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Anti-Oxidant, Anti-Elastase, Anti-Collagenase, Anti-Hyaluronidase, Anti-Breast Cancer Activities of Some Natural Compounds

Nastaran SADEGHIAN[1*]

Highlights:

- Anticancer, collagenase, elastasei and hyaluronidase were studied.
- Clusiacyclol A, Cycloolivil, and Cyclopenol molecules studied.
- molecular docking analysis, molecular dynamics simulation and MM/GBSA calculation studied

Keywords:

- Natural compounds
- Antioxidant
- Enzyme inhibition
- Molecular docking
- Anti-breast cancer

ABSTRACT:

Natural products provide great potential for contemporary medication development due to their diverse biological activity, large range of sources, and structural diversity. In this investigation, the enzymes collagenase, elastasei and hyaluronidase were inhibited by Clusiacyclol A, Cycloolivil, and Cyclopenol molecules with excellent to good IC50 values of 11.35 ± 1.24, 75.36 ± 11.73, and 25.17 ± 3.38 µM for Clusiacyclol A, and 19.42 ± 2.66, 84.16±6.80, and 94.36± 8.46 µM for Cycloolivil, and 0.65±0.04, 50.24±5.07, and 66.01± 10.83 µM for Cyclopenol, respectively. These chemical compounds therefore might be able to suppress cancer cells and enzymes. Moreover, these substances markedly decreased the viability of breast cancer cells, even at low concentrations. Additionally, the impacts of breast cancer cells was significantly reduced at a 100 µM dosage of all compounds. The chemical bioactivities of Clusiacyclol A, Cycloolivil, and Cyclopenol against collagenase, hyaluronidase, and elastase were assessed by performing the molecular docking analysis, molecular dynamics (MD) simulation and MM/GBSA calculation. These substances' anti-cancer properties were evaluated about breast cancer cells, including Hs 281.T, SK-BR-3, MCF7, CAMA-1, MDA-MB-468, and NMU. Using in-silico techniques, they examined a few expressed surface receptor proteins, including the androgen receptor, progesterone receptor, estrogen receptor, and CD47.

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INTRODUCTION

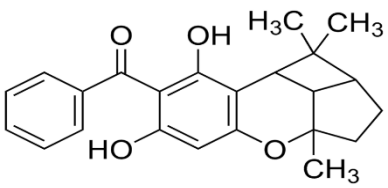
For thousands of years, nature has provided medical materials, and a staggering number of contemporary medications have their roots in it. The design, discovery, and development of new medications have been influenced by the diverse natural compounds found in plants, which have long been utilized as folk herbal medicines to treat a range of illnesses. The development of protein-based molecular targets has raised the need for novel chemical diversification in screening (Atanasov et al. 2021). Since much of the world's biodiversity is yet unknown, natural products will be crucial to satisfying this demand (Figure 1). Finding and utilizing suitable high-throughput screening bioassays, expanding the availability of bioactive compounds, and obtaining plant materials are just a few of the challenges still facing drug development from medicinal plants, which is still a major source of new therapeutic leads. Antioxidants are becoming more and more popular in today's world due to the social economy's quick expansion and the significant advancement in people's living standards. Free radicals are highly reactive molecules that are naturally produced by the body and have unpaired electrons. In numerous physiological functions, free radicals are crucial for controlling the immune system, communication, and cell cycle (Chopra & Dhingra 2021, Rastelli et al. 2020). Normally, a variety of endogenous and exogenous enzymatic or non-enzymatic processes result in the production and transformation of free radicals in cells in a dynamic equilibrium. However, unnecessary free radicals will be produced when the body is agitated by external stressors. High levels of free radicals, particularly reactive oxygen species (ROS), can result in oxidative stress, which can promote the growth of cancer cells, skeletal muscle atrophy, inflammation, and other chronic diseases by destroying redox signaling and regulation. This can also lead to molecular damage, including damage to cellular proteins, lipids, and DNA. Thus, it is critical to prevent the production of unnecessary free radicals in order to preserve cell redox equilibrium and guarantee the regular physiological operation of biological systems (Aware et al. 2022, Porras et al. 2020).

The International Agency for Research on Cancer (IARC) stated in 2020 that breast cancer is the most common cancer among women, overtaking lung cancer in incidence and ranking as the most common disease globally. Roughly 2.3 million new cases are diagnosed each year, accounting for 11.7% of all instances of cancer. Close to 685000 people die from breast cancer annually, making it the fifth most common cause of cancer-related mortality. By 2040, estimates point to a rise in new cases of breast cancer that will surpass 40.0%, with 3 million cases expected annually (Islam et al. 2022). Additionally, it is predicted that mortality rates will increase by more than 50.0%, with 1 million cases reported by 2040. In most nations, breast cancer accounts for 25% of all cancer incidences and 16.7% of all cancer deaths (Barbosa & Martel 2020).

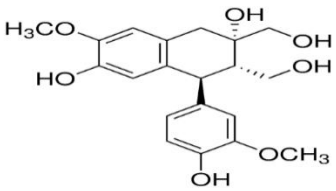
The hydrolase enzyme collagenase disassembles collagen's triple helix structure. Hydrolases carry out hydrolysis reactions, which include breaking down molecules with the help of ions, H^+, and OH from water. After its substrate, this significant enzyme is also known as matrix metallopeptidase-1 or matrix metalloprotease-1. Depending on the kind, collagenases range in size from 50 to 60 kDa, and zinc metal serves as their cofactor. The two categories of extracellular proteolytic enzymes are serine proteases, which have reactive serine amino acids at their active sites, and metalloproteases, which must have bound Ca^{2+} or Zn^{2+} ions to activate (Jiratchayamaethasakul et al. 2020, Hering et al. 2023). By breaking down matrix proteins including collagen, lanin, and fibronectin, serine proteases and metalloproteases promote cell motility. Collagenase is one of these vital enzymes. These enzymes play an important role in both pathological and physiological processes, including the spread of tumor cells to neighboring tissues and their disruption of their normal function. Physiological conditions include

wound healing, normal tissue and system structure, tissue remodeling, and normal developmental processes. Collagenases are endopeptidases that break down the triple-helixed natural collagen, which is the main fibrous component of animal connective tissue. Bacterial collagenases are very selective toward certain substrates, in contrast to vertebrate collagenases (Li et al. 2024). Unlike animal collagenases, bacterial collagenases are capable of breaking down both water-soluble denatured collagen and native water-insoluble collagen. Collagenase of this kind is capable of repeatedly dissolving all forms of collagen within the triple helix structure. Its weight varies based on kind, ranging from 50 to 60 kDa. Zinc metal is its cofactor. Furthermore, the enzymes known as elastases, which are found in the tissues of vertebrates, are members of the serine protease group and degrade elastin. Pancreatic elastase, fibroblast elastase, neutrophil elastase, and macrophage elastase are members of this group. Neutrophil elastase, for instance, is a 30 kDa glycoprotein with serine protease activity like chymotrypsin. In addition to elastin, other intercellular matrix proteins such as collagen, fibronectin, and others can also be broken down by the enzyme elastase. In order to facilitate wound healing under normal physiological settings, elastase enzyme activity is required to degrade foreign proteins produced by neutrophil phagocytosis (Eun-Ho & Young-Je 2020, Wang et al. 2024).

