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Interaction Studies of Olive Oil Component Oleocanthal with Anticancer Targets

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Abstract:

Cancer is a progressive disease. There are number of treatments available such as chemotherapy, radiation and surgery. These treatments are associated with side effects and are not effective completely. Many of the common foods found in grocery stores or organic markets contains molecules that possess cancer-fighting properties, from antioxidants that neutralize the damage caused by free radicals to powerful phytochemicals that researchers are just beginning to explore to develop as new anticancer drug molecule. Oleocanthal is a component of olive oil which has the potential to kill cancer cells. This *in-silico* study sheds some light on the interaction of oleocanthal and anticancer targets by employing several bioinformatics softwares. The structure of Oleocanthal was drawn using the SMILE format and then was saved in PDB format. All the structures of anticancer drug targets were obtained with the help of protein database PDB (protein databank). Oleocanthal was docked with the anticancer drug target receptor proteins with the help of Autodock Vina. Chimera was used to study the docked complex whereas Discovery Studio visualizer was used to study the receptor-ligand interactions. The docked complex of Oleocanthal and 3EIG had the most stable conformation with the docking energy of -10.9 kcal/ml closely followed by 3W8Q with the energy of -10.8 kcal/ml. On the other hand, the complex of Oleocanthal with 1VPF was the least stable with the energy of -6.1 kcal/ml followed by 4KQ5 with the energy of -6.3 kcal/ml. The receptor ligand interaction maps showed that Oleocanthal interacted well with most of the anticancer drug target proteins, making it a potent cancer inhibitor.

Keywords: Cancer, *in silico*, Oleocanthal, Olive oil, clinical nutrition, phytochemicals, Autodock Vina, docking, Bioinformatics.

I. INTRODUCTION:

Cancer is a group of diseases that can metastasize and form tumors. In today's world, people are aware that there are different types or forms of cancers out of which breast and colon cancer are most common [1]. There are various medications and treatments available but can cause disturbing side effects such as hair loss during chemotherapy sessions. There are plenty of natural substances or compounds (antioxidants) that has the potential to inhibit cancer growth by inducing apoptosis. Olive oil plays a vital role in promoting good health as it is an integral ingredient of Mediterranean diet and these people are familiar with olive oil's nutritional and cosmetic gains [2]. It can preserve the brain cells and improve memory as the oil is rich in healthy fats. Olive oil had been used for treatment of several diseases as it has cardio protective and antimicrobial properties [3]. Recent epidemiological studies indicated that regular involvement of olive oil in the diet prevented colorectal and breast cancers [4]. It also reduced the cases of cardiovascular disorders and atherosclerosis [5, 6]. This beneficial effect of oil prolongs or increases the life expectancy of Mediterranean population [7]. The international olive oil council has categorized or classified olive oils based on the acidic levels, such as extra virgin and ordinary virgin olive oil [8]. The so called extra virgin olive oil is extracted by physical or the mechanical method also known as cold-pressed technique from the olive tree. This is regarded as the best method for obtaining the oil as it will not alter its characteristics. Other extraction methods may end up losing the phytochemicals during refining process [8,10]. This study aims to analyze the interactions between the olive oil component oleocanthal and several anticancer targets. A simple combination of different Bioinformatics softwares which were employed for this *in silico* study helped in determining the anticancer properties of oleocanthal that would complement *in vitro* and *in vivo* findings till date.

II. MATERIALS AND METHODS:

Retrieval of anticancer drug information and drug targets structures:

In this study, the lists of anticancer drugs were retrieved using the National cancer institute's website. Most of the anticancer drugs that are currently available in the market are present in its list of anticancer drugs. Drugbank is a database dedicated to inform about the different type of drugs which are under experimental phase. It gives a detailed information about a particular drug including its structure, target etc. In this database, there is also a link to uniprot database dedicated to provide information regarding the protein structure and sequences. A unique uniprot ID is assigned to each protein structure and reveals information about that

particular protein when searched using the assigned ID. The above-mentioned website was referred to note the variety of anticancer drugs and drug targets. The databases (Drugbank and uniprot) were accessed for the additional information about these drug targets. For the retrieval of the anticancer drug targets structures (receptor proteins), PDB database was accessed. This database consists information about the different kinds of proteins and its structures assigned with a unique 4 letter ID. PDB is highly reputed as hypothetical protein structures cannot be submitted to this database.

