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Molecular Docking Analysis of 9-Octadecene, 9,12,15-Octadecatrienoic acid, Methyl Ester, Phytol, 9,12-Octadecadienoic Acid and 9-Octadecenoic Acid with Anticancer Target Enzyme Caspase 3 (PDB: 1CP3)

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Abstract

Computers and computing methods are commonly used in biological research today. In silico-molecular docking is a highly effective technology for identifying novel ligands for proteins with established structures and is crucial in the development of structure-based medicines. Caspase 3 plays a central role in apoptosis and splits many protein substrates in the cell when activated, which leads to cell death. Since it is known that many chemotherapies drugs trigger apoptosis in cancer cells, the promotion or activation of apoptosis through targeted control of apoptosis regulators has been proposed as a promising strategy for the discovery of cancer drugs. Therefore, in this present study in silico-molecular docking was carried out to determine the binding properties of 9-octadecene, 9, 12 and methyl ester, phytol, 15-octadecatrienoic acid, 9 and 12-octadecadien acids and 9-octadecenoic acid and target Protein 1CP3 (Caspase3). The study suggests that methyl ester, 9-octadecene, 9, 12-octadecadinenoic acid, phytol, 9,12,15-octadecatrienoic acid and phytol can inhibit caspase-3. Among the various phyto-compounds, 9,12,15-octadecatrienoic acid has more possible bond interactions than other compounds. Therefore, this study can serve as evidence of in vivo cancer activity that helps these molecules to come onto the market as over-the-counter medicines.

Keywords: *Autodock, Apoptosis, Caspase 3, Ligands, Molecular Docking, Proteins.*

Introduction

Computers and computational techniques are extensively employed in contemporary biological research. In silico-molecular docking is a highly effective technology for identifying novel ligands for proteins with established structures and is crucial in the development of structure-based medicines. Additionally, this technique can be employed for the examination of potential target structures of binding or active sites, the generation of candidate molecules, the assessment of their drug similarity, the targeting and docking of these molecules, the organisation them based on binding affinity, and the further enhancement of the molecules to enhance their binding properties. Approaches to drug design have been observed in silico to significantly minimize the associated costs and the time it takes the molecule to go through the drug discovery pipeline. Virtual screening, a silicon analogue

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of high-throughput screening, also offers great potential for identifying new drug candidates [1, 2].

The computational approach was perceived as a cost-effective and straightforward technique for identifying physiologically active molecules in the context of medication development. This methodology offers a comprehensive evaluation of the pertinent characteristics and interplays of compounds, hence reducing the occurrence of errors and unfavourable outcomes in in vitro investigations [3]. Both structure-based and ligand-based molecular docking has become a powerful and inexpensive method for researching new lead compounds. Molecular docking has succeeded in finding novel anti-cancer compounds against several protein targets. One goal for cancer drugs is apoptosis (programmed cell death). Caspases play an important role in regulating apoptosis [4]. Caspase-3 is an essential part of some apoptotic routes [5]. The study was designed to investigate structure-based molecular interactions between phytochemical components of 9-octadecene, 9, 12 and 15-octadecatrienoic acid, methyl ester and phytol, 9 and 12-octadecadien acids and 9-octadecenoic acid from *Sphaeranthus indicus* and Caspase-3. The compounds were found in the plant *Sphaeranthus indicus* leaves through GC-MS Analysis. Through the GC-MS analysis, these phytocompounds were identified in the plant, *Sphaeranthus indicus* and their pharmacological properties especially the antioxidant properties of the compounds were found through literature studies.

Materials and Method

In Silico Molecular Docking

Recent pharmaceutical research has successfully utilised computational drug discovery technologies to molecularly model compounds employing a range of algorithmic programming software. This approach facilitates the determination of ligand and protein binding values through algorithmic programmes, allowing for the utilisation of various software applications to optimise protein-ligand interactions and get optimal outcomes [6].

Ligand and Protein Preparation

The ligands, including 9,12, and methyl ester, 9 and 12-octadecadeinoic acid and 15-octadecatrienoic acid, 9-octadecenoic acid, and phytol (as shown in Figure 3), were sourced from the PubChem database, Ligand. These ligands were then converted into PDB format using the open-babel software, while the proteins were obtained from the PDB database, specifically Caspase 3 (PDB: 1CP3). During protein preparation, all water molecules and other ligand molecules were typically removed before docking. However, proteins created with Pymol software were retained when forming the PDB files.

In Silico Docking Studies

The protein structure was retrieved from the Protein database (PDB), while the ligands were from PubChem. Grid generation, docking score calculation, and identification of activator conformers tied to the protein's active site were carried out using automated docking tools and graphical user interfaces such as AutoDock Tools. The energy minimization process was performed using ACD/ChemSketch, resulting in a minimized structure used for the docking studies. To facilitate docking, heteroatoms, including additional atoms and water molecules were removed from the protein structure. The Lamarckian algorithm methodology was incorporated in AutoDock 4.1, and was employed for docking. This software estimates energy during ligand-protein interactions and identifies the most energetically favourable

ligand pose. The evaluation function relies on the intermolecular interactions between ligands and proteins throughout the docking process. In line with the genetic algorithm, all ligand rotations were permitted during docking. Grid cards were centred on certain residues of proteins and created by setting up grid dimensions (middle x, middle y, middle z). Lamarck's genetic algorithm and the pseudosoris and wetting method were used to minimize default parameters [7-9]. The modelling software PyMol (Delano Scientific LLC, San Carlos, California), Chimera version 1.10.1 (UCSF Biological Computing Visualization and Informatics Resources modelled with NIH, CA, USA) and Pause View [10].