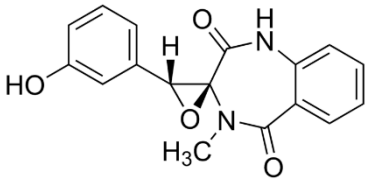
Proteins generally operate by engaging with their substrate within the body (Mihăşan 2012). Comprehending how proteins bind with their substrates or activators is essential for biologists. Molecular docking and MD simulation are practical strategies that allow the choosing of substances for enzymes to act upon. Another way this approach can be employed is to check if what has been obtained from the experiments fits the results (Poustforoosh et al.2022). In silico analysis can be a general technique that can provide this information and be used for discovering enzymatic substrates (Naghiyev et al. 2022). Interpreting empirical data is another benefit of employing computational methods, such as molecular docking study, MM/GBSA calculations, and MD simulation. The interaction between chemical compounds and biomolecules can be monitored at the atomic level via the use of molecular docking and MD (Jahantigh et al. 2023). There is a lot of emphasis on these useful computational methods for their part in drug product. Besides, comprehending which drugs work on certain targets in the body and understanding how they function on a molecular level can make it more effortless to design new drugs.



Clusiacyclol A



Cycloolivil



Cyclopenol

Figure 1. Structures of natural compounds (Clusiacyclol A, Cycloolivil, and Cyclopenol)

MATERIALS AND METHODS

DPPH Radical Scavenging Capacity

Using a previously described methodology, the DPPH scavenging capacity was evaluated. To summarize, 50 µL of the protein hydrolysate or xylo-oligosaccharide sample was combined with 3.9 mL of DPPH solution and left to incubate for 25 minutes in the absence of light. After centrifuging the solution for 10 minutes at 8000 × g, the absorbance at 517 nm was determined. Micromoles Trolox equivalents (TE) per gram of dry weight (µmol TE/g) were used to express the results (Chen et al. 2020).

ABTS Radical Scavenging Capacity

The prior approach was used to measure the ABTS scavenging capabilities of the protein hydrolysates and xylo-oligosaccharides. The sample (50 µL) was mixed with 3.9 mL of ABTS solution and incubated for 10 minutes in the dark. The resultant mixture's absorbance was then measured at 734 nm. Micromoles Trolox equivalents (TE) per gram of dry weight (µmol TE/g) were used to express the results (Takatsuk et al. 2022).

Collagenase Inhibitory Assay

Using a modified approach to measure the hydrolysis of the synthetic FALGPA, collagenase inhibitory activity was assessed. In 50 mM Tricine, 10 mM $CaCl_2$, 400 mM NaCl, pH 7.5) collagenase from Clostridium histolyticum (0.1 units) and FALGPA (1 mM) were produced. Before adding 20 µL of FALGPA, each protein hydrolysate or xylo-oligosaccharide sample (1 mg/mL, 30 µL) in deionized (DI) water was incubated for 15 minutes with collagenase (10 µL) in Tricine buffer (60 µL). Using DI water as a control, the absorbance at 345 nm was measured for 20 minutes. The formula (Absorbancesample – Absorbancecontrol)/(Absorbancecontrol) × 100 was utilized to get the inhibition (%) (Deniz et al. 2021).

Elastase Inhibition Assay

The previously described methodology was used to evaluate the inhibitory action of elsastase. To put it briefly, a solution of Tris–HCl buffer (2 mM, pH 8.0) containing porcine pancreatic elastase was mixed with protein hydrolysate or XOS sample. N-Succ-Ala-Ala-Ala-p-nitroanilide was added and incubated at 30 °C for 20 minutes after 15 minutes of incubation. At 410 nm, the absorbance was then measured. The collagenase inhibitory assay formula was used to calculate the enzyme inhibition activity (Juliana et al. 2020).

Hyaluronidase Inhibition Assay

The inhibitory action of hyaluronidase was ascertained using an earlier technique. In summary, the sample (0.5 mg/mL) was mixed with 0.1 M acetate buffer (pH 3.5) that included Type-1-S bovine testes hyaluronidase. The mixture was then allowed to incubate for 20 minutes at 37 °C. Finally, calcium chloride (20 µL, 12.5 mM) was added and the mixture was incubated for an additional 10 minutes. Then, after an additional 40 minutes of incubation, sodium hyaluronate was added. Sodium hydroxide was then added, and the mixture was incubated for ten minutes. After adding 50 µL of 4-(dimethylamino) benzaldehyde solution, the mixture was allowed to sit for ten minutes. Using the same formula as the collagenase inhibitory assay, absorbance was measured at 585 nm, and the inhibition (%) was calculated (He et al. 2021).

Anticancer assay

This study included anti-cancer assays on tumor breast cancer cell lines (Hs 281.T, SK-BR-3, MDA-MB-468, CAMA-1, MCF7, and NMU). Using the proper medium that contained 10% fetal bovine

serum, the monolayer cell culture was trypsinized and the cell density was increased to 1x105 cells/ml. Twenty-four hours prior to compound treatment, cells were plated on ninety-six-well microscope plates (50,000 cells/well) to encourage cell adhesion to the plate wall. The items under test were dissolved using dimethyl sulfoxide. after twenty-four hours, when a partial monolayer started to form (Sibuh et al. 2021). The leftover residue was drained. After once the monolayer was cleaned with the media, various concentrations of the test chemical (6.25, 12.5, 25, 50, and 100 µl) were added to the cell monolayer. For every concentration, three duplicate wells were created. Monolayer cells were exposed to the chemicals for a whole day at 37 °C with 5% CO2. One day later, 100 µL of dimethyl sulfoxide was added to the growth plate after it had been gently shaken to dissolve the formazan. A microplate reader was used to detect absorbance at 570 nm. Plotting the connection between the survival percentage and drug concentration yields the breast tumour cell line survival curve after the designated period. The formula will be used to determine the percentage of viability. The reference medication 5FU was used to compute the molar concentration (IC50) needed to inhibit cell viability by 50% (Yumei et al. 2023).

