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Anticancer Docking Study Of Selected Compound Of Clove

Dawen D. E[1]. C. A. Amienyo[2], O.B. Okechalu[3], Asogwa N.T[4].
Department Of Plant Science And Biotechnology, University Of Jos [123]
Central Research Laboratory, Ilorin, Kwara State, Nigeria

Abstract
This study was designed to identify the phytochemicals from ethanolic extract of Syzygiumaromaticum(clove) using Gas Chromatography–Mass Spectrometery (GC-MS) analysis against cancer. Various anticancer medications have been discovered due to advances in the health care industry though they have adverse side-effects. Anticancer drugs derived from natural sources have low side effects, making them good for cancer therapy. This study aimed at identifying phytocompounds which were subjected to molecular docking to find the effects of clove buds on cancer (tumor cells) as a potential anticancer agent by using crystal structures of B-Raf (BRAF), epidermal growth factor receptor (EGFR), macrophage colony-stimulating factor (MCSF), mammalian target of rapamycin (mTOR) with PDB IDs 2FB8, 2J6M, 3BEA, 4DRL, 3FRA 1AD4, 4URM and 1A7T respectively obtained from protein dtabank (www.rcsb.org).
Therefore clove bud extract has potential to act as anticancer medication.
Key: *Syzygiumarmaticum, GC-MS, Molecular docking, anticancer*

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I. Introduction

Components of medicinal plants are known to possess bioactive compounds that can be used to treat specific diseases. The functional parts of plants- leaves, fruits, flowers, seeds, rhizomes, stems, barks, buds and sap [1]. *Syzygium armaticum* (clove) have been utilized for ages as spice [2] substantially, clove consist of essential oil (10-20%, stems (5-10%) and leaves [3]. The essential oil of clove has high quality as a result of its yield and eugenol content of 80 – 90% hence, cloves have diverse benefits and have proven effective as additives in food and beverages having high nutritional value, antimicrobial, anti-inflammatory, antiproliferative, antifibrogenic, insecticidal and analgesic properties [4]. Its high eugenol concentration helps it to have significant antioxidant activity [4].

Eugenol and caryophyllene are the major phytochemicals in clove oil. The former has proven anticancer activities against colon, stomach, breast, prostrate, melanoma and leukemia cancers while the latter has anticancer effects on cervical, lymphatic cutanoeus and pancreatic malignancies [5].

The possible contender for future development as aid to current chemotherapeutic therapies is eugenol which is known to inhibit growth and development of tumors, induces apoptosis, increase reactive oxygen species having genotoxic effects on cancer cells [5] Eugenol can be used as adjuvant therapy for individuals undergoing chemotherapy, a combination known to decrease toxicity and increase efficacy [6]. The IUPAC name of eugenol is 4-ally1-2-methoxyphenol with the chemical formular C_{10} H_{12} 0_2. The percentage eugenol oil in clove ranges from 70% - 96% [7]. Advancement in medical field has enabled discoveries of various anticancer medications whose primary objective is to selectively destroy cancer cells without interfering with normal cells [7]. Plants containing anticancer properties are strongly connected with decreased risk of cancer, which was revealed in recent studies [4]. Consequently, there are scarce reports on cloves as anticancer agent therefore, this study is designed to evaluate the anticancer properties of clove extract using AutoDockVina method which is easy, precise and straight forward.

Evaluation of the efficacy of conventional treatments requires scientific investigations such as in toxicology, pharmacology, identification and isolation of active chemical compounds present in plants. Thus, this study intends to establish the anticancer efficacy of ethanolic clove buds extract using molecular docking.

Molecular docking approaches are used in modern drug design to understand interaction between drug–receptor [8]. Computational techniques help and strongly support the design of novel, more potent inhibitors by revealing the mechanism of interaction of drug-receptor. The essence of ligand-protein docking is to predict the predominant binding model ligand with a protein of three-dimensional structure [9].

Thus, this study intends to establish the anticancer efficacy of clove buds extract using molecular docking, which would be a future explorer for designing a novel drug and as possible therapeutic intervention for cancer.

II. Materials And Methods

Collection and identification of plant material

Buds of *Syzygiumarmaticum* (clove) were obtained from FarinGada (Vegetable Market) Jos, Nigeria. The sample was obtained in a sterile polytene bag which was dipped in 5% hypochlorite. It was conveyed to the Department of Plant Science and Biotechnology, University of Jos, Nigeria where it was identified and given a voucher number UJH000319. The sample was washed under running sterile distilled water, air-dried at room temperature for 2 weeksuntil completely dried. The cloves were pulverized and stored in an air-tight sterile container, at refrigeration temperature until required for use.

Preparation of Ethanolic Clove Extract

The pulverized sample weighed (200g) and placed in a sterile conical flask. 70%ethanol and 30% sterile water were dispensed into the pulverized clove and loaded in Soxhlet extractor for extraction. After extraction, the extract was evaporated in a water bath set at 60^0C leaving, pure extract which was stored in aseptic container until required for use.