Results

Active Site of Caspase 3

The striking binding site of the 1N8Z protein was calculated via a CASTP server with ideal parameters. In the CASTp assessment, amino acids, and the surface of the active location (481,025) were observed. Chain A was involved in the active properties identified with the CASTp server. The grid card focuses on a certain residue of the protein and was generated in the A chain (centre x = 14.04, centre y = 0.80, Center z = 33.24) with a prepared grid dimension. The light blue colour indicated amino acids at the binding site (**Table 1**). Also, the active site of Caspase-3 is comprised of amino acid residues as follows: ARG64, SER120, HIS121, GLY122, GLN161, ALA162, CYS163, SER25, TRP206, ARG207, ASN208, SER209, TRP214, MET222, GLN225, TYR226, ARG238, ARG241, LYS242, THR245, GLU246, PHE247, GLU248, SER249, PHE250, SER251 and PHE256. Figure 1 represents the structure of Caspase 3 from PDB with the ID 1CP3.

Studies *in silico* have shown that the docking results are 9-octadecene, 9, 12 and 15-octadecatrienoic acid, methyl ester, phytol, 9 and12-octadecadienoic acid and 9-octadecanoic acid, −5.00, −5.40, −5.20, 6.20 and −5.30. It binds to the active enzyme site with the binding energy of mol (**Table 1**). The best docking model is a conformation cluster with the lowest free binding energy. The lowest binding energy such 9,12-octadecadienoacid (−) 6.20 kcal / mol) has the strongest affinity for caspase 3, which inhibits the expression of caspase 3 (Figure 1 - 8).

Table 1. Energy Values Obtained in Molecular Docking Interactions between Ligands and Caspase 3 (1CP3)

Ligands	**Molecular formula**	**Molecular weight in g/mol**	**Hydrogen bond donor/acceptors**	**Binding affinity in kcal/mol**	**Ligand binding site of target Amino acids**
9-Octadecene	$C_{18}H_{36}$	252.50	0/0	-5.00	**Ser 205**, **Phe 256**, **Cys 163**, **Arg 207**, **Trp 206**, **Tyr 204**, **Ser 251**, Phe 250, Asn 208, Trp 214, Ser 249.
9,12 and 15-Octadecatrienoic acid, methyl ester	$C_{19}H_{32}O_2$	292.50	0/2	-5.40	Gly 122, **Arg 207**, Arg 64, **Ser 205**, **Ser 251**, Ala 162, Ser 120, **Cys 163**, His 121, **Trp 206**,

					Tyr 204, Thr 62, **Phe 256**.
Phytol	$C_{20}H_{40}O$	296.50	1/1	-5.20	**Ser 251**, Ser 209, Asn 208, **Arg 207**, His 121, **Trp 206**, Thr 62, **Cys 163**, **Ser 205**, **Phe 256**, **Tyr 204**.
9 and 12-Octadecadienoic acid	$C_{18}H_{32}O_2$	280.40	1/2	-6.20	Ala 162, Ser 120, Gly 122, His 121, Asn 208, Phe 250, Ser 249, Trp 214, **Ser 251**, **Tyr 204**, **Trp 206**, **Cys 163**, **Phe 256**, **Ser 205**, **Arg 207**, Gln 161, Arg 64.
9-Octadecenoic acid	$C_{18}H_{34}O_2$	282.50	1/2	-5.30	**Ser 251**, Phe 250, Ser 249, **Phe 256**, **Trp 206**, **Tyr 204**, **Ser 205**, **Arg 207**, Arg 64, Gln 161, His 121, Ala 162, Ser 120, **Cys 163**.

* Bold amino acid residues are involved in all ligand interactions.

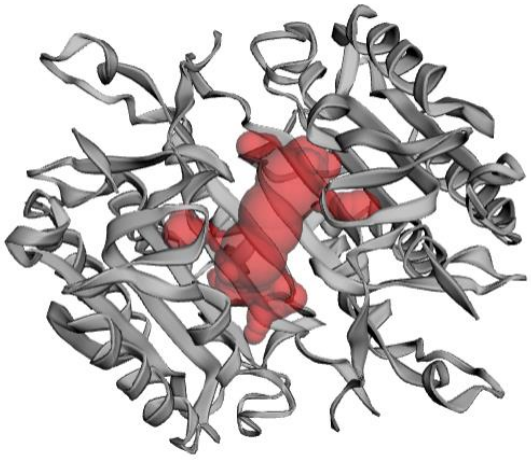

Figure 1.1CP3 Active Site (Red colour area Å2 = 481.025) and Protein 3D View, Using CASTp Server