Molecular docking study

We employed molecular docking to see how a molecule and a protein make interaction with each other. Three structures of the enzymes Collagenase (PDB ID: 4AR1), Hyaluronidase (PDB ID: 2PE4), and elastase (PDB ID: 2Z7F) were obtained from the PDB database and studied. Since the advantages of synthetic and natural compounds, like their possibility to inhibit cancer cells, have been widely recognized (Mehrabani et al. 2020, Fazel et al. 2023), the anti-cancer activities of clusiacyclol A, cycloolivil, and cyclopenol were assessed against some breast cancer cells such as Hs 281.T, MCF7, CAMA-1, MDA-MB-468, SK-BR-3 and NMU. Surface receptors proteins were selected for these cell lines to be examined by computational methods. For Hs 281.T (Alibolandi et al. 2016), the folate receptor (PDB ID: 4LRH) was selected; for MDA-MB-468 (Dustin and Brian 2008), EGFR (PDB ID: 5WB7) was selected; for MCF7 (Bajalovic et al. 2022), progesterone receptor (PDB ID: 1A28) was selected; for CAMA-1 cells (Scott et al. 2020), estrogen receptor (PDB ID: 3OS8) was selected; for SK-BR-3 (Menck et al.2017), CD47 (PDB ID: 2JJS) was selected; androgen receptor (PDB ID: 2Q7I) was selected for NMU (Joly and Rochefort 1981). The molecular docking was done using Schrödinger's Maestro software (Maestro2021). Initially, the protein preparation tool was operated to confirm that the proteins were prepared correctly (Epik2016). The box was centroid of the protein active site (Poustforoosh et al. 2021). The chemical structures of clusiacyclol A, cycloolivil, and cyclopenol retrieved the PubChem database, in the SDF format. We used the LigPrep module within the Schrödinger software to prepare the compounds for investigation (LigPrep 2021). After all the preparations, the interactions among the molecules and the protein amino acids were analyzed using Glide ligand docking (Poustforoosh et al. 2022). The calculations were conducted by the OPLS force field (Poustforoosh et al.2024).

Binding free energy calculation (MM/GBSA)

The MM/GBS method was operated for the estimate of the binding free energy. Schrödinger's Prime was used for that, and the settings were left as they were. The solvation model of VSGB was utilized for the calculation, while the OPLS was set as the force field (Poustforoosh et al. 2022, Poustforoosh et al. 2022).

Molecular dynamics (MD) simulation

Molecular dynamics simulation was used to evaluate the interaction between protein and ligand molecules (Türkan et al. 2022, Taysi et al. 2023). Desmond of Schrödinger was used to perform the MD

simulation. Using the OPLS force field to lower the bond energy between the proteins and the ligand was the first step in the molecular dynamics simulation (Poustforoosh et al. 2022). After the chemical was put inside an orthorhombic box with a 10 Å partition to create a water box, the structure was equilibrated by adding a certain amount of Na^+/Cl^- ions (Rashedinia et al. 2023). The study used specific measurements, such as temperature, pressure, time, and distance, to investigate how a chemical substance interacts with a protein (Sadeghian et al. 2024). The cut-off distance was set at nine angstroms, and each step was separated by two femtoseconds (Poustforoosh, 2024). The pressure was kept at 1.01325 bar and the initial temperature was set at 310.15 K, or 37 °C (Poustforoosh 2024). Van der Waals and electrostatic interactions were studied during the 100 ns simulation (Poustforoosh et al. 2023). RMSD, contacts, ligand properties, and the protein's secondary structure were among the metrics that were analyzed.

RESULTS AND DISCUSSION

Antioxidant results

For thousands of years, people have turned to medicinal and fragrant plants, particularly those with ethnopharmacological applications, as a natural source of healing and wellness. Poultices, decoctions, tinctures, inhalations, teas, macerations, infusions, percolation products, and other herbal preparations were among the early forms of these widely used drugs. Oral tradition has been used to pass down the exact dosage of a plant and the method of administration for treating particular ailments from one generation to the next. Eventually, traditional pharmacopeias recorded the knowledge of medicinal plants (Diniz do Nascimento et al. 2020). The process of finding drugs from medicinal plants has involved a variety of research approaches and disciplines. Ethnobotanists, botanists, ethnopharmacologists, and plant ecologists often collect and identify the plants of interest. Thanks to recent technological developments, plants can now be utilized as "factories" to create natural medicinal compounds for use in the development of biotech medications, cures, and treatments. One of the few stable organic nitrogen radicals is the DPPH radical test, which has a rich purple hue (Gaber and Shalaby2021). The foundation of this process is the evaluation of antioxidant compounds' capacity to reduce DPPH radicals. The half maximal radical scavenging concentrations (IC50) of cycloolivil, cyclopenol, clusiacyclol A, and Trolox, a reference radical scavenger, are displayed in Figure 2. The results showed that the IC50 values for cycloolivil, trolox, clusiacyclol A, and cyclopenol were 7.61 mg/mL, 8.55 mg/mL, 9.36 mg/mL, and 10.66 mg/mL, respectively. Additionally, ABTS scavenging activity is another enhanced method for identifying radical scavenging. When ABTS and $K_2S_2O_8$ were combined, ABTS radicals were produced. The IC50 values of cycloolivil, clusiacyclol A, Trolox as a reference radical scavenger, and cyclopenol are displayed in Figure 2. The results showed that the IC50 values for cycloolivil, clusiacyclol A, Trolox, and cyclopenol were 4.53 mg/mL, 5.75 mg/mL, 6.73 mg/mL, and 9.08 mg/mL, respectively.

