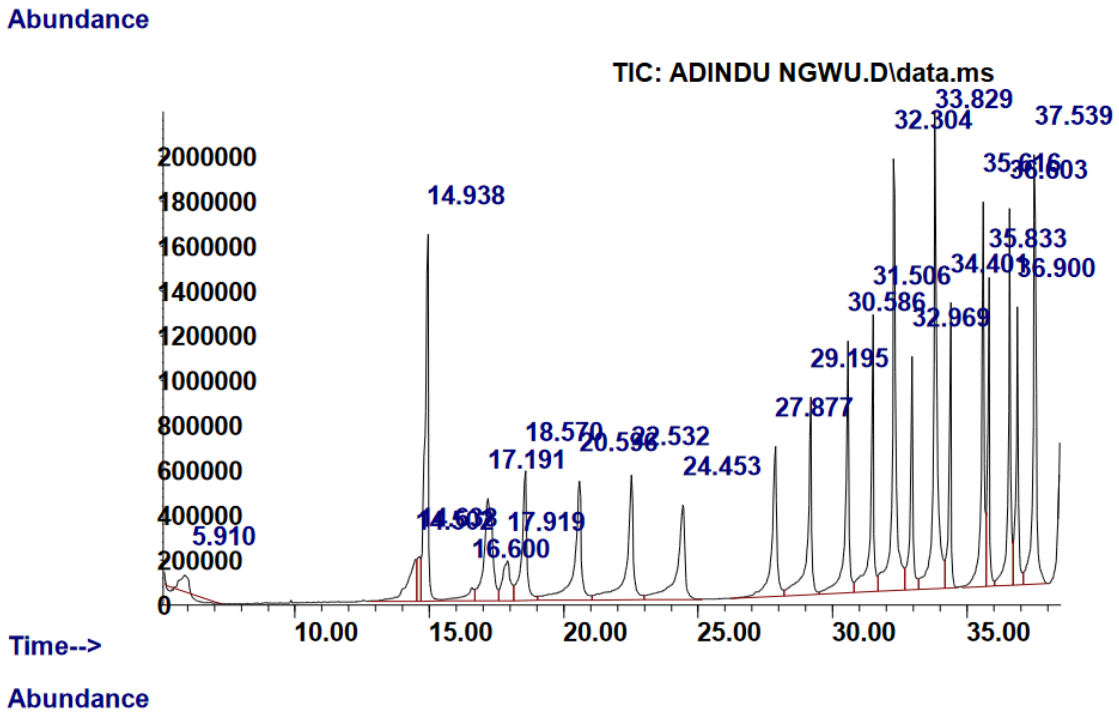

Figure 3: Chromatogram of the n-hexane extract of *Elaisiguineenses*

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Table 2 Compounds identified in the hexane extract of *Elaisiguineenses* oil

S. No.	Compound	Area Peak	Pubchem Id	Structure	Molecular formula	Molar weight (g/mol)
1.	cis-Vaccenic acid	28.5039	5282761		$C_{18}H_{34}O_2$	282.50
2.	13-Octadecenal, (Z)-	3.1848	5364497		$C_{18}H_{34}O$	266.5 0
3.	Palmiticacid	6.6054	985		$C_{16}H_{32}O_2$	256.42
5	Oleic Acid	27.5039	445639		$C_{18}H_{34}O_2$	282.5 0
6	trans-13-Octadecenoic acid	4.1679	6161490		$C_{18}H_{34}O_2$	282.50
7	9-Octadecenal, (Z)-	4.8017	5364492		$C_{18}H_{34}O$	266.50
8	Hexadecenoic acid, Z-11-	8.983	5312414		$C_{16}H_{30}O_2$	254.41
9	1,4,10,13-tetraoxa-7,16-dithiacyclooctadecane	9.0417	675277		$C_{12}H_{24}O_4S_2$	296.5
10	Z-2-Octadecen-1-ol	7.2167	5365011		$C_{18}H_{36}O$	268.5