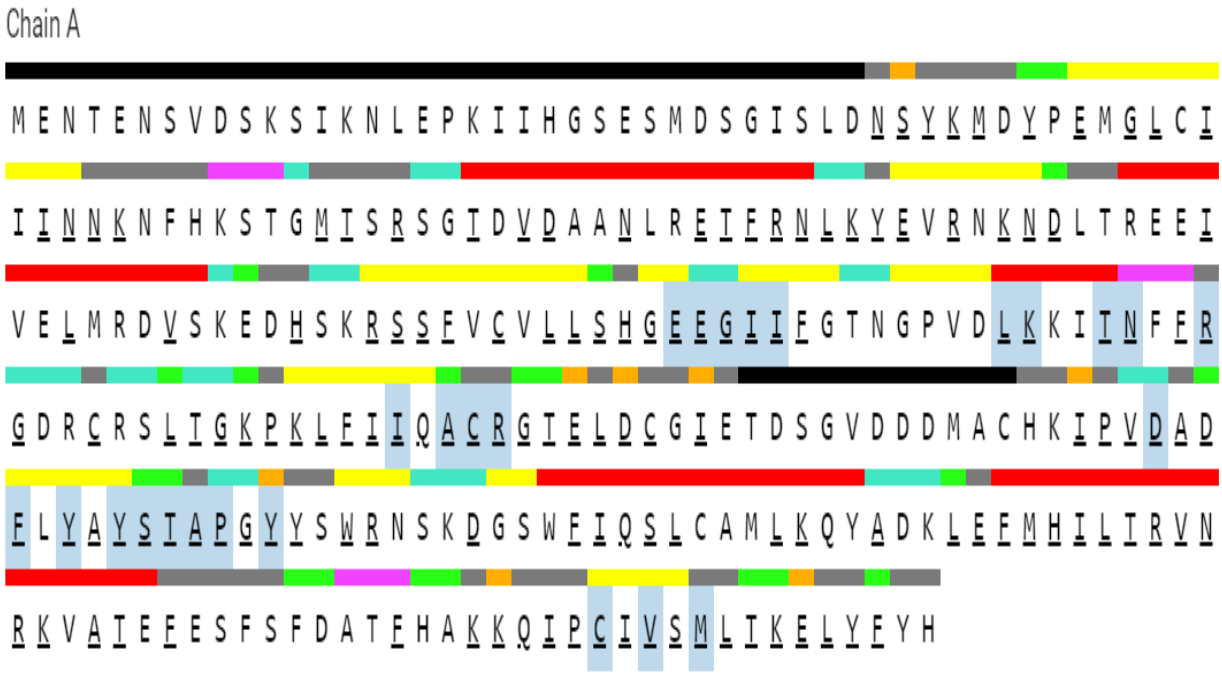

Figure 2.1CP3 Active Site 3. (A) 9,12,15-Octadecatrienoic Acid, Methyl Ester, (B) 9,12-Octadecadienoic Acid, (C) 9 Octadecene, (D) i. 9-Octadecenoic acid, ii. Phytol

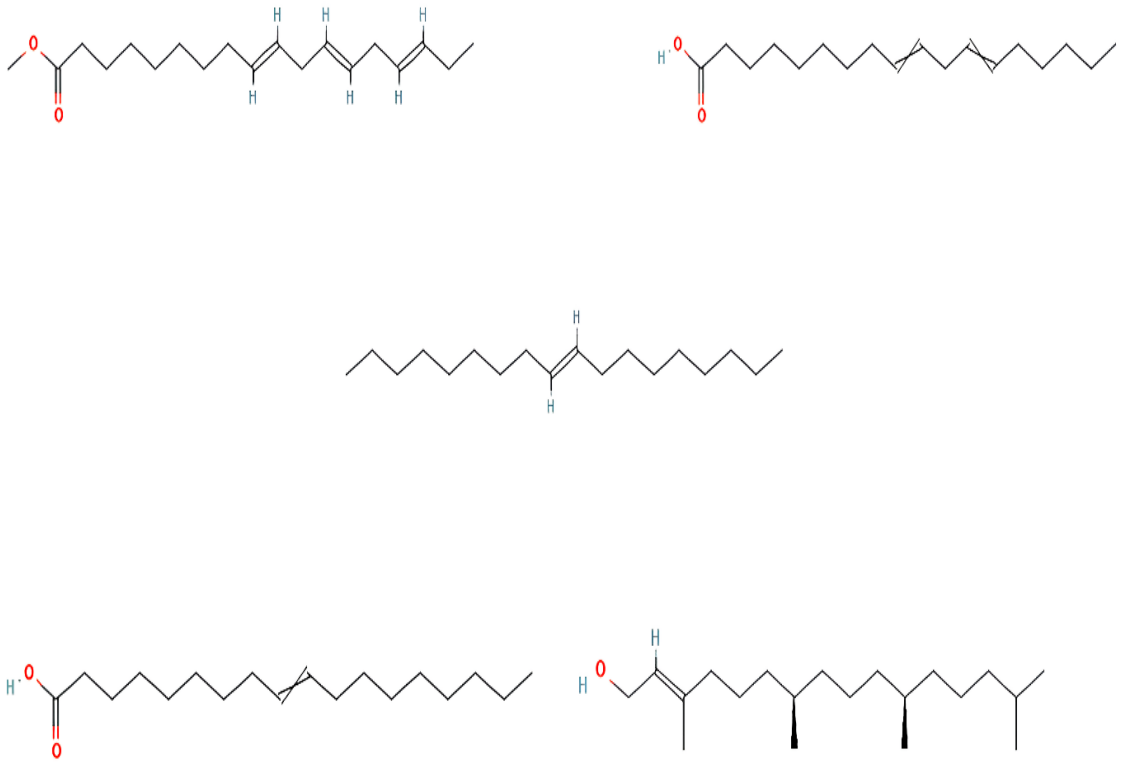

Figure 3. 3.(A) 9,12,15-Octadecatrienoic Acid, Methyl Ester, (B) 9,12-Octadecadienoic Acid, (C) 9 Octadecene, (D) i. 9-Octadecenoic Acid, ii. Phytol