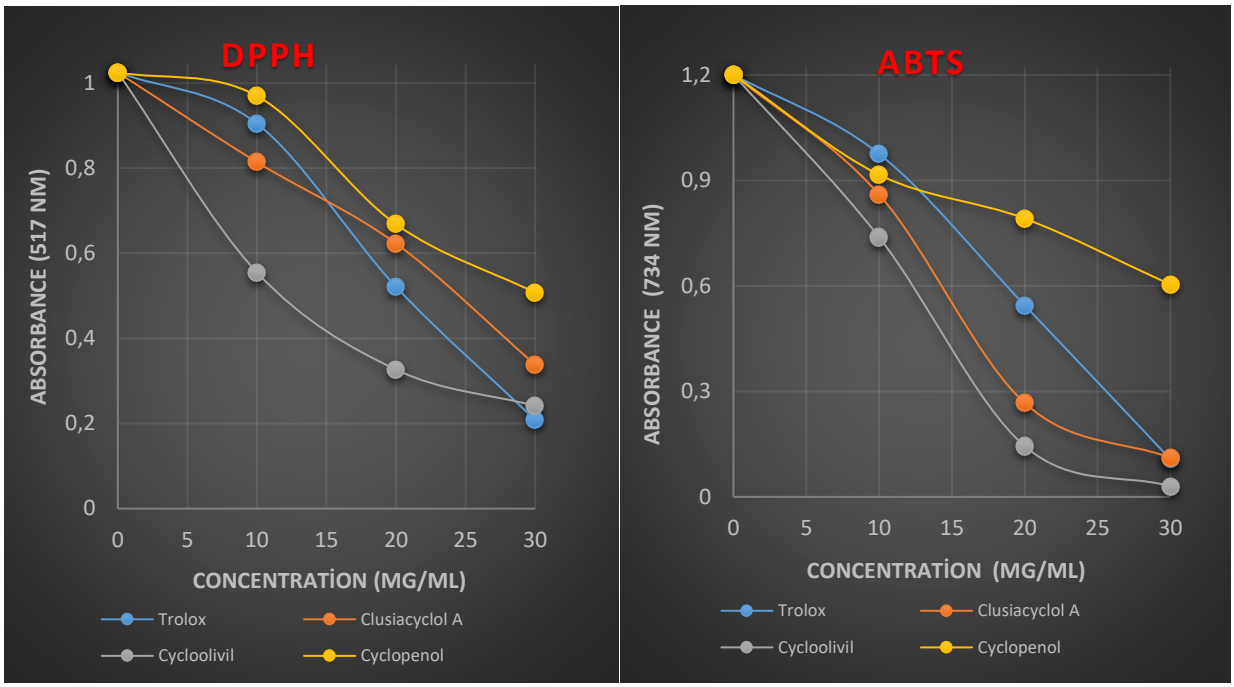


Figure 2. Antioxidant graphs of studied naturals compounds

Enzymes inhibition results

For collagenase enzyme, the inhibitory effects of all the natural substances on collagenase activity were greater than those of the reference compound, oleanolic acid (IC_{50} 21.54 ± 3.13 µg/mL). Based on the IC_{50} values that were computed for the natural substances presented in Table 1, Cyclopenol had the good anti-collagenase activity, measuring 0.65±0.04 µg/mL. Out of all the substances, Cycloolivil showed the weak amount of influence on collagenase activity (IC_{50} 19.42 ± 2.66 µg/mL), but it was still higher than the standard. Even though a lot of research has been done to find new synthetic and natural collagenase, elastase, and hyaluronidase enzyme inhibitory compounds, there is still a great need for new inhibitors of these enzymes because the current inhibitors have either low efficacy or side effects. Furthermore, there are currently very few of these enzyme inhibitors on the market, and the cosmetics and wound-healing industries are the key markets for novel inhibitors (Go et al.2020). Through a variety of in vitro experiments, we have examined a great deal of natural chemicals to date. As a result of these investigations, we have discovered various inhibitors of the enzymes collagenase, elastase, and hyaluronidase, including Clusiacyclol A, Cycloolivil, and Cyclopenol. The inhibitory effects of all the natural substances on hyaluronidase activity were greater than those of the reference substance, oleanolic acid (IC_{50} 101.36 ± 7.83 µg/mL). Based on the IC_{50} values that were computed for the natural substances presented in Table 1, Clusiacyclol A had the powerful anti-hyaluronidase activity, measuring 25.17 ± 3.38 µg/mL. Out of all the substances, Cycloolivil showed the least amount of influence on hyaluronidase activity (IC_{50} 94.36± 8.46 µg/mL), but it was still higher than the standard. Additionally, the inhibitory effects of all the natural substances on elastase activity were greater than those of the reference substance, oleanolic acid (IC_{50} 91.38 ± 6.20 µg/mL). Based on the IC_{50} values that were computed for the natural substances presented in Table 1, Cyclopenol had the powerful anti-elastase activity, measuring 50.24±5.07 µg/mL. Out of all the substances, Cycloolivil showed the weak amount of impact on elastase activity (IC_{50} 84.16±6.80 µg/mL), but it was still higher than the standard.

Table 1. Anticancer results of some natural compounds Clusiacyclol A, Cycloolivil, and Cyclopenol

Enzymes / Compounds	Clusiacyclol A	Cycloolivil	Cyclopenol
	(IC_{50} as µM)		
Collagenase	11.35 ± 1.24	19.42 ± 2.66	0.65±0.04
Elastase	75.36±11.73	84.16±6.80	50.24±5.07
Hyaluronidase	25.17 ± 3.38	94.36± 8.46	66.01± 10.83

Anticancer results

The FDA has approved fulvestrant, lapatinib, eribulin mesylate, pertuzumab, everolimus, and many additional medications for the treatment of breast cancer, including different subtypes of the disease. These medications' tendency to develop resistance has limited their use, and we still need some alternatives to provide a 100% effective treatment for breast cancer. It is necessary to create a new class of anticancer drugs that specifically target breast cancer cells despite in-depth study and swift advancements in cancer treatment. These days, there are many strong bioactive substances that can be employed as effective anticancer agents in breast cancer treatment. These substances can come from both synthetic and natural sources (AbdulJabar et al.2021).

The chemical performed well on breast cancer cell lines (micromolar). One by one, the breast results show that MDA-MB-468 (0.58± 0.04 µM) from breast cell lines performed well when treated with Cycloolivil. In terms of breast cancer outcomes, the top performers were also MDA-MB-468 with Clusiacyclol A (1.15±0.14 µM) and CAMA-1 cell line with Cycloolivil (1.74± 0.14 µM). The IC50 values of Cycloolivil as a natural chemical for breast cancer cell lines are provided in the following order, respectively Table 2: MDA-MB-468 (0.58± 0.04 µg/mL) < CAMA-1 (1.74± 0.14 µg/mL) < SK-BR-3 (2.46± 0.41 µg/mL) < Hs 281.T (3.29 ± 0.15 µg/mL) < NMU (6.03±0.30 µg/mL). Additionally, the IC50 values for 5FU for breast cancer cell lines are provided in the following order, respectively: MDA-MB-468 (1.06±0.28 µg/mL) < CAMA-1 (3.58±0.24 µg/mL) < SK-BR-3 (7.54±0.66 µg/mL) < Hs 281.T (16.05±1.36 µg/mL) < MCF7 (19.47±3.57 µg/mL).