Docking:

Autodock Vina was employed for a flexible docking because it has an accuracy of 78% which makes it preferable over other available software tools. It uses PDBQT as its input and output file, also used by its predecessor Autodock. This tool was used to dock the oleocanthal with different anticancer drug target receptor proteins.

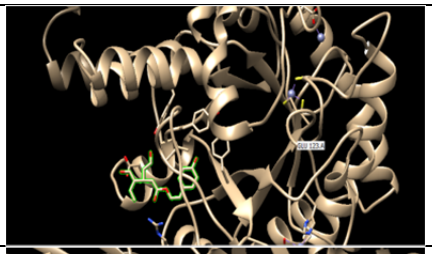
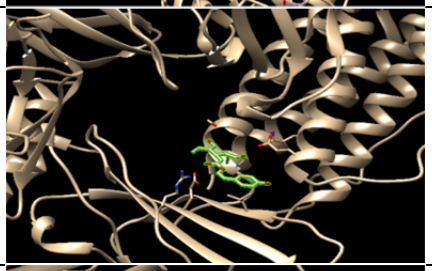
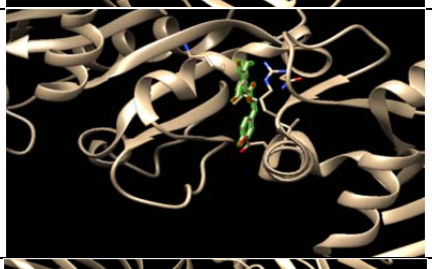
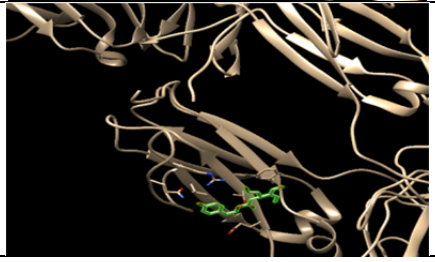
Visualization and editing of drug target structures:

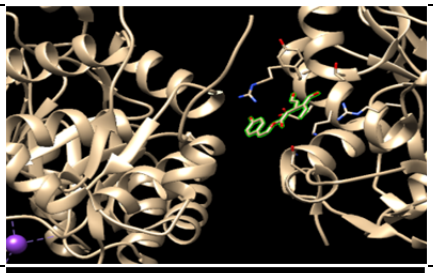
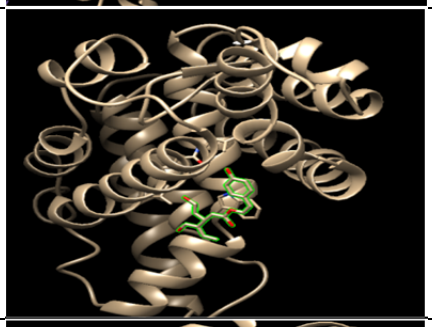
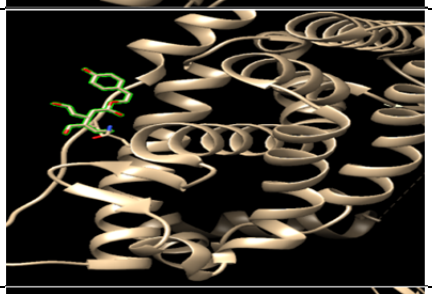
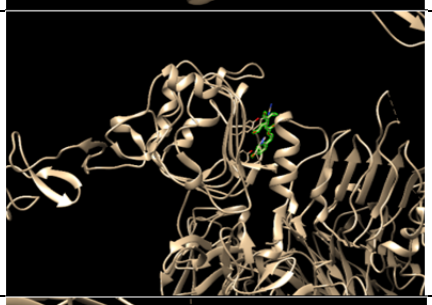
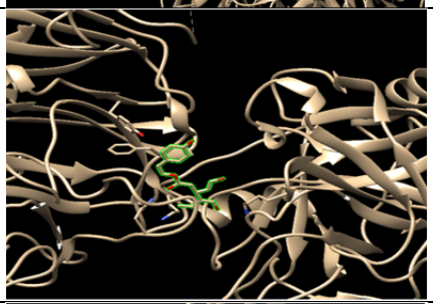
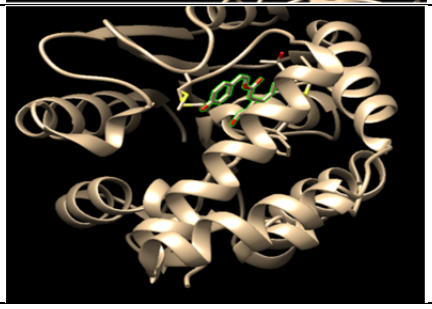
Chimera is a tool that can be employed to visualize different protein structures of interest that are retrieved from PDB. Structures can also be edited and saved to different file formats supported by chimera. Chimera can also be used to visualize the docked poses given by the Autodock Vina. The anticancer drug target proteins were edited using this tool. The discovery studio Visualizer is a tool that possess similar features as chimera. However, it has some special features such as the ligand design, QSAR, pharmacophore mapping, SBDD etc. The ligand-receptor interactions that occurred were studied and the interaction maps of the docked poses were generated using this discovery studio visualizer.