Gas Chromatography – Mass Spectrometry (GC-MS) Analysis

GC – MS of ethanolic extract of *Syzygiumarmaticum* was performed using GC – MS Agilant 6890 gas chromatograph equipped with an on-column automatic injector, flame ionization detector, HP capillary column (100m x 0.25um film thicknes) CA, USA detector temperature. The procedure was carried out as described by the manufacturer.

Fixed setting: the gas flow was adjusted to the column, the in lets, the detectors and the split ratio. In addition, the injector and detector temperatures were set. The detectors were held at the high end of the over temperature range to minimize the risk of analyte precipitation. All parameters were double-checked to ensure the values were correct.

Detector A: 250^0C

Injector Temperature, both injectors 220^0C integrator chart speed: 2cm/min. the oven temperature was set at 180^0C and the GC was allowed to warm us. While warming, the following were set:

SIG 1	**A**	**Final value**	**181^0C**
INIT value	181^0C	final time	1min
	INIT time	15 minutes	
	Rate	O^0C/min	

When the instrument was ready, the "Not ready" light was turned off, and the run was started. A microliter (1µl) of sample was injected into column A, using proper injection technique. After the completion of the analysis, the result was automatically printed out.

In Silico Studies

Ligand Selection

From the GC – MS analysis of the ethanolic extract of clove, compounds were identified and analyzed. The 3D structure of the major compounds were retrieved from database and used in this study.

The crystal structures of B-Raf (BRAF), epidermal growth factor receptor (EGFR), macrophage colony – stimulating factor (MCSF), mammalian target of rapamycin (mTOR), Dihydrofolatereductase (DHFR), Dihydropteroate synthase (DHPS), DNA gyrase and B-lactamase with PDB IDs 2FB8, 2J6M, 3BEA, 4DRI, 3FRA, 1AD4, 4URM and 1A7T respectively were obtained from protein databank (www.rcsb.org). The existing ligands and water molecules were removed and hydrogen molecules were added. SDF structures sorafenib, lapatinib, pexidartinib, dactolisib, trimethoprim, sulfamethoxazole and amoxicillin, were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The molecules were converted to mol2 chemical format using open babel (O' Boyle *et al* ., 2011). The protein and ligand molecules were further converted to pdbqt chemical format using AutoDock tools. Docking of ligands to various protein targets and determinations of binding affinities was carried out using Vina (Trott and Olson, 2010). Molecular interactions between proteins and ligand with higher binding affinity compared to the standard drug were viewed with Discovery Studio 2020.

III. Results

GC – MS Analysis

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Table 1: Binding affinity of ligands to BRAF

		Binding affinity (kcal/mol)
S/N	Compounds	BRAF
S	Sorafenib	-11.3
1	9-Hexadecenoic acid, methyl ester (Z)	-7.8
2	13-Octadecenal, (Z)-	-7.9
3	cis-11-Hexadecenal	-8.2
4	Erucic acid	-8.9
5	Eugenol	-6.8
6	Oleic acid	-8.8
7	Oleyl alcohol, heptafluorobutyrate	**-11.5**
8	Phenol, 2-methoxy-3-(2-propenyl)-	-6.8
9	Phenol, 2-methoxy-4-(2-propenyl)-, acetate	-7.8
10	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	-8.6

