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Original article

Marine-derived sea urchin compounds as potential anti-cancer drug candidate against colorectal cancer: *In silico and in vitro* studies

Mohammad Habibur Rahman Molla, Mohammed Othman Aljahdali *

Department of Biological Sciences, Faculty of Science, King Abdulaziz University, P.O. Box 80203, Jeddah 21598, Saudi Arabia

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ABSTRACT

Sea urchin-derived compounds are potential candidates for the development of effective drugs for the treatment of cancer diseases. In this study, 19 compounds derived from sea urchin (*Diadema savignyi*) were used to treat colorectal cancer using the HCT116 cell line. However, molecular docking, ADME (absorption, distribution, metabolism, and excretion), toxicity, molecular dynamic (MD) simulation, and molecular mechanics generalized Born surface area (MM-GBSA) were used to confirm the ligand–protein interaction. Interactions of Importin-11 receptor with sea urchin compounds reveal that four compounds have higher binding affinities (ranging from -8.6 to -7.1 kcal/mol). In vitro testing revealed that the CID 6432458 compound was effective (docking score of −8.6 kcal/mol) against the HCT116 cell line. The cytotoxicity of HCT116 has been documented, with an IC50 value of 6.885 ± 4. MTT assay, apoptosis analysis, and cell cycle assay were utilized to examine cell death in colorectal cancer. In the MTT experiment, 15 μM and 20 μM dosages were associated with 77% cell death; however, flow cytometry analysis using the IC50 value revealed that the selected chemical induced greater apoptosis in the HCT116 cell line (58.5%). The gene expression data revealed that the apoptotic gene BAX is expressed at a higher level than the BCL-2 gene. The IPO11 gene was downregulated during treatment. In the experiment involving the cell cycle, the S phase for the 30 μM dose showed 75.1% apoptosis, which was greater than the other concentrations used alone. These in silico *and in vitro* analysis will not only provide new information about Importin-11 receptor and insight into colorectal cancer but will also facilitate the development of natural compounds in a significant and worthwhile manner.

1. Introduction

The distinct biodiversity of the Red Sea is a result of its high salinity range, and more than 1,000 species of invertebrates have been documented (Al-Mur, 2020). However, it is crucial to exploring bioactive substances created by marine invertebrates. Sea urchins consist of a wide range of bioactive compounds and have a great deal of chemical diversity due to their unique characteristics (Romano et al., 2022). The small molecule created by living creatures as a secondary metabolism. However, the discovery of a novel chemical structure in the marine product provided an unlimited source of anticancer therapeutics. The pharmacophore diversity has been growing for the last few decades, but still, natural products from the Red Sea are not being discovered properly (Khalifa et al., 2019). Therefore, it is important to investigate small molecules from marine invertebrates in the Red Sea, Saudi Arabia.

Sea urchin, a bottom-dwelling marine invertebrate belonging to the phylum Echinodermata and class Echinoidea, is having high nutritional and medicinal properties. It exhibited anticancer activities on different CRC cell lines (Khalifa et al., 2019). The chemical-based drug shows a severe threat to humans, although sea urchins may be promising for selecting pharmaceutical candidates. Its different organs, such as shell, gonad, and spines are consisted of valuable bioactive compounds (Chamika et al., 2021). Cancer treatment relies heavily on the use of conventional therapies, including surgery, chemotherapy, and radiation. However, developing an effective cancer therapy is difficult due to the disease's resistant nature and associated adverse effects (Aljahdali et al., 2021b). Therefore, discovering a new cancer therapy is a time-consuming

* Corresponding author at: King Abdulaziz University, Faculty of Science Biological Sciences Department, P.O. Box 80203, Jeddah 21589, Saudi Arabia.
E-mail addresses: mrahmanmolla@stu.kau.edu.sa (M.H.R. Molla), moaljahdali@kau.edu.sa (M.O. Aljahdali).