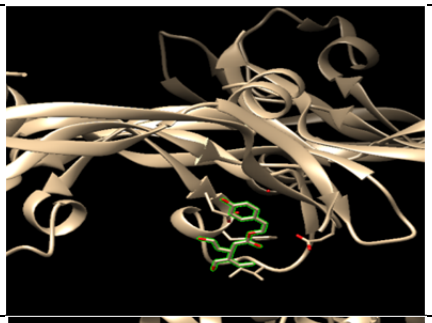
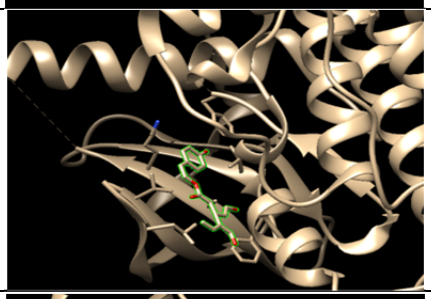
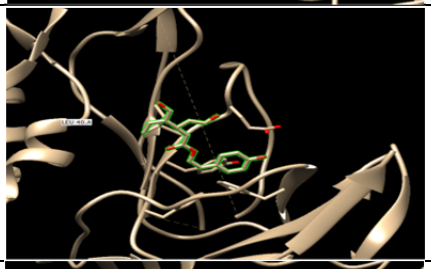
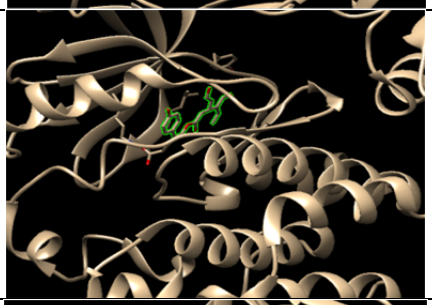
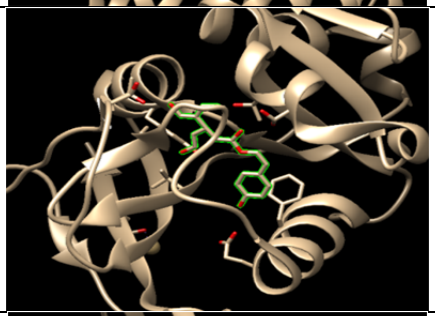
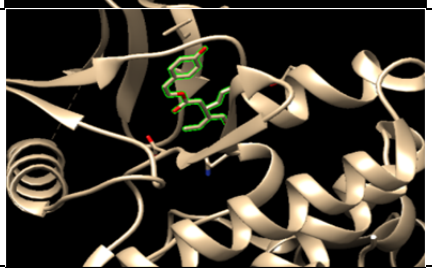
III. RESULTS:

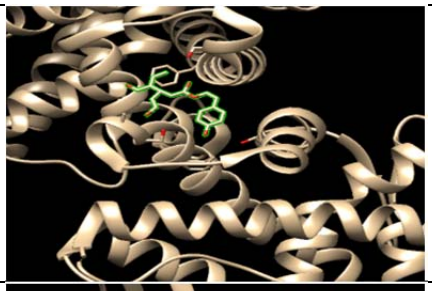
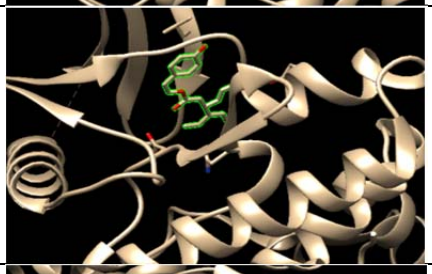
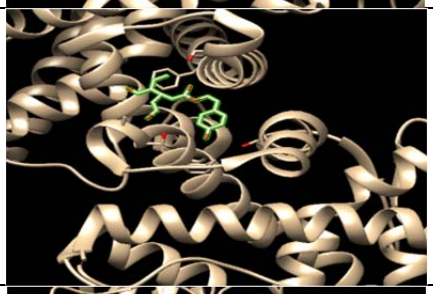
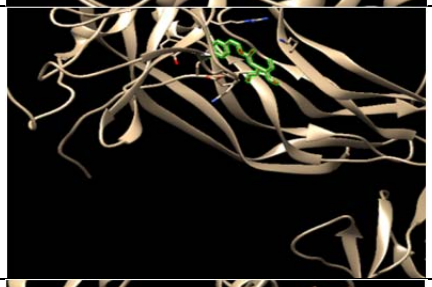
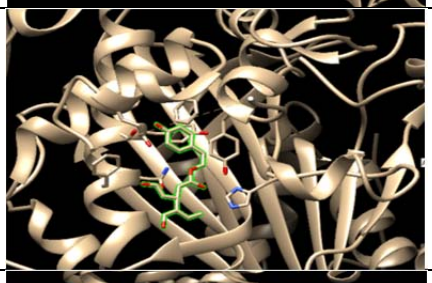
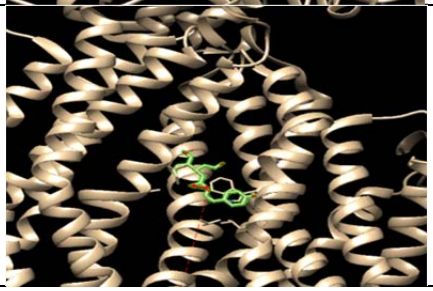
The interactions that occurred between the ligand oleocanthal and various anticancer drug targets receptors were analyzed and for the record screenshots were taken as presented in (**Error! Reference source not found.**)

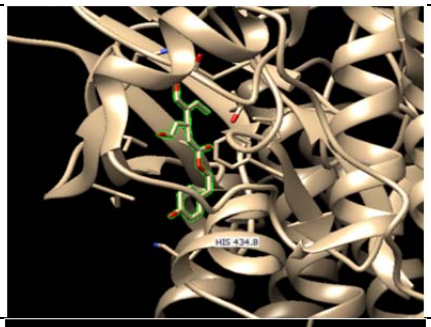
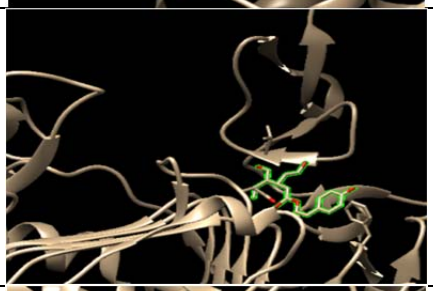
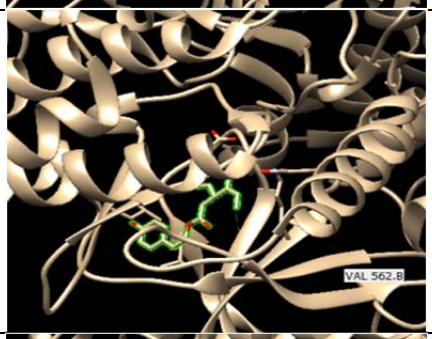
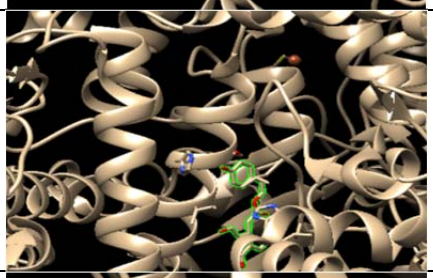
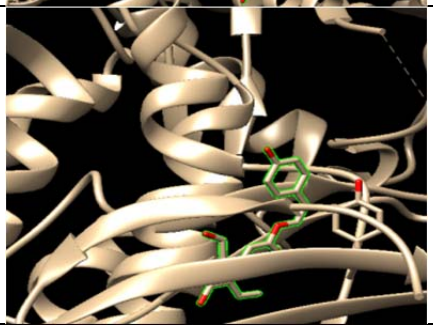
Table 1. Depicts the values achieved after in silico interaction of oleocanthal and anticancer drug targets.