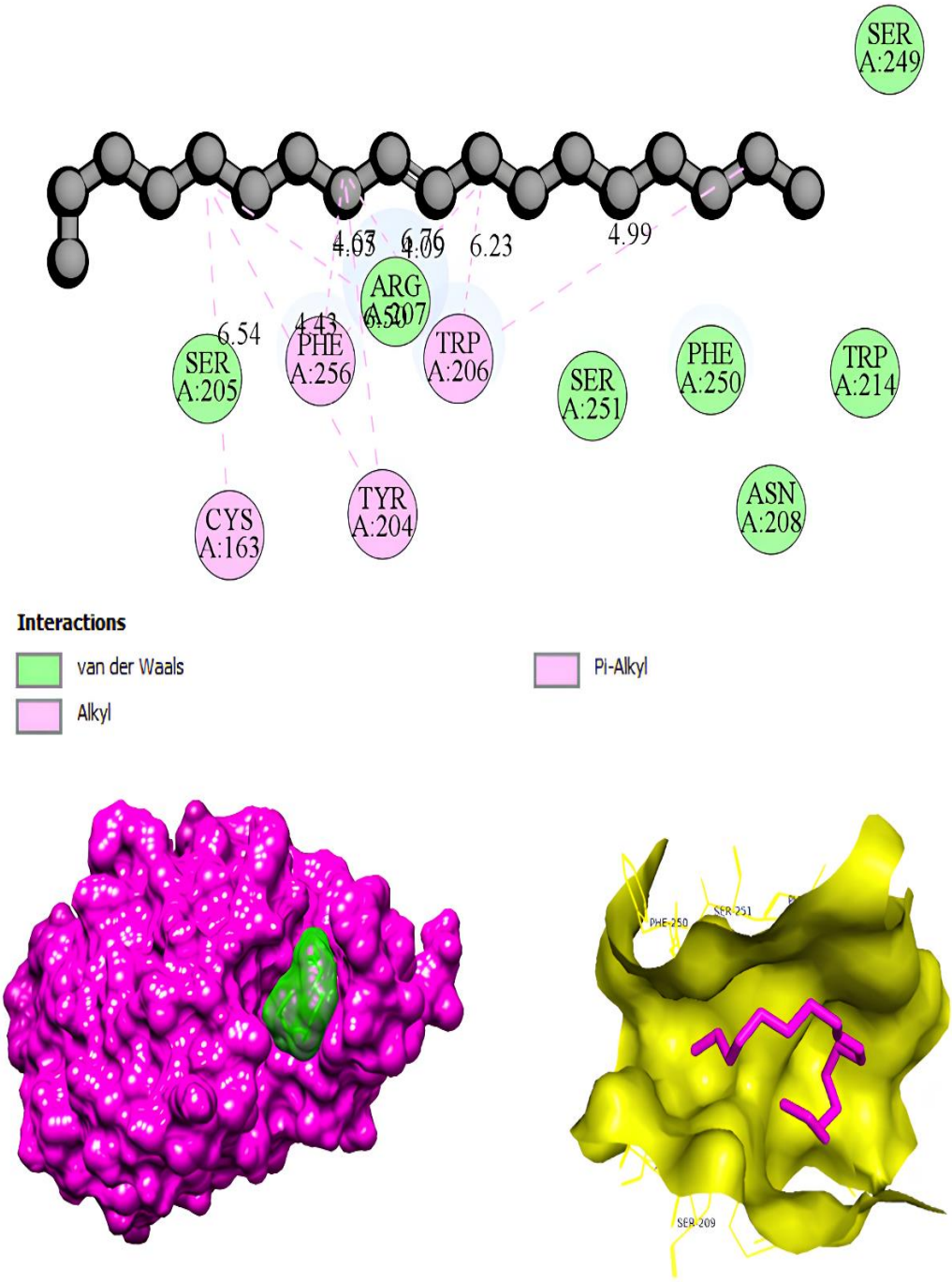

Figure 4. (A) Two-Dimensional View of 9-Octadecene Interaction with Protein of ICP3, (B) Three-Dimensional View of Protein Active/Binding Site with Ligand