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endeavor. The sea has been a valuable source for numerous drugs, and the majority of antitumor medications derive from marine sea urchins. Due to their bioactive properties, they have cast a spotlight on colorectal malignancy therapy and are attracting an increasing amount of attention (Zuo and Kwok, 2021).

Colorectal cancer may occur based on geographic area, and it varies from females to males. The mortality rate of cancer-affected people is higher for males than females in the world (Xi and Xu, 2021). There are different signaling pathways for malignant tumors occurring in the body, and Wnt-βcatenin pathway is one of them, which is responsible for CRC cancer initiation (Zhao et al., 2022). Moreover, APC mutations are more prevalent in colorectal cancer than other cancer-progression genes, such as KRAS, TP53, and SMAD4. It not only increases the level of βcatenin but also helps several genes that accelerate cell proliferation and cell growth in CRC (Colussi et al., 2013). Nevertheless, the molecular contrivance of βcatenin nuclear transport is not completely implicit, although several studies have found that the IPO11 gene is responsible for nuclear import. IPO11 encodes a protein called importin-11 that bind to βcatenin and escort to the nucleus of colorectal cancer for mutations in APC (Mis et al., 2020). The study suggested that preventing βcatenin by blocking importin-11 activated their target protein. Importin-11 is triggers the growth and cell proliferation of CRC. As a result, it is crucial to inhibit importin-11 in order to slow the spread of colorectal cancer, which results from APC gene mutations.

The computer aided drug design helps to determine suitable candidates against diseases. It is a time-consuming and reasonable way to develop the right candidates (Molla et al., 2023). The bioinformatics approach, such as molecular docking can choose compounds based on their high docking score, and ADMET properties provide the toxicity profile for each compound (Molla and Aljahdali, 2022). MD simulations confirm the stability of the binding interaction. Moreover, molecular process with a widespread Born and surface area solvation (MM/GBSA) is a well-known method for calculating the free energy of ligand–protein binding (Hasan et al., 2022; Aljahdali et al., 2022). In an *in vitro* study, MTT assays the cellular activity, and flow cytometry shows cell apoptosis. However, it is difficult to select a new protein class with a focus on small molecules for the colorectal cancer cell line. Therefore, in this study, we investigate new compounds from the sea urchin that inhibit the importin 11 receptor (Islam et al., 2022).

2. Materials and methods

2.1. Sea urchin capture and sample preparation

Sea urchins (*D. savignyi*) were collected from Red Sea coast near Saudi Arabia during summer seasons (Aljahdali and Molla, 2022). They were collected through scuba diving, and the season of natural reproduction was chosen. After all the samples were taken, they were sent right away to the biological science lab at King Abdulaziz University. Before using the samples, the urchins were dissected and cleaned with fresh distilled water (Shamim Parvez et al., 2020). Also, the internal organs of sea urchins were dried and ground into a powder before ethanol was added to reduce them. The sea urchin was mashed and combined with 500 ml of ethanol. Because of its high polarity and volatility, the sample size was reduced using a rotary evaporator.

2.2. Compound isolation (Gas Chromatography-Mass Spectrometry)

The prepared sample was analyzed with a capillary column expending gas chromatography-mass spectrometry. The sample was injected into the GC–MS from 0 to 45 min prior to the full scan. The helium concentration was recorded to be at 99.99% at 280 °C. The temperature was 50 °C for the first minute before rising to 200 °C for the second. The temperature was increased by 300°after seven minutes of sample analysis (Yusuf et al., 2020).

2.3. Protein preparation, refinement and validation

The importin-11 structure in PDB format was obtained from the recognized database UniProtKB with ID Q9UI26. In addition, the PDB structure was sent to GalaxyRefine for refinement, and a structure with RMSD values and an energy score were provided. Errors in the 3D-dimensional structure were identified using the ProSA-web server. Finally, the Ramachandra plot was used to document allowable and disallowable regions. To validate the protein required to merge polar and nonpolar hydrogen bonds and to calculate the gasteiger charge. The elimination of metal ions and cofactors was recorded (Aljahdali et al., 2021a).