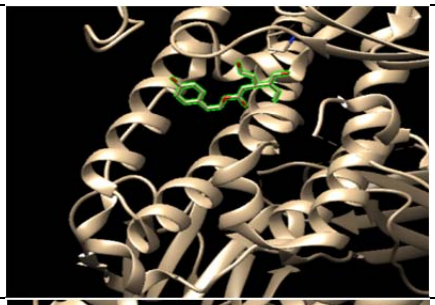
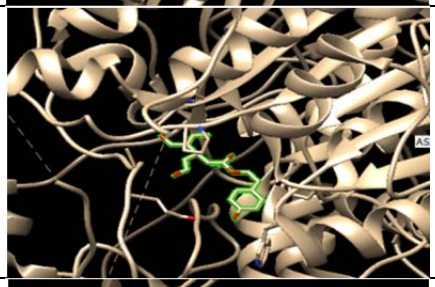
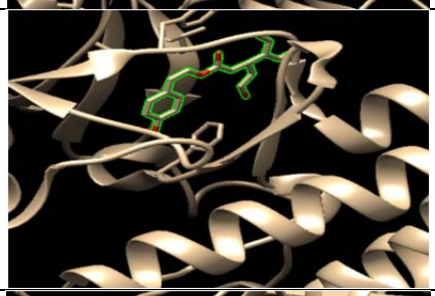
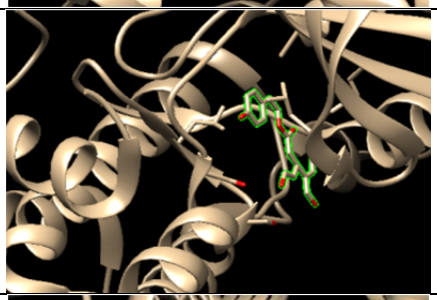
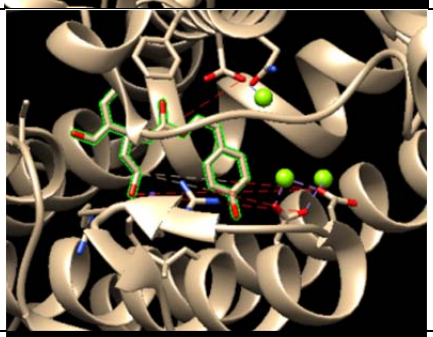
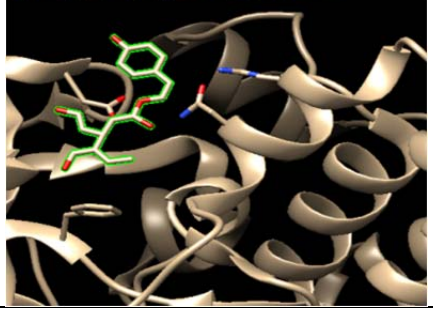
PDB ID	Compound name	Docked Output (first pose)	Binding and docking Energy (kcal/ml)	Co-ordinates
1B4E5	AMINOLEVULINIC ACID DEHYDRATASE		-8.6	X = 15.412 Y = 24.935 Z = 14.494
1CD9	GRANULOCYTE COLONY-STIMULATING FACTOR		-7.2	X = 53.694 Y = 36.766 Z=129.105
1DHF	DIHYDROFOLATE REDUCTASE		-9.3	X = 16.687 Y = 25.844 Z = 44.429
1E4K	IMMUNOGLOBULIN GAMMA FC		-7.7	X = 55.212 Y = 35.08 Z = 3.931