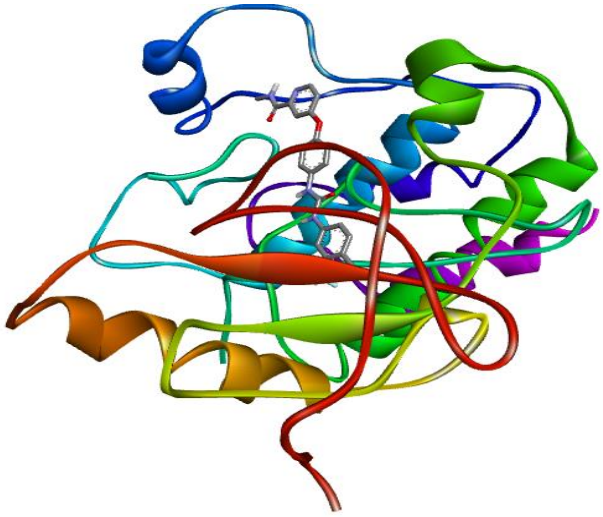

Figure 1: 3D view of the interaction between sorafenib and BRAF

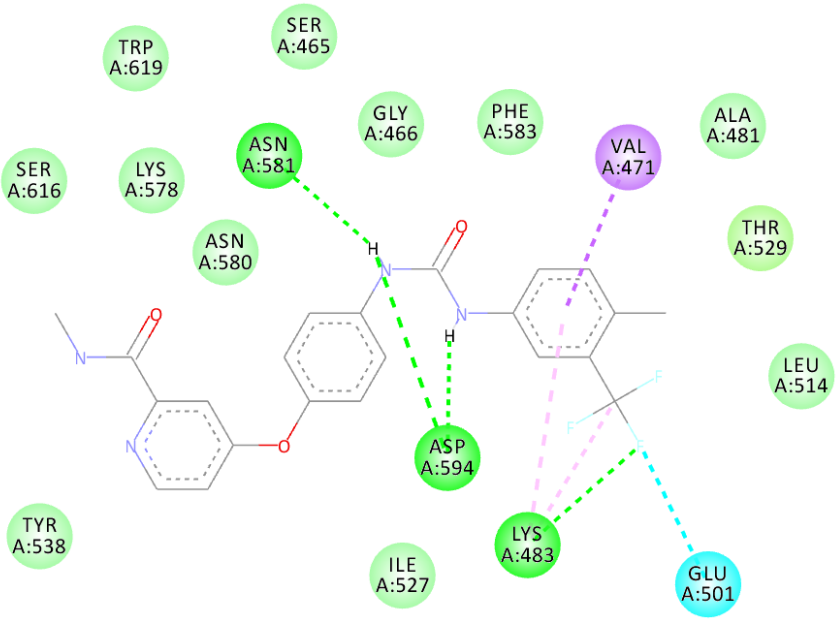

Figure 2: 2D view of the interaction between sorafeniband amino acids in the binding site of BRAF

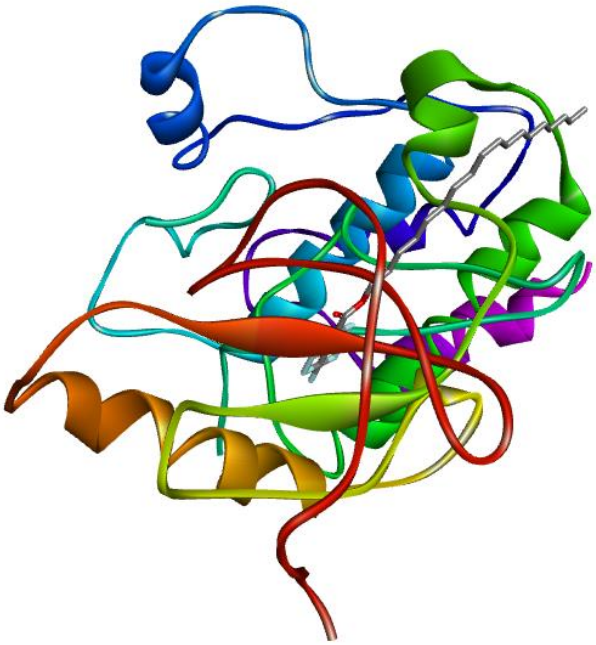

Figure 3: 3D view of the interaction between oleyl alcohol, heptafluorobutyrate and BRAF

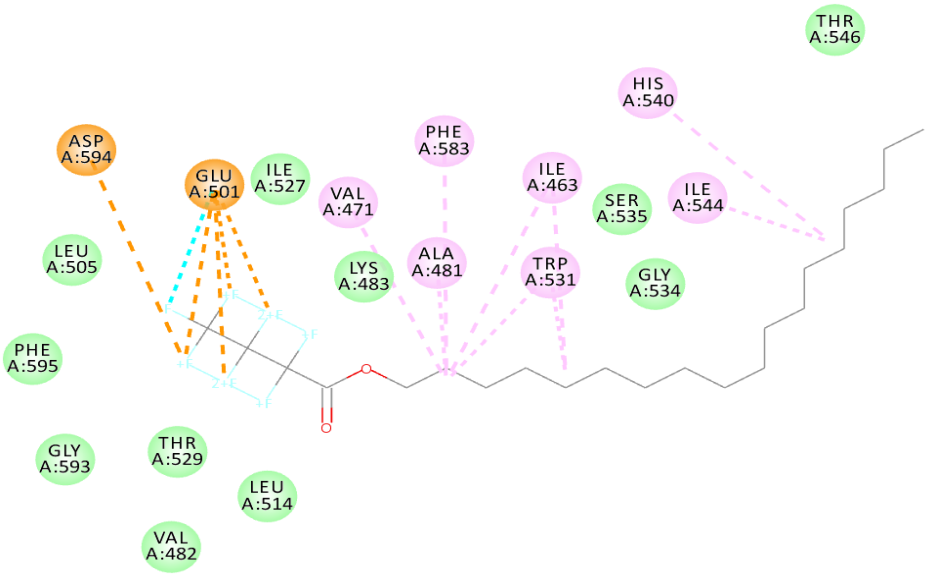

Figure 4: 2D view of the interaction between oleyl alcohol, heptafluorobutyrate and amino acids in the binding site of BRAF

Table 2: Binding affinity of ligands to EGFR

		Binding affinity (kcal/mol)
S/N	Compounds	EGFR
S	Lapatinib	-10.4
1	9-Hexadecenoic acid, methyl ester (Z)	-6.7
2	13-Octadecenal, (Z)-	-6.8
3	cis-11-Hexadecenal	-6.3
4	Erucic acid	-7.8
5	Eugenol	-6.0
6	Oleic acid	-7.0
7	Oleyl alcohol, heptafluorobutyrate	**-10.7**
8	Phenol, 2-methoxy-3-(2-propenyl)-	-5.8
9	Phenol, 2-methoxy-4-(2-propenyl)-, acetate	-6.5
10	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	-6.6