2.4. Documented active sites and generated receptor grids

The importin-11 receptor was submitted to the CASRp 3.0 web server for active site generation. The active sites have a document after receiving server output from the accumulated surface. It was represented visually using the BIOVA Discovery Software (Samad et al., 2020).

2.5. Molecular docking between sea urchin compounds and receptor

Molecular docking study used 19 sea urchin compounds and the importin-11 receptor. It was recorded in order to find the greatest binding affinity. For molecular docking, the PyRx program was utilized. The AutoDocVina tools were used to validate the docking score and interact with ligands and receptors (Aljahdali et al., 2022).

2.6. ADME and toxicity analysis

The primary consideration in choosing any molecule as a therapeutic candidate is its analogue. *In vivo* and *in vitro* failure rates may be lowered with careful chemical selection. Swiss-ADME is a web-based system that evaluates bioavailability and solubility to locate promising compounds. Additionally, the compounds' safety profiles are evaluated using CADD methods. It is a crucial part of determining whether or not a substance is dangerous to humans or animals. Quantitative and qualitative evaluations of mutagenicity, carcinogenicity, LD50 value, and immunotoxicity were performed. Toxicological profiles were calculated using the ProTox-II web server (Opo et al., 2022).

2.7. MD simulation

The somatic sign of atoms and molecules in the selected substances was investigated using MD simulation. For default time, selective compounds are allowed to interact. The Schrodinger software is utilised to analyse protein–ligand interactions. Multiple temperature scales were used in conjunction with the free energy perturbation (FEP) calculation to narrow down the pool of potential candidates. The molecular dynamic simulation phase was ran with a constant force field from OPLS 2005. To record the simulation phase, complicated structure of an orthorhombic periodic boundary was used. During the simulation, sodium ions were electronically stabilized and neutralized. In the periodic boundary condition, NPT assemblies were used with the buffer box technique. The simulation interaction diagram (SID) was analyzed using the Schrödinger program (Dain Md Opo et al., 2022).

2.8. MM-GBSA analysis

The bound free energies were calculated from the molecules and proteins. It is a complex system, and score calculated the free energy of binding from the path of an MD simulation. MM-GBSA was used to find the free energy of binding (Gbind) for certain chemicals with the importin-11 protein. The Schrodinger Maestro package was utilized for the calculation of MM-GBSA (Forouzesh and Onufriev, 2020).

2.9. In-vitro studies with HCT-116

2.9.1. Preparation stock solution

The brassicasterol molecule (B864420) was used to obtain 5 mg of the chemical, as shown in Table S1. DMSO and pure water were used to water down the powder. The total amount of active powder was then used to make 10 mM stock for the experiment.

2.9.2. Cell culture and subculture

The cell line HCT116 was collected from the patient and keep it 37 °C in a water bath until thawing. Cells were melted and then centrifuged for 4 min at 1200 rpm in a 15 ml Falcon tube to fix them. A 2 ml DMEM medium was used to mix with cell after becoming liquid. Moreover, a cell culture plate (T25 cm^2) was used to take a small number of cells from the DMEM medium and then trypsin was added in it. Finally, cells were centrifuged at 1200 rpm and kept in a CO_2 incubator at 37 °C.

2.9.3. Cell toxicity assay

Cell toxicity test is a reliable technique for documenting membrane deterioration in the presence of an inhibitor. We calculated (1×10^4) tumor cells from the HCT116 cell line through the confocal microscope and seeded them in 96-well plates. Later, it was transferred to a 24-hour CO_2 incubator. The same drug concentration was used for incubation for 48 h. Before eliminating the old culture medium, MTT compounds were applied to cells. It was incubated to produce a purple hue. The cell was then analyzed using a 570 nm microplate reader.