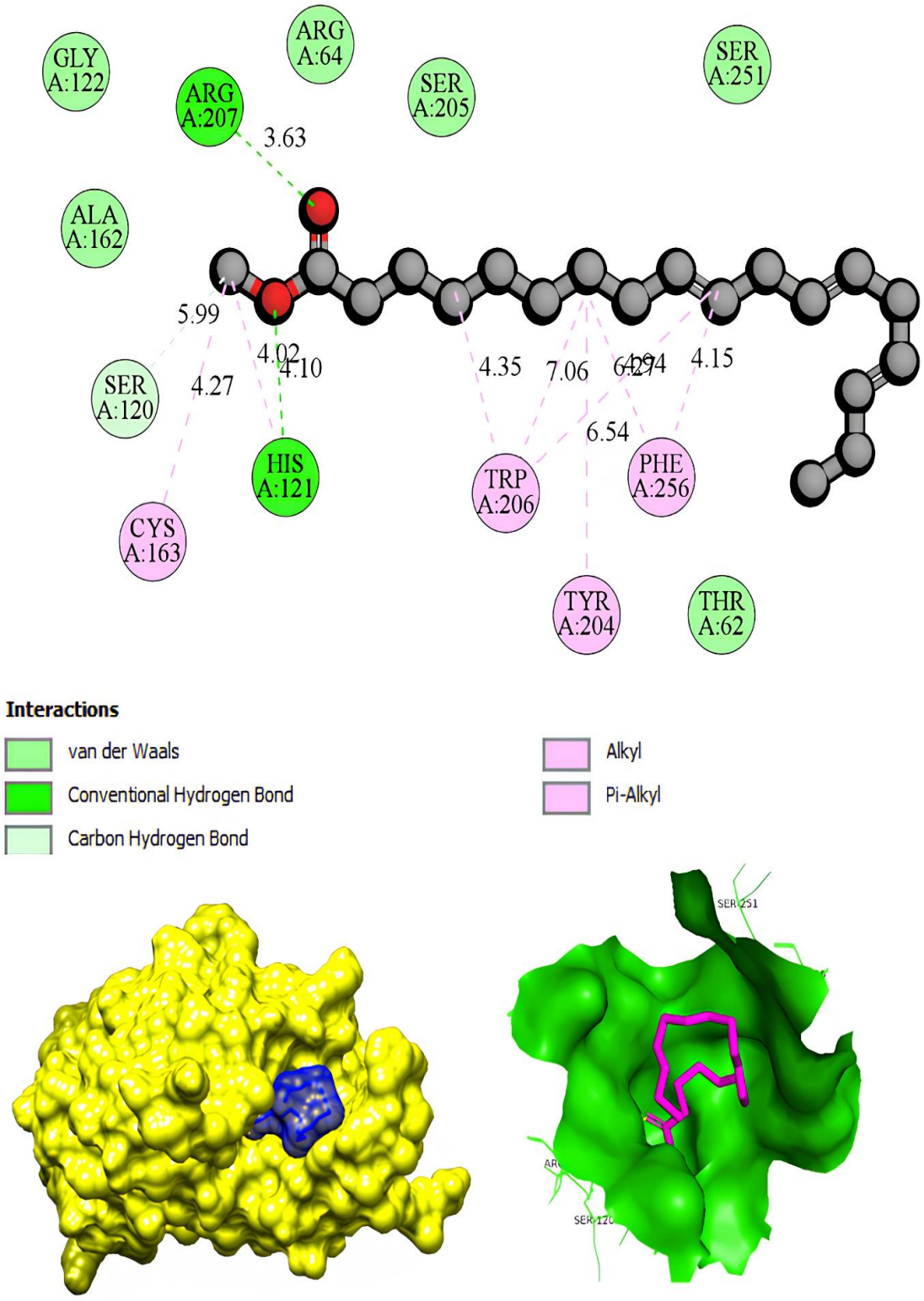

Figure 5. (A) 2D View of 9, 12, 15-Octadecatrienoic Acid, Methyl Ester Interaction with Protein of 1CP3, (B) 3D Surface View of Protein Active/Binding Site with Ligand.