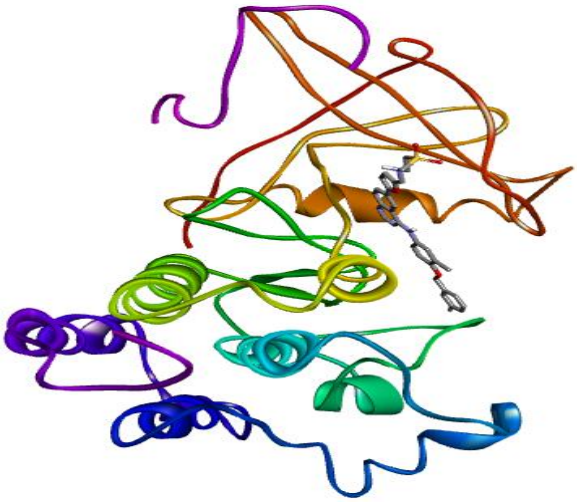

Figure 5: 3D view of the interaction between 1apatinib and EGFR

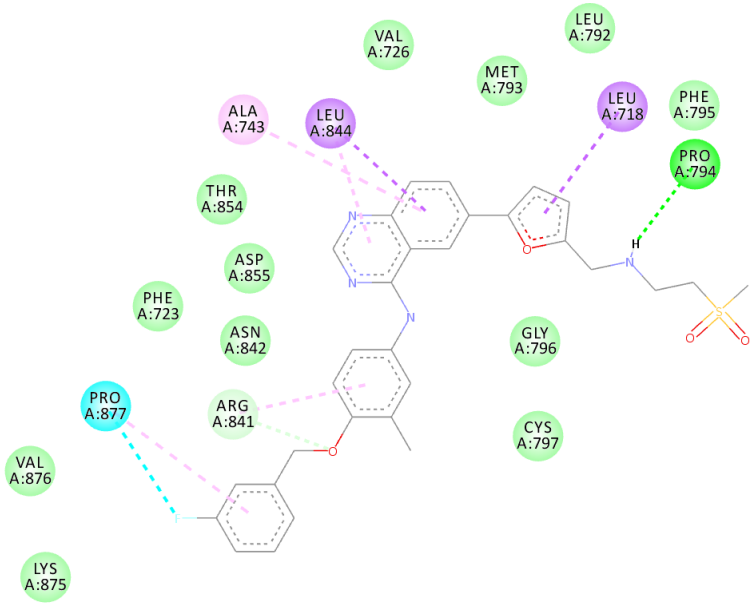

Figure 6: 2D view of the interaction between 1apatinib and amino acids in the binding site of EGFR

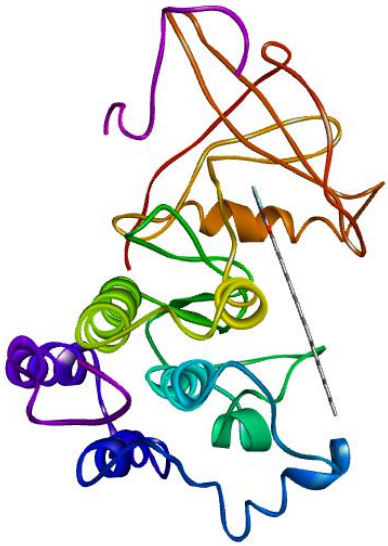

Figure 7: 3D view of the interaction between oleyl alcohol, heptafluorobutyrate and EGFR