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3.3. ADMET behavior

The Absorption, distribution, metabolism and elimination and toxicity prediction which show their pharmacokinetics and pharmacodynamics properties are summarized in Table 3. pfizer's rule of five by Lipinksi [24] was used to evaluate the drug-likeness of the identified compounds. The rule helps to determine if a compound with given pharmacological activity has chemical and physical

properties that would likely make it an orally active drug in humans. In compliance to the rule, none of the compound has more than 5 hydrogen bond donors, none has more than 10 hydrogen bond acceptors, Table 2 showed that all have molecular massesof(< 500),According to Lipinski, an orally active drug should not violate more than one of the rules. The result revealed that all the compounds studiedwere in compliance with the rule,the higher human intestinal absorption (HIA) results indicated that the compounds may be better consumed from the gastrointestinal tract upon oral administration, none of the compounds was found to be carcinogenic. With the observed results, the compounds may be classified as good drug candidates.

Table 3. ADMET characteristics of the docked compounds

S/NO.	Compound	HIA	AOT	BBB	WS	H-bond acceptor	H-bond donor	Rotatable bonds	Carcinogenicity
1.	Osimertinib	+	2.893	-	-3.549	8	2	10	-
2.	cis-Vaccenic acid	+	1.238	+	-4.04	1	1	15	-
3.	13-Octadecenal, (Z)-	+	1.535	+	-2.949	1	0	15	-
4.	Palmitic acid	+	1.16	+	-3.502	1	1	14	-
5.	Oleic Acid	+	1.246	+	-4.04	1	1	15	-
6.	trans-13-Octadecenoic acid	+	1.228	+	-3.791	1	1	15	-
7.	9-Octadecenal, (Z)-	+	1.228	+	-3.791	1	1	15	-
8.	Hexadecenoic acid, Z-11-	+	1.268	+	-3.791	1	1	13	-
9.	1,4,10,13-tetraoxa-7,16-	+	1.853	+	-1.002	6	0	0	-

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	dithiacyclooctadecane								
10	Z-2-Octadecen-1-ol	+	1.297	+	-2.349	1	1	15	-

HIA=Human intestine Absorption, AOT=Acute oral Toxicity, BBB=Blood Brain Barrier, WS=Water Solubility

3.4 Molecular docking result

Molecular docking was performed onepidermal growth factor receptor lung cancer protein (4zau) using the identified compounds and a control (**Osimertinib**). The 3D and 2D protein–ligand interactions of the compounds are presented in Figure 4 while the binding scores,forces of interaction and amino acid residues involvedin the interactions with the compounds and the control drug Osimertinib are shown in Table 4. The main forces involved in the interactions were Van der Waals, conventional hydrogen bond, alkyl, unfavorable acceptor-acceptor. The results from the docking studies showed that all the compounds either bound exactly at active site or close to the active site and their binding scores were equally close to that of the control. 1,4,10,13-tetraoxa-7,16- dithiacyclooctadecanegave the closest binding score (-5.8 kcal mol^{-1})to the control.