For Clusiacyclol A, IC50 values of some cancer cell lines are given in the following order respectively (Table 2): MDA-MB-468 (1.15±0.14 µg/mL) < CAMA-1 (4.42 ± 0.34 µg/mL) < SK-BR-3 (10.17 ±0.88 µg/mL) < Hs 281.T (10.66 ± 1.35 µg/mL) < MCF7 (22.68± 3.50 µg/mL).

Table 2. Anticancer results of some natural compounds Clusiacyclol A, Cycloolivil, and Cyclopenol

Cell lines / Compounds	Clusiacyclol A	Cycloolivil	Cyclopenol	5FU
	(IC_{50} as µM)			
Hs 281.T	10.66 ± 1.35	3.29 ± 0.15	43.15±3.55	16.05±1.36
MDA-MB-468	1.15±0.14	0.58± 0.04	1.94±0.23	1.06±0.28
MCF7	22.68± 3.50	17.10±1.08	39.13 ± 7.02	19.47±3.57
CAMA-1	4.42 ± 0.34	1.74± 0.14	45.13± 6.31	3.58±0.24
SK-BR-3	10.17 ±0.88	2.46± 0.41	11.26±0.98	7.54±0.66
NMU	23.56 ± 3.71	6.03±0.30	27.65 ±4.78	20.15±3.28

Computational study

Evaluating the efficacy of a molecule in advance allows for enhancements to be made prior to its experimental application. Molecular docking is frequently employed to investigate the interactions between a molecule and a protein. A useful analogy for comprehending molecular modeling calculations is to consider the interaction between biomolecules and protein molecules as akin to the mechanism of a key fitting into a lock. The behavior of these molecules is predominantly determined by these crucial interactions. Scientists observed that an increase in the frequency of these connections resulted in enhanced efficacy of the molecules against proteins. Some of these interactions are polar, hydrophobic

interactions, hydrogen bonds, and π-π (Kafa et al.2022). The computations illustrate how the molecules interact with enzyme. These docking scores are an indication of the binding affinities of the compounds to the enzymes, which can be found in Table 3, and the interactions between the ligands with higher binding affinity for each enzyme are presented in Figures 3-5. Table 4 shows the docking scores of these compounds against the receptor proteins.

The docking score's significance lies in its capacity to demonstrate the relative performance of one molecule compared to others. The lowest docking score between the ligand-enzymes belongs to Clusiacyclol A-hyaluronidase with the value of -7.807. The lowest docking scores for collagenase and elastase are -5.557 and -3.615, respectively, which belongs to Cycloolivil. These results show that Clusiacyclol A sticks to hyaluronidase more strongly than other enzymes. Figure 3 illustrates the establishment of four hydrogen bonds in the investigation of the interaction between Clusiacyclol A and four residues of hyaluronidase. These residues are Tyr75, Glu131, Tyr247, and Trp321. Moreover, there exists one Pi-Pi stacking interaction involving the compound and Tyr202. Figure 3 displays the presentation of the pose and interactions between Clusiacyclol A and hyaluronidase. Figure 4 shows the pose and interactions of Cycloolivil with collagenase. Cycloolivil has constructed five hydrogen bonds with Arg464, Asp388 (two bonds), Thr650, and Arg652 of collagenase. There are also three Pi-cation interactions between Cycloolivil and collagenase (Figure 3). The residues with Pi-cation interactions are Lys375, Lys641, and Arg652. Cycloolivil has also formed two hydrogen bonds with the residues of elastase. These residues are Cys168 and Arg129. Figure 5 shows the pose and created interactions. Table 4 displays the results for these substances and surface receptors.

The binding free energies were estimated by MM/GBSA method. The results for the compounds against the enzymes are presented in Table 5. This value for Clusiacyclol A is -34.58, -35.51, and -47.08 kcal/mol for hyaluronidase, collagenase, and elastase, respectively. The results for Cycloolivil and Cyclopenol are presented in Table 5. Data presented in Table 6 are the binding free energies of these three compounds against the surface receptor proteins.

Table 3. Docking scores of the compounds against the enzymes

	Hyaluronidase	Collagenase	Elastase
Clusiacyclol A	-7.807	-2.967	-1.919
Cycloolivil	-6.646	-5.557	-3.615
Cyclopenol	-3.333	-2.188	-0.113

Table 4. Docking scores of the compounds against the receptor proteins

	Folate receptor (Hs 281.T)	EGFR (MDA-MB-468)	Progesterone receptor (MCF7)	Estrogen Receptor (CAMA-1)	CD47 (SK-BR-3)	Androgen receptor (NMU)
Clusiacyclol A	-8.549	-4.655	-0.611	-2.478	-4.028	-
Cycloolivil	-9.018	-6.536	-3.430	-11.981	-4.638	-
Cyclopenol	-3.684	-3.523	-0.504	-6.123	-2.470	-6.328

Table 5. The results of MM/GBSA for the compounds against the enzymes

	Hyaluronidase	Collagenase	Elastase
Clusiacyclol A	-34.58	-35.51	-47.08
Cycloolivil	-44.63	-33.43	-42.28
Cyclopenol	-34.12	-40.31	-35.74

Table 6. The results of MM/GBSA for the compounds against the receptor proteins

	Folate receptor (Hs 281.T)	EGFR (MDA-MB-468)	Progesterone receptor (MCF7)	Estrogen Receptor (CAMA-1)	CD47 (SK-BR-3)	Androgen receptor (NMU)
Clusiacyclol A	-57.63	-43.73	-52.37	-38.92	-18.58	-
Cycloolivil	-61.61	-41.20	-34.09	-64.41	-30.43	-
Cyclopenol	-42.54	-44.65	-40.53	-57.41	-21.34	-47.49