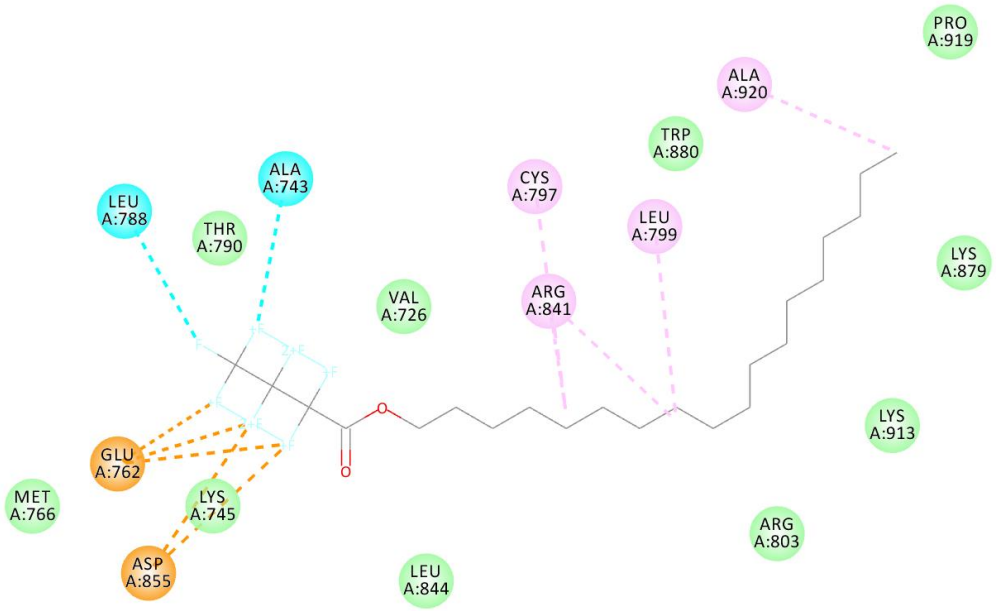

Figure 8: 2D view of the interaction between oleyl alcohol, heptafluorobutyrate and amino acids in the binding site of EGFR

Table 3: Binding affinity of ligands to MCSF

		Binding affinity (kcal/mol)
S/N	Compounds	MCSF
S	Pexidartinib	-10.4
1	9-Hexadecenoic acid, methyl ester (Z)	-8.0
2	13-Octadecenal, (Z)-	-8.6
3	cis-11-Hexadecenal	-8.0
4	Erucic acid	-8.6
5	Eugenol	-7.3
6	Oleic acid	-8.7
7	Oleyl alcohol, heptafluorobutyrate	**-11.1**
8	Phenol, 2-methoxy-3-(2-propenyl)-	-7.0
9	Phenol, 2-methoxy-4-(2-propenyl)-, acetate	-8.1
10	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	-8.3

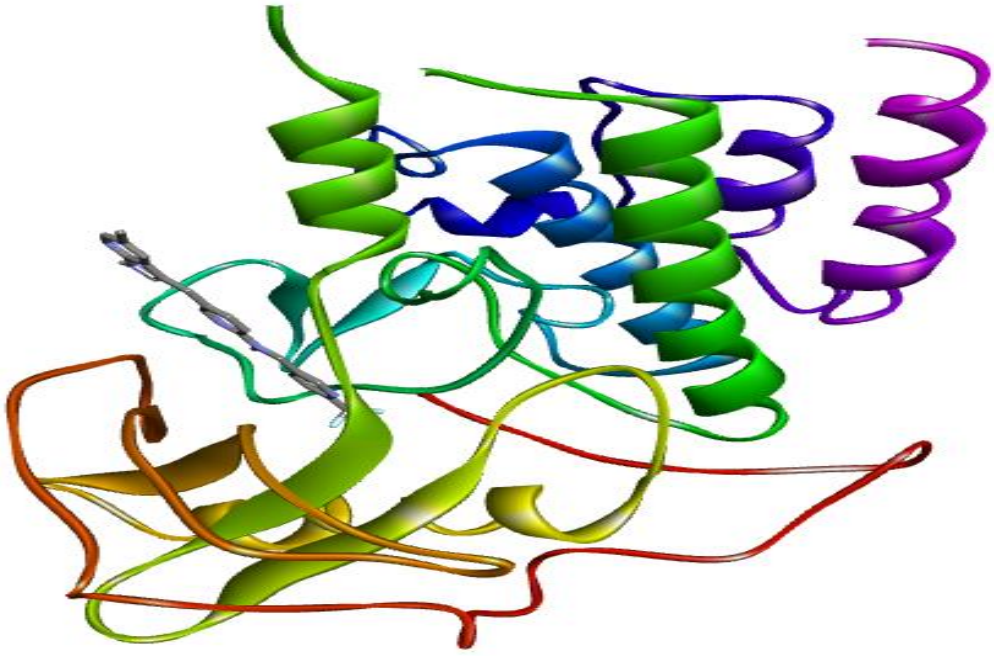

Figure 9: 3D view of the interaction between pexidartinib and MCSF1

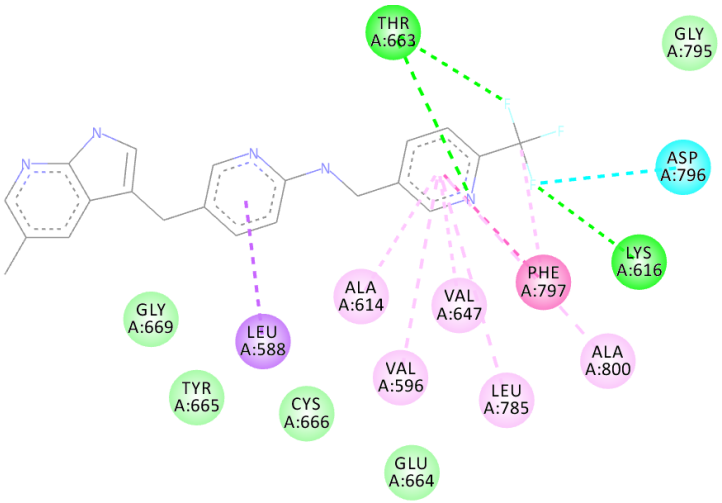

Figure 10: 2D view of the interaction between pexidartinib and amino acids in the binding site of MCSF1