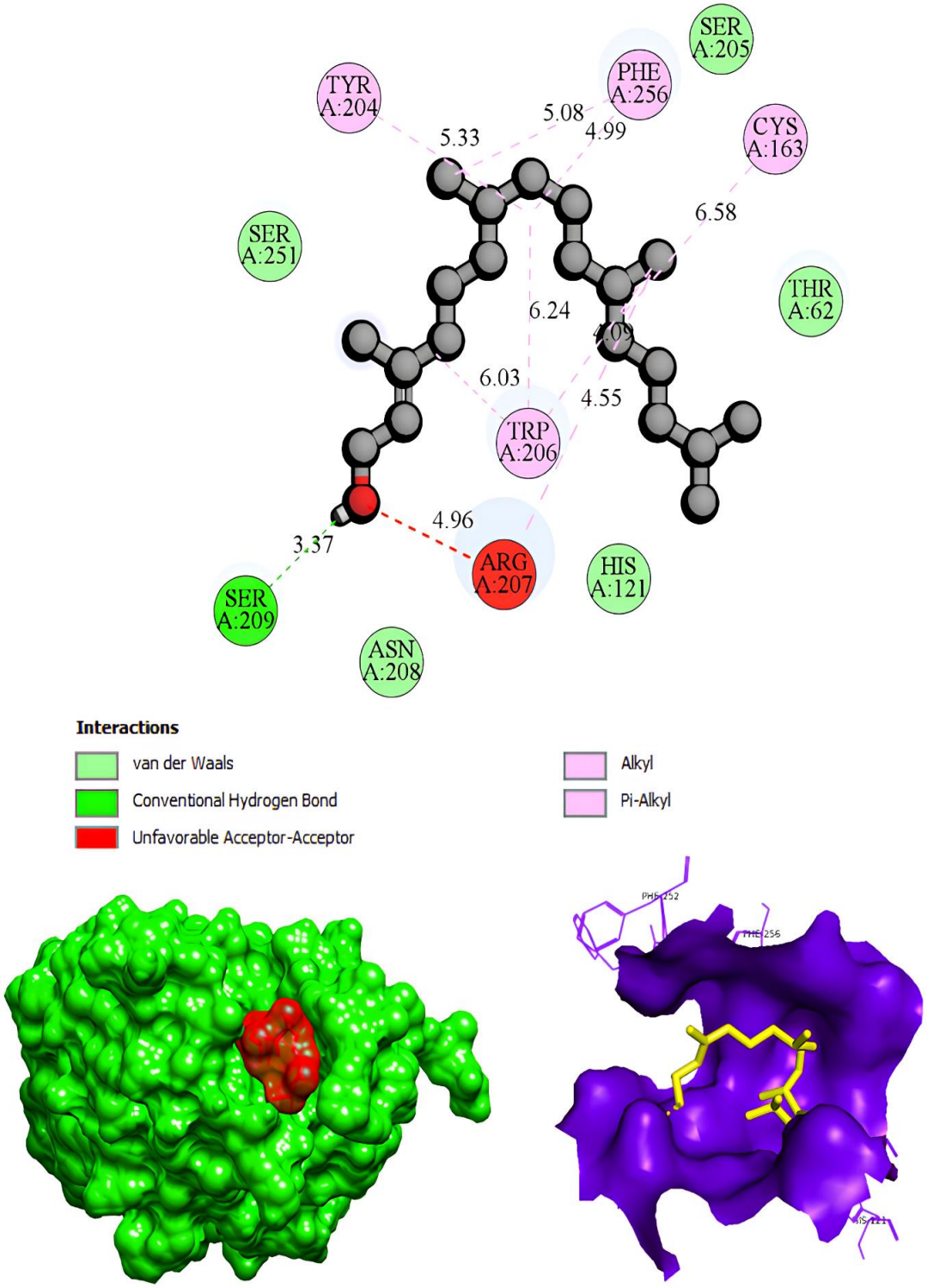

Figure 6. (A) 2D View of Phytol Interaction with Protein of 1CP3, (B) 3D Surface View of Protein Active/Binding Site with Ligand