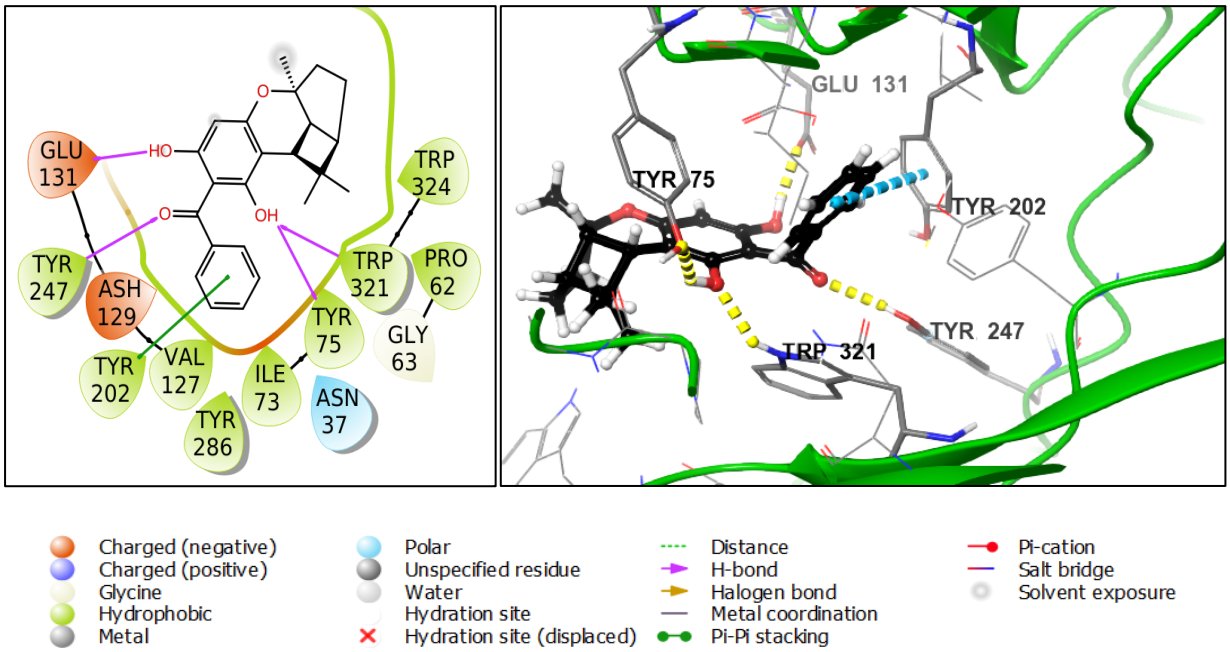


Figure 3. Clusiacyclol A's docking position (right) and created contacts (left) in the hyaluronidase

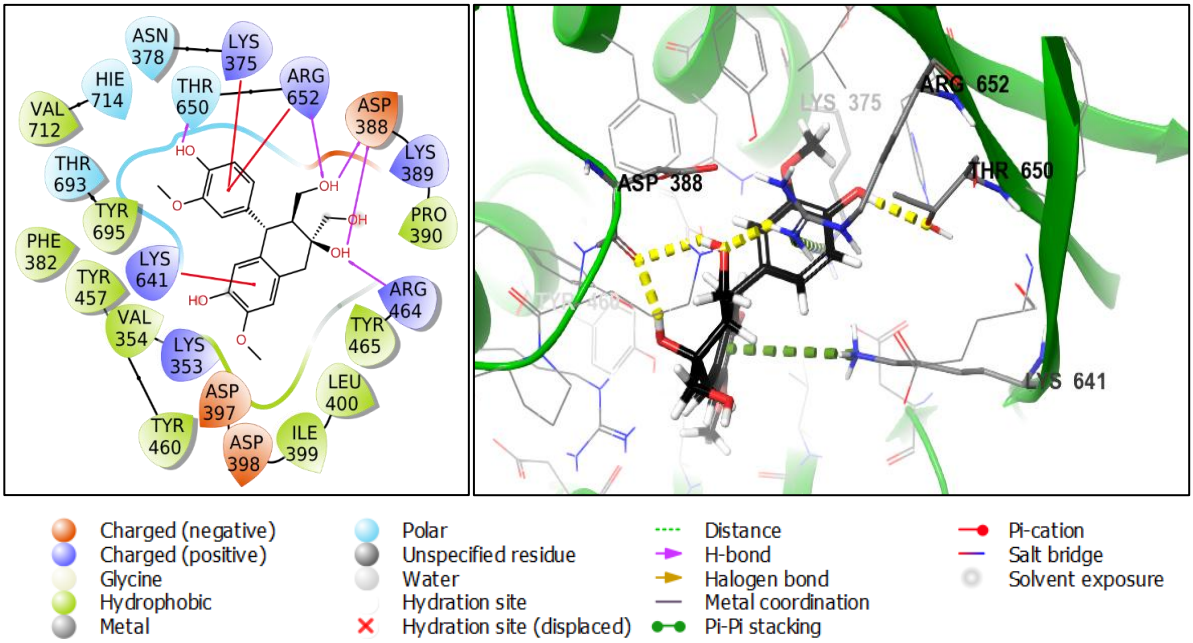


Figure 4. Cycloolivil's built interactions (left) and docking position (right) in the collaactive sitegenase active site

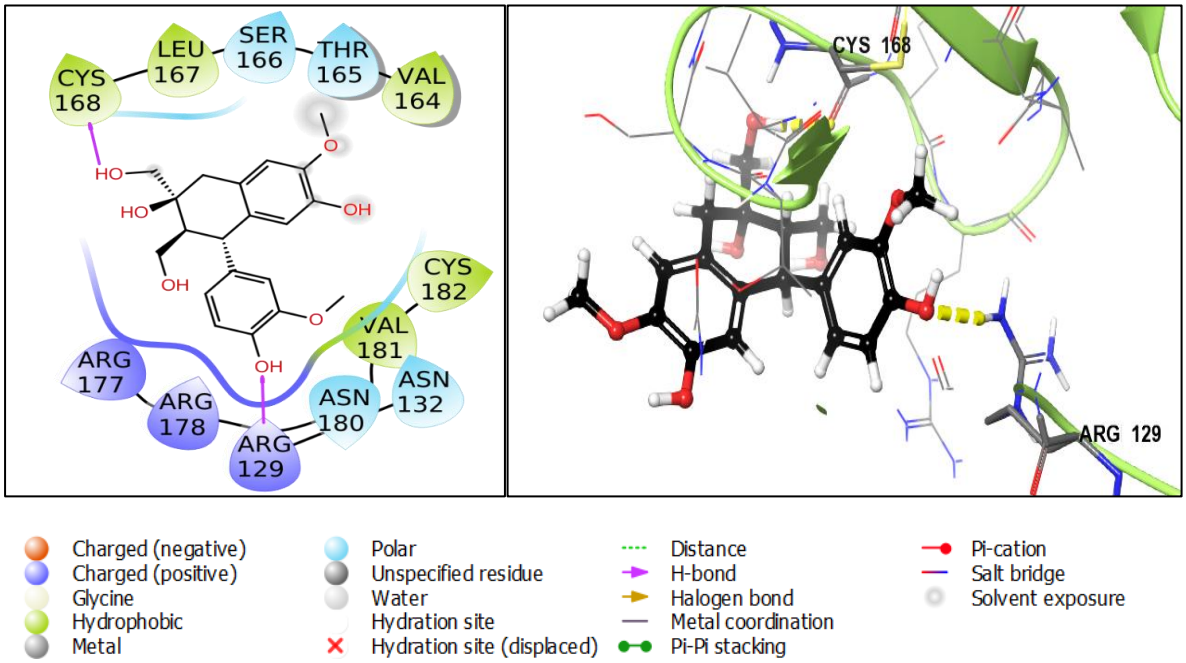


Figure 5. Cycloolivil's built interactions (left) and docking position (right) in the elastase active site