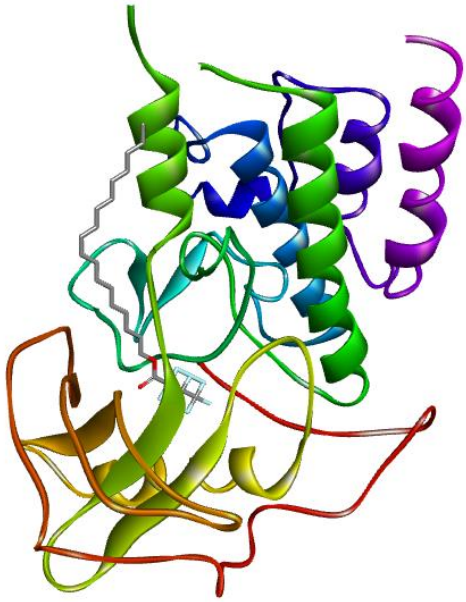

Figure 11: 3D view of the interaction between oleyl alcohol, heptafluorobutyrate and MCSF1

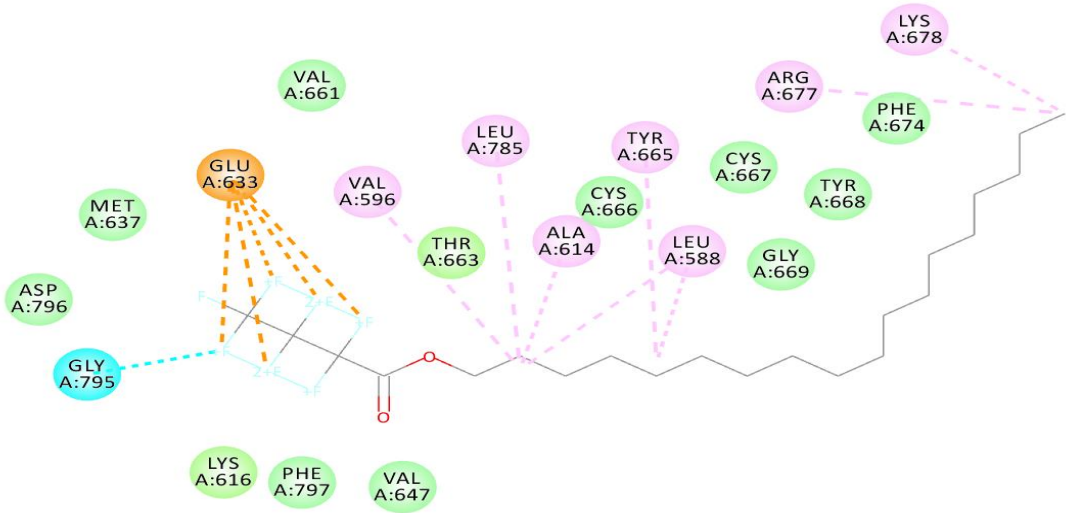

Figure 12: 2D view of the interaction between oleyl alcohol, heptafluorobutyrate and amino acids in the binding site of MCSF1

Table 4: Binding affinity of ligands to mTor

		Binding affinity (kcal/mol)
S/N	Compounds	mTor
S	Dactolisib	-11.1
1	9-Hexadecenoic acid, methyl ester (Z)	-6.9
2	13-Octadecenal, (Z)-	-6.3
3	cis-11-Hexadecenal	-6.7
4	Erucic acid	-6.8
5	Eugenol	-6.9
6	Oleic acid	-6.9
7	Oleyl alcohol, heptafluorobutyrate	-8.9
8	Phenol, 2-methoxy-3-(2-propenyl)-	-7.2
9	Phenol, 2-methoxy-4-(2-propenyl)-, acetate	-7.9
10	Z-(13,14-Epoxy)tetradec-11-en-1-ol acetate	-7.6