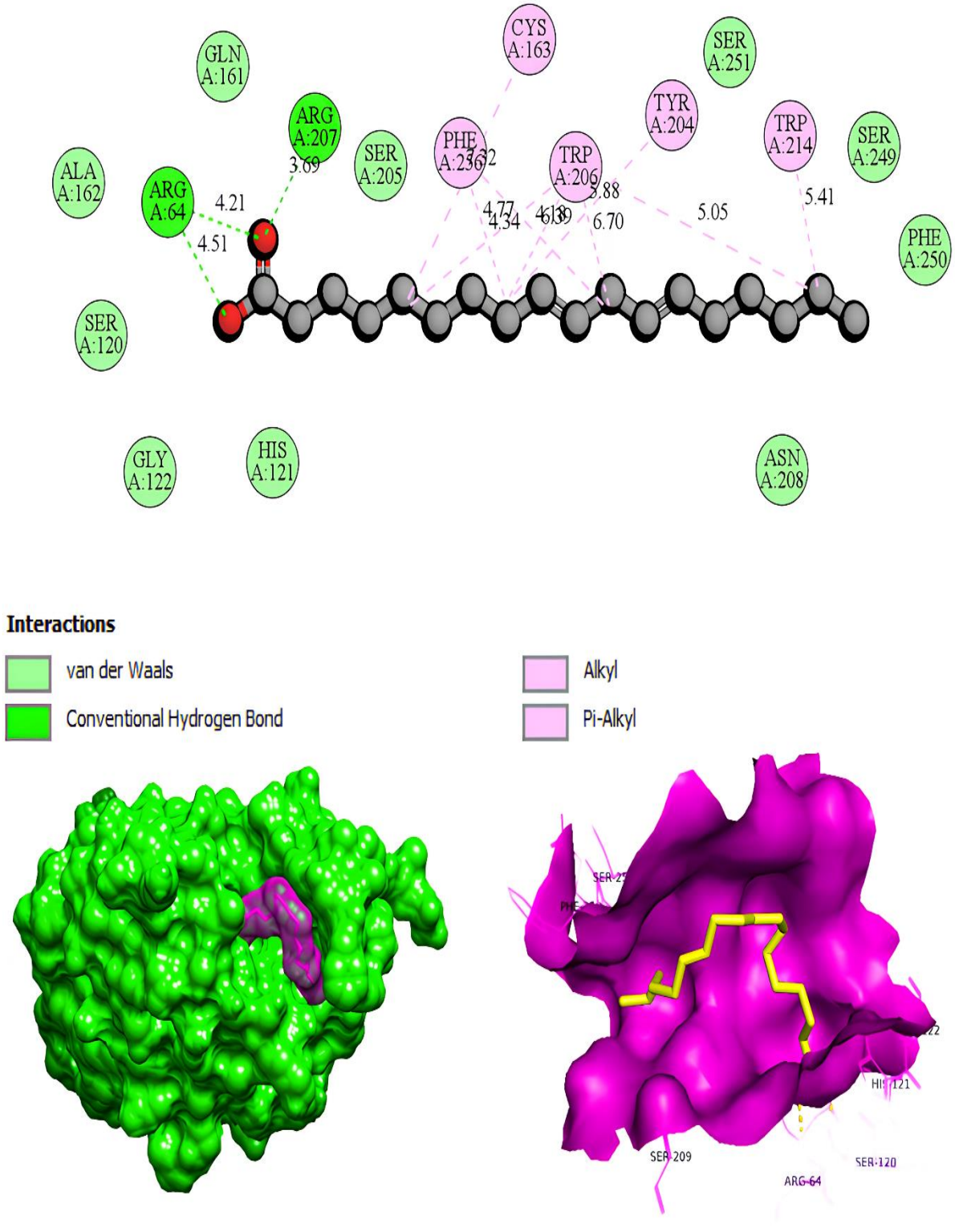

Figure 7. (A) 2D View of 9, 12-Octadecadienoic Acid Interaction with Protein of 1CP3, (B) 3D Surface View of Protein Active/Binding Site with Ligand.

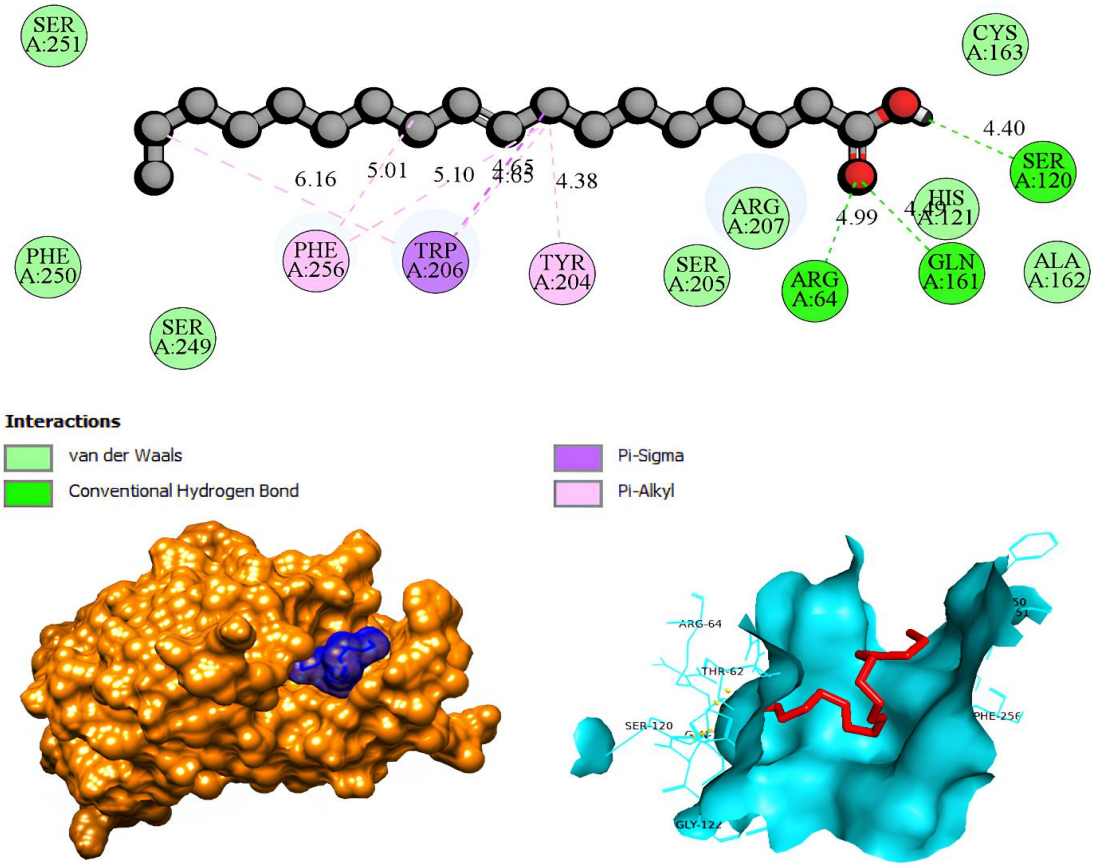

Figure 8. (A) 2D View of 9-Octadecenoic Acid Interaction with Protein of 1CP3, (B) 3D Surface View of Protein Active/Binding Site with Ligand

Discussion

Molecular docking is a technique used to predict the binding mode and interaction energy between a target protein and a ligand. It is an in-silico modelling approach that helps to significantly reduce the time and resources required for chemical synthesis and biological testing. By simulating the molecular interactions between the protein and ligand, molecular docking aids in the identification of potential drug candidates and the optimization of their binding affinity, thereby accelerating the drug discovery process. Computing models and simulations are therefore necessary to facilitate, accelerate and rationalize the discovery and development of medicinal products and to save time, money, and resources. Computer modelling and simulation currently account for approximately 10% of research and development (R&D) expenditure in the pharmaceutical industry. However, it is projected that by the year 2016, this percentage will double, reaching 20% of the total R&D expenditure. This increase underscores the growing importance and utilization of computational techniques in drug discovery and development processes within the pharmaceutical sector [11]. The application and usefulness of this virtual screening approach in combination with an activity-induced fractionation of medicinal plants was also recently demonstrated, which leads to the coined word “in the combination screen.”