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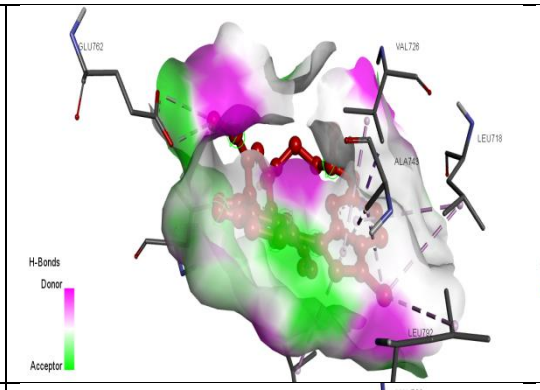
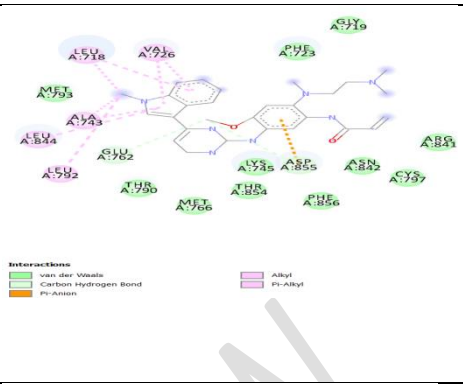
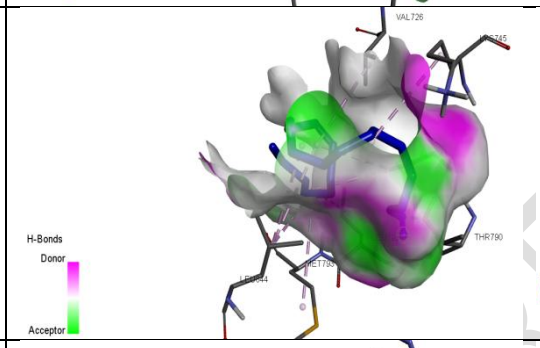
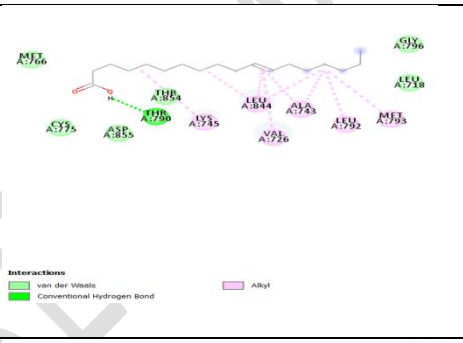
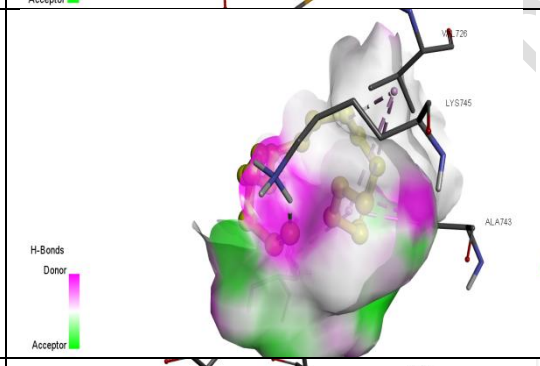
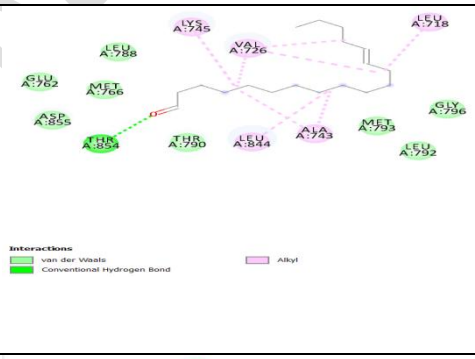
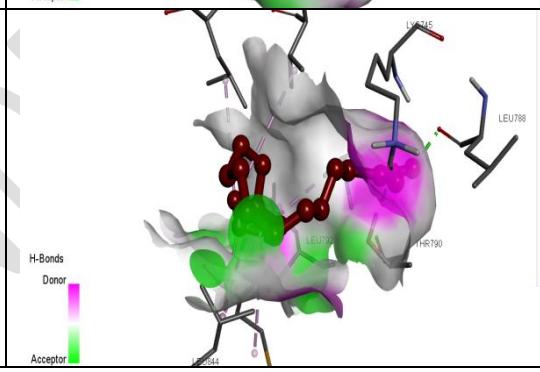
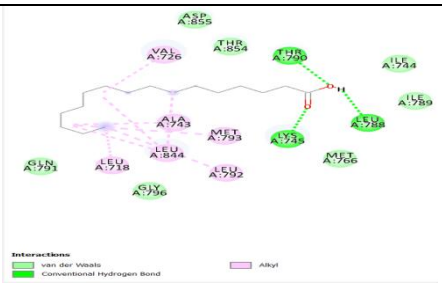
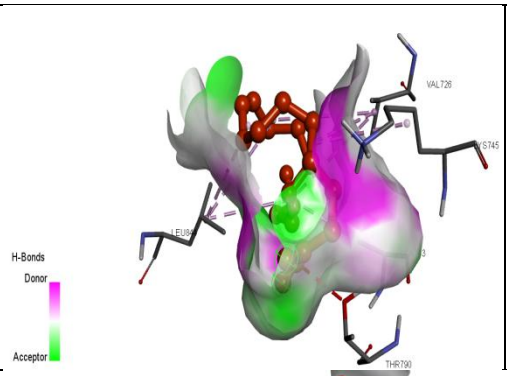