2.10. Apoptosis assay by flow cytometry

Cancer cells (HCT116) in a fixed quantity (1×10^5) was put in a flask of 25 cm^2 and cultured for one day in the CO_2 incubator at 37° C. The IC_{50} concentrations for CID6432458 chemical was determined after incubating cancer cells for 24 h. Cell death was measured after 48 h in these flasks, with untreated cells as control. The dead cells in the tube and the ones removed by trypsinization were both collected. Cells were centrifuged at 600 × g for 5 min after being rinsed with 1X Annexin V binding buffer. After 15 min of incubation, cells were stained with 5 ul of fluorochrome-conjugated annexin. Once again, 1 mg/ml of PI solution was used to counterstain all the cells. The BD FACSCanto TM Flow Cytometer examined a tube containing cells after keeping it in the dark at 4° C for a predetermined amount of time.

2.10.1. Cell cycle assay

FACS is a popular method for determining whether an inhibitor is causing cell cycle inhibition. In six-well plates, 1×10^5 cells were dispersed in various proportions and put in the incubator until attachment. The cells were washed with phosphate buffered saline (PBS) before adding the compound. All the containers were treated for 48 h with various IC_{50} concentrations. However, HCT116 cell lines were collected and centrifuged for five minutes. The cells were stabilized through the vortexing continuously. Vessels were retained at 20° C overnight, and the cells were washed the following day. Each tube received 500 µl of RNAse and 500 µl of propidium iodide and was stored in the dark at ambient temperature. All the sample were examined using a BD FACSCanto TM flow cytometer, and the findings were intended using FACSDiva software.

2.10.2. Identification of apoptotic genes

The apoptotic gene was expressed from the extraction of RNA sample through qRT-PCR analysis (Molla et al., 2019). RNA was isolated by using Purelink RNA mini Kits, Thermo Scientific. The compound was applied to the cell and kept incubating for 48 h, 72 h, and 96 h. The samples were moved to centrifugation at 4° C for mixing properly. The NanodropTM spectrophotometer is used to determine for collecting RNA sample. In the experiment, GAPDH was selected as the reference gene for the HTC116 cell line. The gene expression analysis was evaluated by working with TB Green ™ Premix Ex Taq (TAKARA BIO INC.). The Livak ($2^{-\Delta\Delta CT}$) method was used to quantify each gene's expression (Sumon et al., 2022). The human IPO11/IPO-11 qPCR primer pair was used to show gene expression, and the purchasable cat number was HP102086.

3. Result

3.1. Sea urchin compound isolation through the GC–MS

In total, 19 different compounds were identified in sea urchins through the gas chromatography and mass spectrometry investigations. The retention period, chemical formula, and area were recorded up to the 50-minute retention interval. (Table S1). Most substances were detected between 4.0 and 39.0 min within the assays.

3.2. Validation, refinement and receptor preparation

The protein was found in the well-known databank UniPotKb, and the Ramachandra plot was used to figure out which areas were allowed and which were not. The favorable region, the allow region and the deny region were found to be 95.1%, 2.97%, and 92% respectively. To construct the protein, molecular docking methods were used, and the protein was kept in a pdbqt format for molecular docking (Fig. S1).

3.3. Active sites searching

The active sites of protein were identified through the CASTpi server. Four active pockets were selected to determine active sites in the importin-11 protein. The first active pocket showed the binding sites at the position of THR22, LEU25, LYS26, GLU29, GLU30, LYS33, GLU36, TRP60, LEU61, LEU64, TYR65, LYS67, HIS68, ILE70, ASP71, ARG72, TYR73, TRP74, ARG75, ALA78, and PRO79. The second active pocket posed several residues position at TYR152, LYS156, ALA159, LYS162, ARG167, PHE170, TYR171, LYS216, ARG219, VAL223, and PHE226. Furthermore, the amino acid residues compiled with at the position of third active sites at GLU902, PRO903, VAL905, GLU909, GLU910, PRO912, ASP916, LYS917, LYS919, LYS920 and ALA923. Finally, the fourth active pocket showed amino acid residue positions at TYR373, LEU375, THR377, GLU379, GLU380, MET383, PHE391, GLU394, GLU395, SER404, LEU405, ARG406, PRO407, and THR409 (Fig. 1). In the process of molecular docking simulation, the binding sites were documented to construct a receptor grid with an angstrom (Å) dimension of X = 85.75, Y = 91.72, and Z = 96.45.