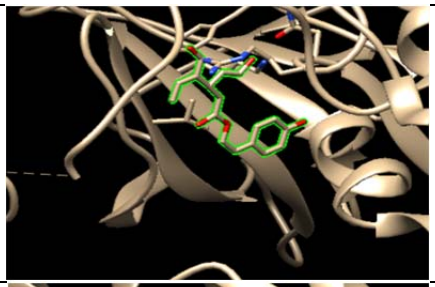
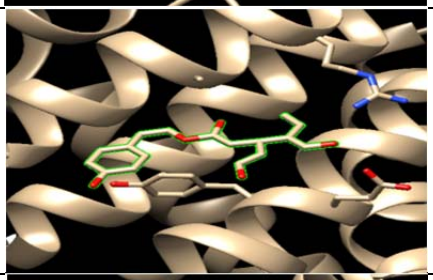
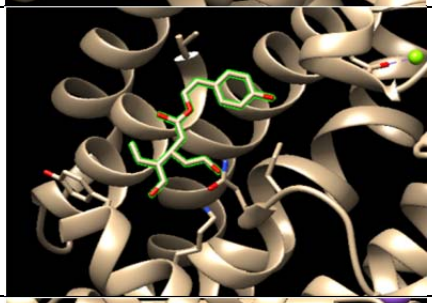
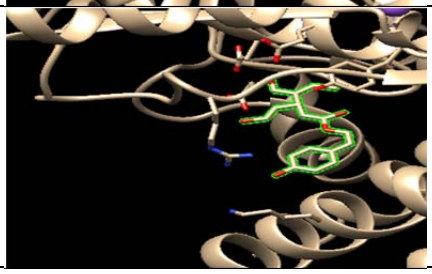
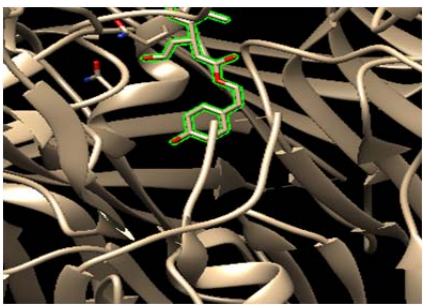
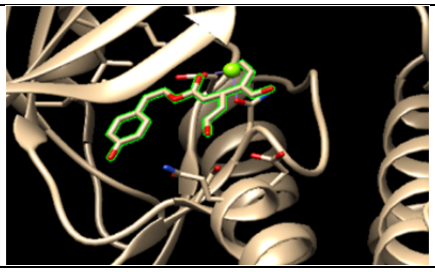
PDB ID	Compound name	Docked Output (first pose)	Binding and docking Energy (kcal/ml)	Co-ordinates
1JRI	INOSINE MONOPHOSPHATE DEHYDROGENASE		-7.1	X = 82.631 Y = 69.357 Z = 61.101
1LBD	HUMAN NUCLEAR RECEPTOR RXR-ALPHA		-9.0	X = 16.948 Y = 80.36 Z = 67.428
1NHZ	GLUCOCORTICOID RECEPTOR		-6.6	X = 3.392 Y = 20.1 Z = 0.448
1S78	PERTUZUMAB		-7.6	X = 40.327 Y = 56.447 Z = 199.719
1SHY	HGF BETA-CHAIN		-8.3	X = -6.934 Y = 24.439 Z = 39.431
1TQH	CARBOXYLESTERASE EST30		-8.3	X = 37.788 Y = 22.642 Z = 19.721

PDB ID	Compound name	Docked Output (first pose)	Binding and docking Energy (kcal/ml)	Co-ordinates
1VPF	VASCULAR ENDOTHELIAL GROWTH FACTOR		-6.1	X = 5.986 Y = 2.598 Z = 28.64
1Y6A	VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR		-9.2	X = 5.991 Y = 38.86 Z = 23.089
1Z92	INTERLEUKIN-2		-8.3	X = 4.013 Y = -31.967 Z = 2.912
2H8H	SRC KINASE		-10.3	X = 19.59 Y = 33.246 Z = 66.752
3EIG	METHOTREXATE-RESISTANT MUTANT		-10.9	X = 12.297 Y = -3.551 Z= -10.523
3EYG	TYROSINE-PROTEIN KINASE		-10.0	X = 10.32 Y = 4.051 Z =-16.631