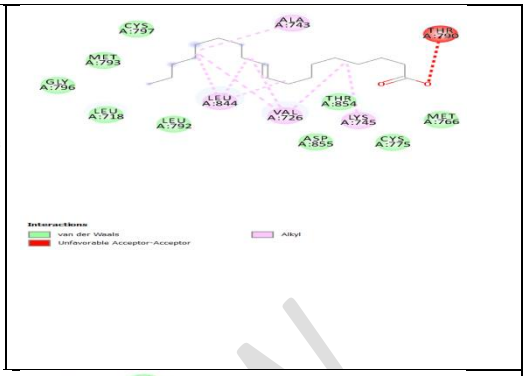
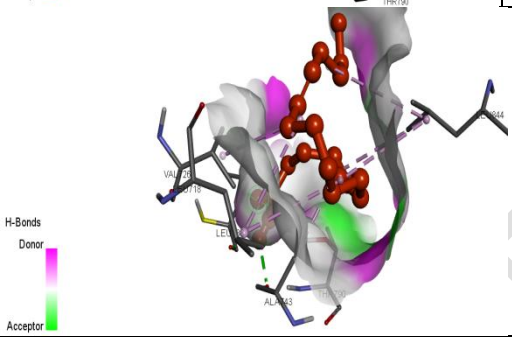
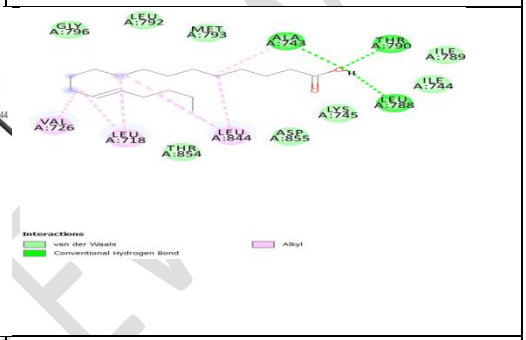
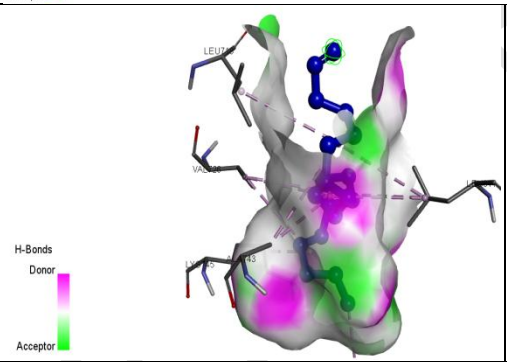
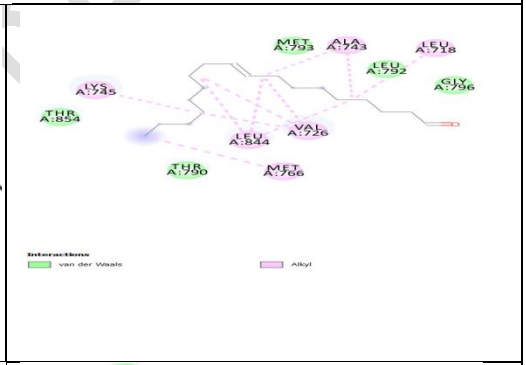
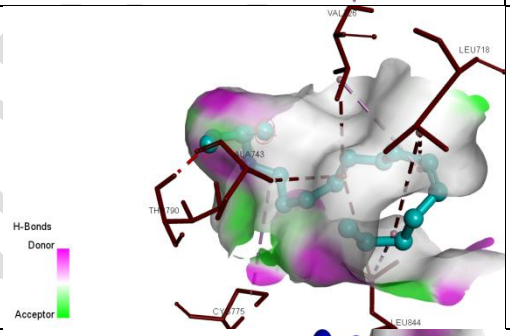
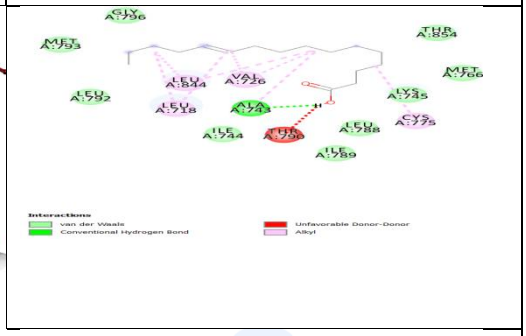
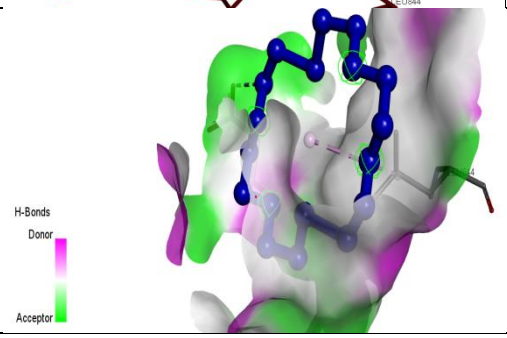
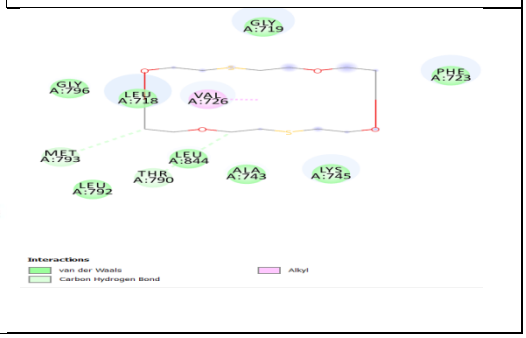
	Compound	3D Molecular interactions (a)	2D Molecular interaction (b)