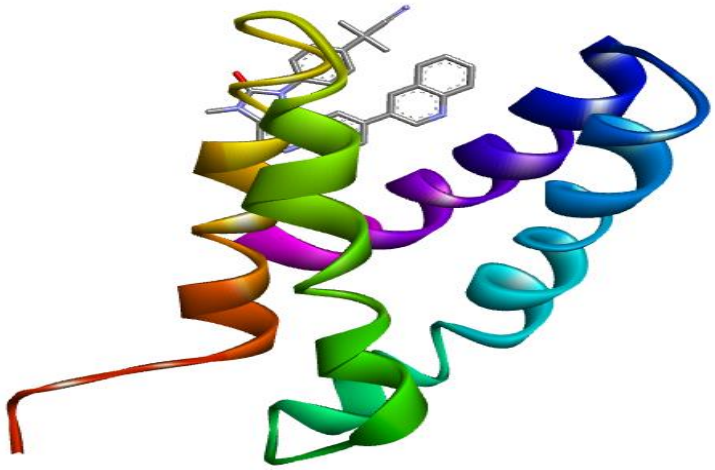

Figure 13: 3D view of the interaction between dactolisib and mTor

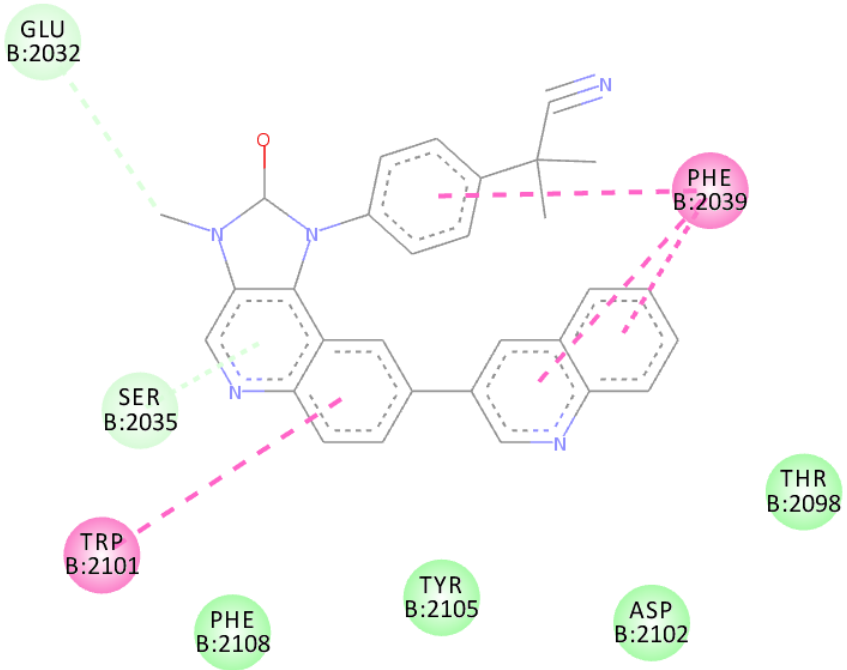

Figure 14: 2D view of the interaction between dactolisib and amino acids in the binding site of mTor

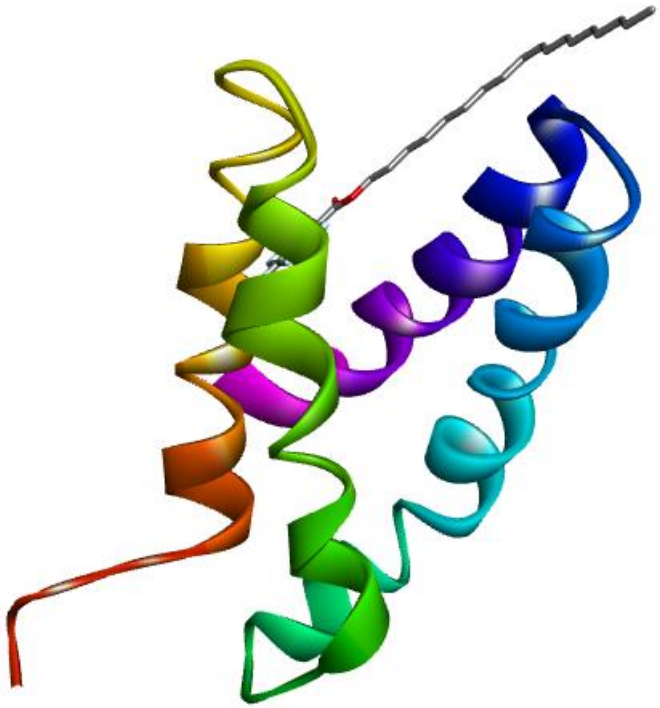

Figure 15: 3D view of the interaction between oleyl alcohol, heptafluorobutyrate and mTor