PDB ID	Compound name	Docked Output (first pose)	Binding and docking Energy (kcal/ml)	Co-ordinates
3G8O	PROGESTERONE RECEPTOR		-7.9	X =10.508 Y = 0.03 Z =17.681
3EYG	TYROSINE-PROTEIN KINASE		-10.0	X = 10.32 Y = 4.051 Z =-16.631
3G8O	PROGESTERONE RECEPTOR		-7.9	X =10.508 Y = 0.03 Z =17.681
3MJG	PLATELET-DERIVED GROWTH FACTOR		-9.2	X =20.364 Y = -33.76 Z = -1.815
3N5E	HUMAN THYMIDYLATE SYNTHASE		-8.8	X = -48.931 Y = 0.05 Z =11.859
3ODU	CHEMOKINE RECEPTOR		-8.6	X = 3.591 Y = 6.89 Z =40.116

PDB ID	Compound name	Docked Output (first pose)	Binding and docking Energy (kcal/ml)	Co-ordinates
3OMV	C-RAF (RAF-1)		-10.3	X = 16.921 Y = 29.112 Z = 35.864
3QWQ	EPIDERMAL GROWTH FACTOR RECEPTOR		-7.6	X = -12.219 Y = -9.171 Z = 26.274
3RI1	FGFR2 KINASE		-9.6	X = -33.813 Y = 24.486 Z = -10.463
3RUK	CYTOCHROME P450 CYP17A1		-8.1	X = 8.625 Y = 16.72 Z = 43.444
3RZF	KAPPAB KINASE BETA		-8.9	X = 73.464 Y = -19.736 Z = 38.978

PDB ID	Compound name	Docked Output (first pose)	Binding and docking Energy (kcal/ml)	Co-ordinates
3SE3	HUMAN IFNA2-IFNAR TERNARY COMPLEX		-7.9	X = 34.498 Y = -28.909 Z = -5.789
3SWR	HUMAN DNMT1		-10.2	X = -21.949 Y = -4.298 Z = 25.683
3VHE	HUMAN VEGFR2 KINASE		-9.8	X = -21.512 Y = -1.059 Z = -3.438
3W8Q	HUMAN MITOGEN-ACTIVATED PROTEIN KINASE KINASE 1		-10.8	X = 22.383 Y = 10.503 Z = 13.476
4DEM	FARNESYL PYROPHOSPHATE SYNTHASE		-8.4	X = 8.912 Y = 29.997 Z = -6.627
4HVS	KIT KINASE		-8.1	X = 23.669 Y = -0.572 Z = 6.528

PDB ID	Compound name	Docked Output (first pose)	Binding and docking Energy (kcal/ml)	Co-ordinates
4I21	EGFR KINASE DOMAIN		-9.4	X = -3.92 Y = 10.235 Z = -24.653
4IEH	BCL-2		-7.3	X = 6.497 Y = 19.969 Z = 19.176
4KQ5	FARNESYL PYROPHOSPHATE		-6.3	X = 9.698 Y = 30.244 Z = -6.511
4LXZ	HDAC2		-8.6	X = 21.218 Y = -19.37 Z = -28.325
4TZ4	HUMAN CEREBLON		-8.7	X = -18.761 Y = 23.343 Z = -53.202
4U7Z	MITOGEN-ACTIVATED PROTEIN KINASE KINASE		-9.5	X = -30.301 Y = 21.413 Z = 9.036

(Grid size X=40, Y=40, Z= 40)

Oleocanthal is an active Olive oil compound and was found to interact better with most of the anticancer drug target proteins. The first pose is often regarded as the best pose as observed after the docking study by using Autodock Vina. The docked complex of Oleocanthal and 3EIG had the most stable conformation with the docking energy of -10.9 kcal/ml closely followed by 3W8Q with the energy of -10.8 kcal/ml whereas, the complex of Oleocanthal with 1VPF was the least stable with the energy of -6.1 kcal/ml followed by 4KQ5 with the energy of -6.3 kcal/ml. The ligand interaction maps generated using Discovery studio also showed that Oleocanthal had better interactions with the active site residues especially with the ones having a stable conformation while it shows weak interactions with the ones having lower energy conformations.