1.	**Osimertinib**		
2.	cis-Vaccenic acid		
3	13-Octadecenal, (Z)-		
4	Palmitic acid		

5	Oleic Acid		
6	trans-13-Octadecenoic acid		
7	9-Octadecenal, (Z)-		
8	Hexadecenoic acid, Z-11-		
9	1,4,10,13-tetraoxa-7,16-dithiacyclooctadecane		

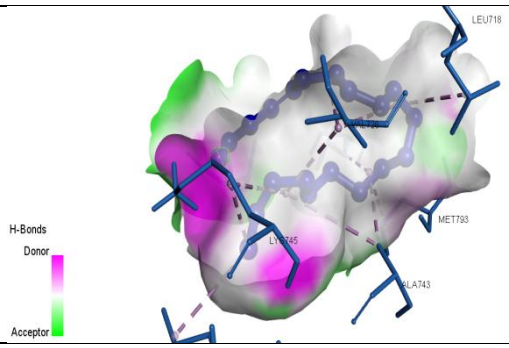
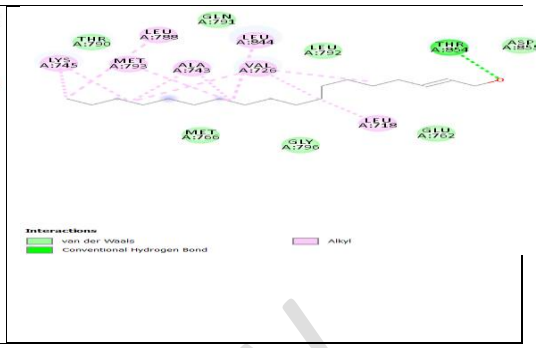
10	Z-2-Octadecen-1-ol		