In molecular docking simulations, it is assumed that the ligand molecules exhibit flexibility, allowing them to adjust their binding orientation to the receptor, whereas the receptor proteins are generally considered to be static and immobile. The protein needs to exhibit sufficient mobility to accurately evaluate the interaction and impact of the drug on its energy levels and movement. In order to gain further insights into the dynamics and structural characteristics of the protein, we conducted a molecular dynamics simulation lasting 100 nanoseconds for Clusiacyclol A-Hyaluronidase, which has the lowest docking scores among the enzymes. Utilizing this simulation allowed us to gain deeper insights into the mechanisms that govern the long-term stability, dynamic mobility, and conformational alterations of the protein complex (Poustforoosh et al. 2023). We analyzed root mean square deviation (RMSD) metrics and corresponding graphical representations illustrating the interactions between ligands and amino acids within this framework. Furthermore, a range of characteristics of the ligand was examined. These are shown in Figures 6-8. The stability of the complexes was assessed through the calculation of the RMSD. The variations noted in the root mean square deviation (RMSD) indicate alterations in structure throughout the simulation (Poustforoosh et al. 2023). Within the simulation, this computation determines the mean variation in the positions of the Cα backbones from the initial state to the final state. The RMSD of the hyaluronidase has stabilized at approximately 0.8 Å, suggesting that the protein maintains a stable conformation. A small molecule binds to a designated site on a protein, facilitating the protein's recognition process by forming distinctive interactions (Figure 6). However, the substance is unable to engage in a similar manner with any other protein. The Molecular Surface Area (MolSA) of the protein exhibited continuous variation within a defined range, as illustrated by the van der Waals surface representation (Figure 7). The dimensions of the solvent-accessible surface area (SASA) frequently reflect the interaction dynamics between a ligand interacting with a solvent and the corresponding behavior of a protein. The system's stability is improved by the minimal value. The polar surface area (PSA) of the molecule is the solvent's ability to readily interact with other substances. The examination is confined to the portions of the molecule that include oxygen and nitrogen. These occurrences can be attributed to the interaction that has been formed between the protein and the molecule (Figure 7). During the course of the simulation, the changes to the protein's secondary structure

were also evaluated. Figure 8 illustrates the alterations in the secondary structure elements (SSE) of hyaluronidase in response to the presence of Clusiacyclol A. he percentage of SSE throughout the MD simulation has remained relatively constant, indicating the stability of the protein during the simulation. The findings indicated that Clusiacyclol A has the capability to bind to the active sites of enzymes and receptors, thereby inhibiting their functionality. Nevertheless, for this compound to exhibit optimal inhibitory activity, it must be transported to the intended target site. Modified vesicular nanoparticles represent one of the most suitable approaches to tackle this issue (Poustforoosh et al.2022, Asadikaram et al. 2021).

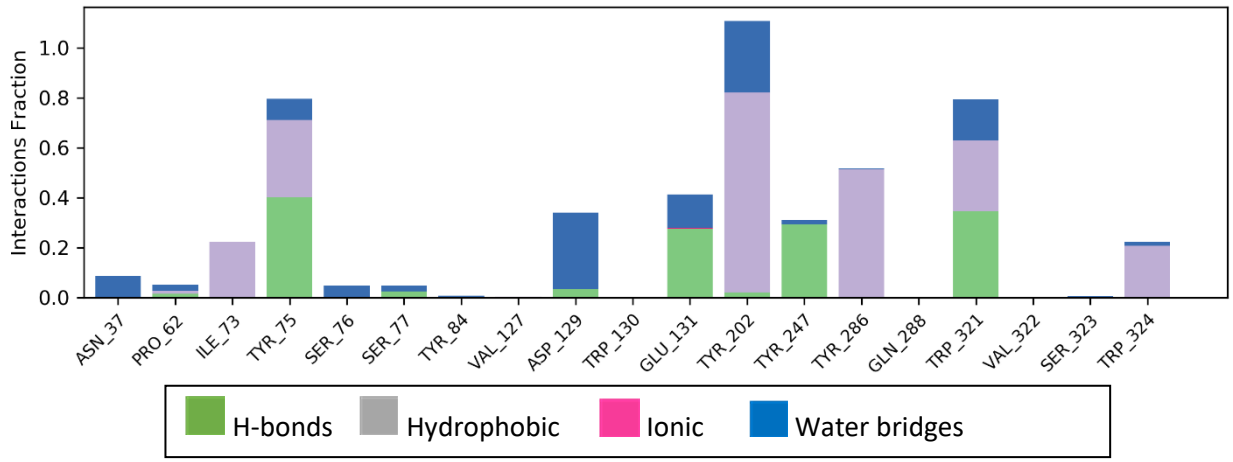


Figure 6. The interactions of Clusiacyclol A and hyaluronidase constructed between this compound and the enzyme during the MD simulation