IV. DISCUSSION:

Strategies keep showing up to tackle this fatal disease. The majority of cancers occur due to the problems in P53 gene which is responsible for tumor suppression and hence called as guardian of the genome. The P53 gene is triggered in case of DNA damage in the cell and it arrests the cell cycle, causes apoptosis of the cell and DNA repair. Any defects in the P53 gene affects its functionality and the downstream effects would not occur. As a result, tumor formation may occur, which if malignant then can turn cancerous. There are several carcinogenic compounds or chemicals that can cause mutations such as some antibiotics like chloramphenicol [9]. Oleocanthal is one of the phenols present in olives that can function as a cancer inhibitor [10].

A team of researchers led by Javier Menendez (Catalan institute of oncology) and Antonio segura-carretero (university of Granada, Spain) investigated the compounds of olive oil that were most active against cancer [11]. This study revealed that the complex phenols in extra virgin olive oil dramatically suppressed overexpression of HER2 gene in breast cancer cells. They carried out oil separations into fractions and tested it with the human breast cancer cells *invitro*. The fractions consisting of phytochemical polyphenols such as lignans and secoiridoids played a vital role in suppressing HER2 gene [11]. These findings indicated that olive oil rich in polyphenols inhibits human breast cancer that is HER2-dependent. It can be a huge risk when the laboratory results are applied to *invivo* or actual human situations because the phytochemicals (lignans and secoiridoids) showed tumoricidal properties against cultured breast cells at high concentrations which is not possible to achieve by normal daily oil consumption through food. However, the fact that there is awareness about the benefits of olive oil, now people safely consume large amount of these phytochemicals through consumption of olives and by using extra virgin olive for cooking. These polyphenols are a ray of hope for the development of new and safe anti-breast cancer drugs [11].

A study at the University of Barcelona and Granada proved that the maslinic acid is a triterpenoid compound that is present in the skin of an olive. It was determined that this acid has the potential to slowdown the growth of the cells and is also capable of causing cell apoptosis in human HT29 colon cancer cells through an intrinsic mitochondrial pathway. A significant number of studies are suggesting that the triterpenoid compounds can prevent the normal cells from transforming into cancerous cells by interfering in certain key pathways such as carcinogen activation and DNA repair [12, 13,14]. The concentration of maslinic acid in the olive is around 80%. A study conducted by Myriam Fezai and her team showed that extra virgin olive oil exhibited analgesic, anti-inflammatory and anticancer activity and supported it as a traditional medicine [10].

Rutgers University and Hunter College published their findings supporting the claim that olive oil compounds are anticancer in nature. They discovered that almost all types or forms of cancer cells under their study died within an hour. It was determined whether oleocanthal was the one responsible for triggering the protein that caused these cell deaths. They confirmed that oleocanthal could induce apoptosis by destroying the waste centre called the lysosomes which are more fragile and large as compared to the healthy cells. Oleocanthal slightly ruptures the lysosome then the acid and the recycling enzymes cause further damage and this leads to apoptosis or programmed cell death. This process can be called as lysosomal membrane permeabilization. It was interesting to know that the oleocanthal only inflicted damage to the cancer cells and the rest normal healthy cells were left untouched, they were put to sleep or underwent a temporary halt (hibernation) and then resumed their cycles [15].

Future study would be to find that, why oleocanthal targets only cancer cells? and why the cancer cells are extremely sensitive to oleocanthal when compared to a non-cancerous healthy cell? Also, it would be interesting to know that whether it is safe to administer highly purified oleocanthal dose therapeutically?

V. CONCLUSIONS:

From the obtained results and upon its analysis it is evident that oleocanthal present in olive can prevent cancer. The docking studies and the ligand interaction maps have suggested that oleocanthal interacts well with most of the anticancer drug target proteins. The implication was to find the most stable conformations among oleocanthal and anticancer drug targets in this study. Oleocanthal can be a very useful compound, that can be used to design a potential anticancer lead which is the need of the hour.

CONFLICT OF INTEREST:

The authors state no conflict of interest.

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