Figure 4 Thethree and two dimension protein–ligand interactions

Table 4.Binding affinity, forces of interactions and amino acid residues involved in the docking studies

S. No.	Compound	Pubchem ID	BA	Types of interaction	amino acids involved
1.	**Osimertinib**	71496458	-7.6	Van der Waals, carbon hydrogen, pi-anion , pi-sigma, alkyl , pi-alkyl.	Leu 718,Val 726, Ala 743, Glu 762, Leu 792, Leu 844 Asp 855
2.	cis-Vaccenic acid	5282761	-5.1	Van der Waals, Conventional hydrogen bond, alkyl.	Val 726, Ala 743, Lys 745, Thr 790, Leu 792, Met 793, Thu 844,
3.	13-Octadecenal, (Z)-	5364497	-4.6	Van der Waals, Conventional hydrogen bond, alkyl.	Leu 718, Val 726, Ala 743, Lys 745, Leu 844, Thr 854
4.	n-Hexadecanoic acid (Palmitic acid)	985	-4.9	Van der Waals, Conventional hydrogen bond, alkyl.	Leu 718, Val 726, Ala 743 Lys 745, Leu 788, Thr 790, Leu 792, Leu 844, Met 793
5	Oleic Acid	445639	-5.0	Van der Waals,	Val 726, Ala 743, Lys 745, Thr 790,

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				unfavorable acceptor-acceptor	Leu 844, Thr 854
6	trans-13-Octadecenoic acid	6161490	-5.3	Van der Waals, Conventional hydrogen bond, alkyl	Leu 718,Val 726, Ala 743, Leu 788, Thr 790, Leu 844
7	9-Octadecenal, (Z)-	5364492	-4.2	Van der Waals, alkyl	Leu 718, Val 726, Ala 743, Lys 745, Leu 792, Met 766, Leu 844
8	Hexadecenoic acid, Z-11-	5312414	-5.4	Van der Waals, Conventional hydrogen bond, alkyl, unfavorable acceptor-acceptor	Leu 718, Val 726, Ala 743, Cys 775, Thr 790, Leu 844, Lys 745
9	1,4,10,13-tetraoxa-7,16-dithiacyclooctadecane	67527	-5.8	Van der Waals, Conventional hydrogen bond, alkyl	Leu 718, Val 726, Met 793, Leu 844
10	Z-2-Octadecen-1-ol	5365011	-4.7	Van der Waals, Conventional hydrogen bond, alkyl	Leu 718, Val 726, Ala743, Lys793, Leu 788, Thr 854, Leu 844, Met 793

BA= Binding Affinity in ($kcalmol^{-1}$)

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Conclusion

The chemical constituents of *Elaisiguineenses* wereextracted in n-hexane, analyzed with FTIR and GC-MS. The FTIR result showed that the compound contained some functional groups which had been associated with drug properties and the GC–MS analysis revealed that it contained many compounds with known biological and therapeutic activities. ADMET properties showed that the constituents have good pharmacological and pharmacodynamics properties in the human system. The molecular docking study of the identified compound against the epidermal growth factor receptor lung cancer protein showed that the constituents of the African palm oil (*Elaisiguineenses*)were good candidates in the inhibition of the activity of thelung cancer protein.

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