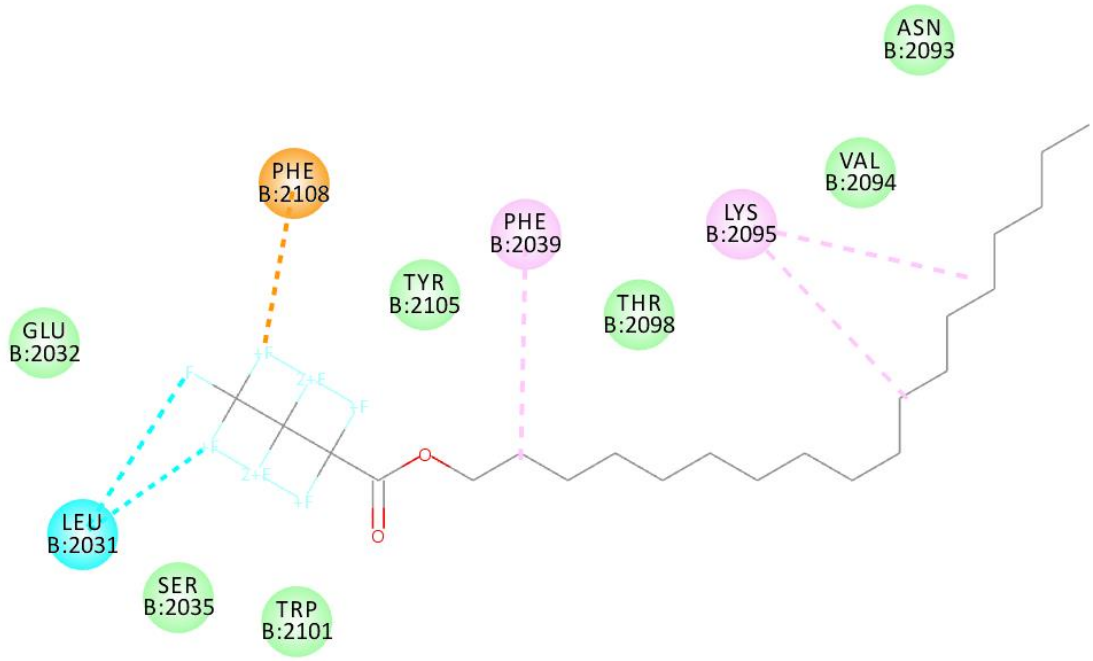

Figure 16: 2D view of the interaction between oleyl alcohol, heptafluorobutyrate and amino acids in the binding site of mTor

IV. Discussion

In the discovery of anticancer based lead molecules for cancer treatment, natural products are gaining relevance [12]. Computational advances play important role in drug development process though, application in the natural phytochemical compounds remain sparsely explored.

Cost and time of drug development is minimized through virtual screening approaches to predict ligand-receptor complex structure [13]. Molecular docking is a technique used to virtually identify on library store of compounds which are arranged based on scores and structural hypothetical theories playing significant role in structure-based drug design [14]. The compound and receptor plays important role in drug formulation

[15]. Products from natural sources such as plants can play curative role in wide range of human diseases including cancer. Given the rise of drug resistance to various diseases, plant-based medicines are the best potential options with tolerable and bearable side effects of traditional treatments [16]. The binding capacities of bioactive compounds from *Syzygiumarmaticum*was investigated with targets on anticancer because previously, ailments had been treated with *Syzygiumarmaticum* traditionally. Docking with higher negative score represented a high binding affinity between the receptor and ligand molecule, clove revealed higher efficiency of bioactive compounds. The anticancer docked ligand scores ranged from -6.3 to – 11.5 for anticancer. The present study revealed that Oleyl, alcohol, heptafluorobutyrate, trucic acid BRAF, EGFR, MS SF had higher binding affinity to Oleyl alcohol, heptafluorobutyrate (-11.5, 10.7 and -11.1 respectively) in sorafenib, lapatinib and pexidar-tinib respectively (-11.5, -10.7 and -10.4). However, Dactolisib, showed greatest affinity to MT or compared to all the compounds dictated.

The different types of bonds, particularly hydrogen bonds, binding affinity and amino acid residue interaction with ligand, the length of bond between the ligand atom and protein a target were observed. The phytocompounds (ligands) were used to determine the binding affinities in kcal/mol. [17]. In this present study, the residual interaction showed where the ligand exactly binds to particular amino acid of the protein. Out of 10 phytocompounds docked, oleyl alcohol, heptafluorobutyrateshowedbest binding affinity of -11.5kcal/mol in BRAF compared to the standard compound sorafenib with binding affinity of -11.3 kcal/mol, EGFR (-10.4) compared to laptinib which showed -10.7 kcal/mol binding affinity and the best binding affinity for MCSF against oleyl alcohol, heptafluoro butyrate was -11.1 greater than the compound pexidartibin with -10.4 kcal/mol binding affinity. mTor did not show good binding affinity to any of the phytocompound compared to Dactolisib (-11.1 kcal/mol) compound. The occurrence of hydrogen bond indicates that the ligand had high binding affinity to protein, whereas, a high negative score showed good binding affinity with target proteins [18, 4]. Phytocompounds from *Syzygiumarmaticum* showed better binding affinity compared with the synthetic drugs sorafinib, lapatinib and pexidartinib.

The physicochemical properties of importance are molecular weight, polarity, solubility, lipophilicity, saturation of carbon fraction and flexibility which are represented by rotatable bonds are necessary to prove its closeness.

The present study therefore, concludes that the phytocompounds identified from the ethanolic extract of *Syzygiumarmaticum* using GC – MS analysis suggest the potential ability of clove to treat cancer. The seventh compound olely, showed excellent binding affinity compared to the standard drugs used for cancer except doctatlisib.

Based on computational assessment, the ADMET parameters of the lead compound outweigh high cost, save time and preventing the unnecessary use of resources. Valuable information is made available through computational method of the compounds likely to be drug out of the array of compounds identified [19]. The activities of the clove extracts assessed are suitable to be used as drugs in treating cancer.

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