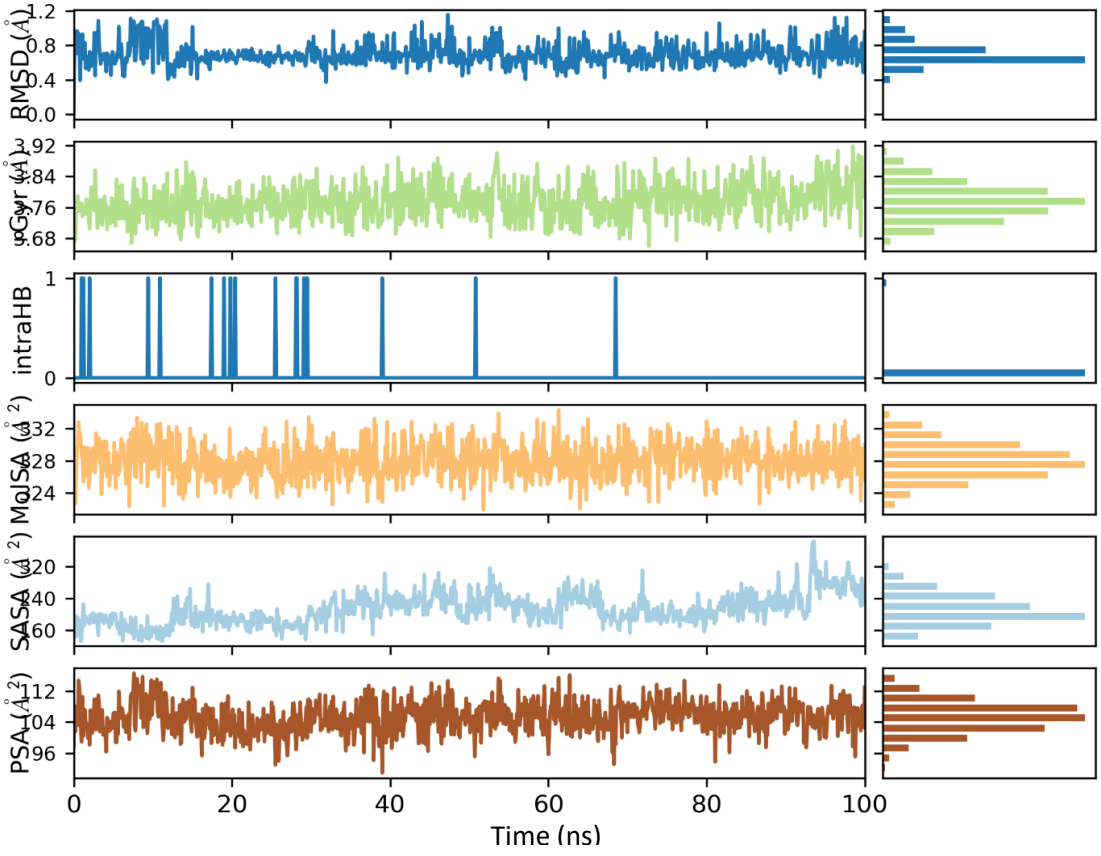


Figure 7. The ligand properties of Clusiacyclol A during the simulation

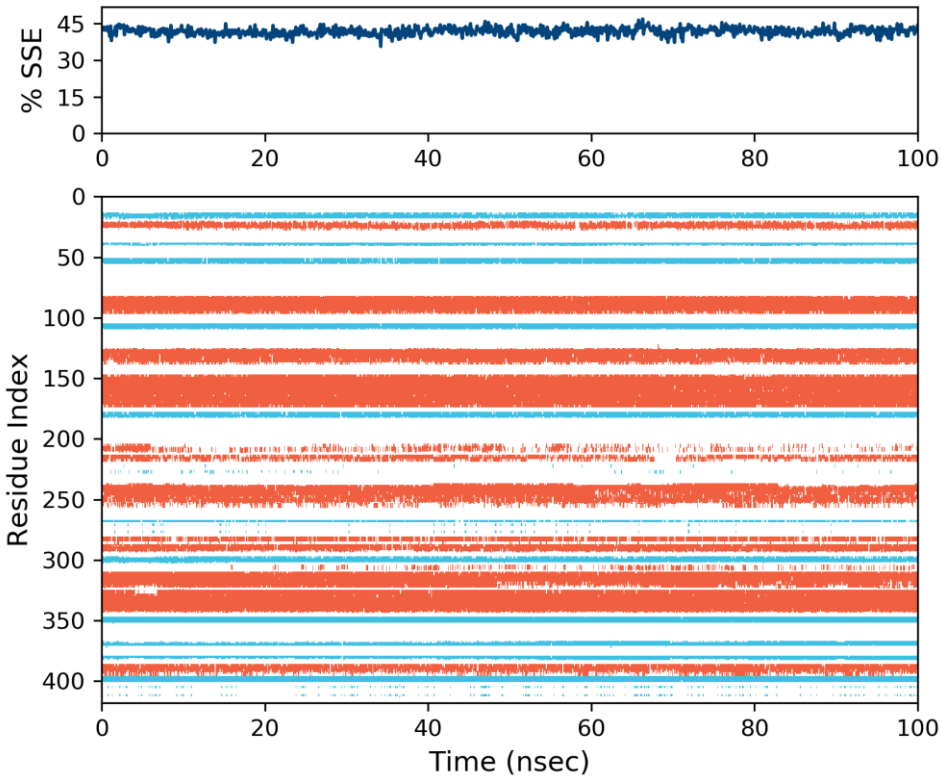


Figure 8. The changes of the secondary structure of hyaluronidase throughout the simulation in the presence of Clusiacyclol A

CONCLUSION

In this study, the anticancer results of Cycloolivil compound were good according to the standard, so we recommend this compound for future studies (especially for in vivo studies) because it may have the potential to be used in drug design studies. Natural products and their animal and plant derivatives have been immensely beneficial to humanity in recent decades, especially as sources of targeted treatments. M. R. Islam (Islam et al., 2022) and colleagues assessed a number of natural compounds and confirmed their efficacy in comparison to traditional breast cancer treatments. They discovered that these compounds have the potential to improve outcomes and lower mortality from breast cancer, and that the development of these natural products may result in a cure for the disease. By influencing multiple cellular processes such proliferation, migration, angiogenesis, invasion, differentiation, and metastasis, these products have shown notable anticancer effects. molecule 3b, a methoxy-substituted molecule, was discovered to have an IC50 of 39.77 µg, which indicates that it is a good candidate for a medication. This shows that compound 3b has anti-cancer activity. Due to their pro-health qualities and capacity to fend off numerous severe systemic ailments, natural antioxidants are in high demand these days. The steady degeneration of the human body is caused by inadequate nutrition and a life marked by stress, and is further intensified by oxidative stress resulting from numerous external sources. The number of diseases affecting civilization is rising because to factors including smog, UV rays, smoking, and eating a lot of manufactured food. The need for novel plant nutrition sources and for the rediscovery of well-known ones is currently increasing as oxidative stress guardians. The search includes sources of cosmetic compounds that can shield the body from UV radiation and free radicals outside in addition to nutraceuticals. Additionally, the results of computational studies could practically demonstrate the interactions between atoms of the ligands and proteins. The compound established strong interactions

with both the enzymes and receptors. These compounds have the potential to inhibit the activity of these enzymes as well as the proliferation of cancer cells. Based on the obtained results, Clusiacyclol A possesses the ability to interact with the active sites of enzymes and receptors, consequently impeding their functional activity. Amongst the enzymes, hyaluronidase can be considered the main target for Clusiacyclol A, and folate receptor is a potent target for this compound. Due to the results, the main receptor protein targets for Cycloolivil and Cyclopenol are estrogen receptor and androgen receptor.

Conflict of Interest

The article author declares that there is no conflict of interest.

Author's Contributions

N.S: Investigation, Methodology, Validation, Data Curation, Formal analysis, Resources, Software, Validation, Enzyme inhibition and Writing - Review & editing.

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