Programmed cell death, commonly referred to as apoptosis, is a vital physiological process essential for the development and maintenance of organisms' health. This process is regulated by a family of cysteine proteases called caspases, which are cysteine-dependent

aspartate proteases. Caspases play a central role in initiating and executing apoptosis by cleaving specific cellular substrates, ultimately leading to controlled cell death [12]. Caspase is a strict endonuclease [13]. Dysregulation of apoptosis is implicated in a wide range of chronic diseases, encompassing neurodegenerative diseases, autoimmune disorders, stroke, myocardial infarction, and various types of cancer [14]. Developing drugs capable of modulating the apoptosis process has proven challenging for both researchers and pharmaceutical companies aiming to address pathological conditions stemming from abnormal apoptosis [15]. Among the 14 members comprising the Caspase family, Caspase 3 (also known as apopain) stands out as a critical executive enzyme pivotal in apoptosis. It oversees the physiological and morphological alterations characteristic of apoptosis and is notably expressed at relatively high levels across nearly all tissues [17-20]. The caspase of the hangman, caspase-3, plays an essential role in apoptosis and is a primary goal for cancer treatment. The expression of abnormal caspase 3 protein has been extensively studied in many types of cancer, including hepatocellular carcinoma [16, 21-22].

Results of the present study showed that the highest activation energy (-5.30 Kcal/mol) was found with 1,2-Benzenedicarboxylic acid, diethyl ester followed by Heptadecane, 8-methyl (-4.90 Kcal/mol), Tetradecane (-4.70 Kcal/mol), and Hexadecanoic acid (-4.00 Kcal/mol) while standard Doxorubicin (-6.90 Kcal/mol).

The 9-Octadecene docked with caspase 3 protein were found to be surrounded by the active site amino acid residues that are Ser 205, Phe 256, Cys 163, Arg 207, Trp 206, Trr 204, Ser 251, Phe 250 and are Asn 208, Trp 214, Ser 249. Among the active site residues, it was found that the fat block of amino acids interacts with proteins through non-covalent hydrogen bond interactions. It was found that the caspase 3 protein and the docked 9,12,15 octadecatrienoate methyl ester of gly 122, arg 207, arg 64, ser 205, amino acid residues in the active area, ser 251, Ala 162, Ser 120 and are surrounded by Cys 163, His 121, Trp 206, Tyr 204, Thr 62, Phe 256. Among the active site residues, it was found that these amino acids interact with proteins through non-covalent hydrogen bond interactions.

It was found that the caspase 3 protein and the docked phytol are surrounded by amino acid residues in the active area are Ser 251, Ser 209, Asn 208, Arg 207, His 121, Trp 206, Thr 62, Cys 163, Ser 205 and are Phe 256, Tyr 204. Among the active site residues, it was found that the fat block of amino acids interacts with proteins through non-covalent hydrogen bond interactions. It was found that 9,12-octadecadienoic acid, which is docked with the caspase 3 protein, is surrounded by amino acid residues in the active area, the Ala 162, Ser 120, Gly 122, His 121, Asn 208, Phe 250, Ser 249 and are Trp 214, Ser 251, Tyr 204, Trp 206, Cys 163, Phe 256, Ser 205, Arg 207, Gln 161, Arg 64. Among the active site residues, it was found, that which lies above the amino acid, interacts with proteins through non-covalent hydrogen bond interactions. It was found that the caspase 3 protein and the docked 9-octadecenoic acid are surrounded by amino acid residues in the active area are Ser 251, Phe 250, Ser 249, Phe 256, Trp 206, Trp 206, Tyr 204, Ser 205 and are Arg 207, Arg 64, Gln 161, His 121, Ala 162, Ser 120, Cys 163. Among the active site residues, it was found that the above amino acids interact with proteins through non-covalent hydrogen bond interactions.

The results of the silico docking show that compounds of plant origin have great potential for anti-cancer activity of the cancer mediator protein 1JPW. Among the four ligands, 2-benzene dicarboxylic acids, diethyl ester has a possible anti-cancer activity. These compounds with high binding activity exhibited strong binding affinity with caspase

3. Hence, they possess the potential to serve as primary agents in the therapeutic management of cancer.

Conclusion

In silico-molecular docking is a highly effective technology for identifying novel ligands for proteins with established structures and is crucial in the development of structure-based medicines. Hence, the present study employed silico-molecular docking to investigate the binding properties of 9-octadecene, 9,12, and 15-octadecatrienoic acid methyl ester, phytol, 9 and 12-octadecadienoic acid, and 9-octadecenoic acid with the target Protein 1CP3 (Caspase3). This study recommends that 9-octadecene, 9, 12 and 15-octadecatrienoic acid, methyl ester, phytol, 9 and 12-octadecadienoic acid and 9-octadecenoic acid can inhibit caspase-3. Among the various phyto-compounds, 9,12,15-octadecatrienoic acid has more possible bond interactions than other compounds. Therefore, this study can serve as evidence of *in vivo* cancer activity that helps these molecules to come onto the market as over-the-counter medicines.

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Ethical Approval

This study does not involve experiments with animals or human subjects.

Consent for Publication

Not applicable.

Availability of Data and Materials

Data and materials used/analyzed during the current study are available from the corresponding author upon reasonable request.

Conflict of Interests

The authors declare that there are no conflicts of interest.

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Not Applicable.

Author's Contributions

V.R.- Formal analysis; P.A.- Conceptualization, Supervision, writing-review and editing; R.V.- Project administration; M.J- Project administration. All authors have contributed equally to the article.

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