3.4. Molecular docking

The structure-based drug design technique was utilised through molecular docking. It is a popular strategy to find an accurate drug

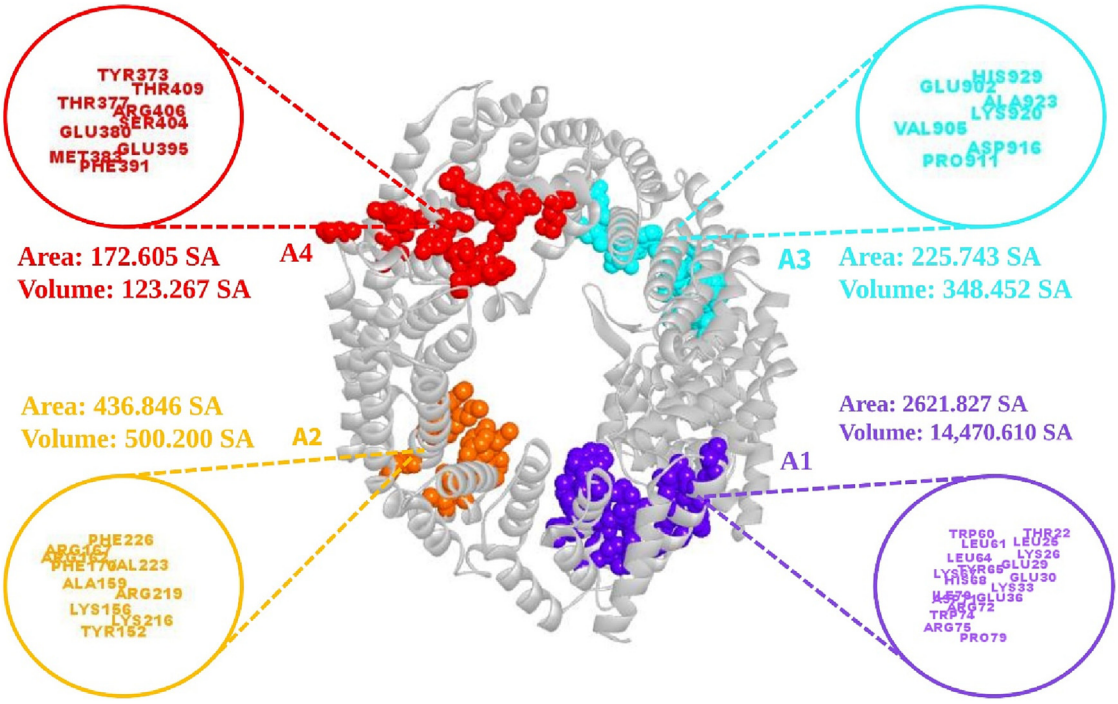


Fig. 1. Active pocket showing the amino acid residue, volume and area. The first active pocket are marked in A1 with purple, A2 with yellow, A3 with blue, and A4 with red colour, respectively.

candidate. Molecular docking confirmed 3D small molecules from the sea urchin within the importin-11 receptor binding sites. The PyRx tools and AutoDock Vina were used to dock sea urchin with importin-11 and give binding affinity values between −3.2 and −8.6. These four molecules were picked for molecular docking (Table 1). It is also compiled in the Supplementary Table S2. The final compound underwent the relocking technique, and the RMSD value was considered for the similar score.

3.5. Importin-11 and compound (CID643258) interaction

The Discovery Studio Visualizer tool BIOVIA was utilized for visualization of protein and ligand interactions. The compound CID643258 had the highest binding affinities and interaction with importin-11 at the positions of VAL227, LYS337, ARG167, LYS168, HIS284 and GLU345 (Fig. S3 and Table S3). The compound CID605775 was found to interact with Importin-11 at various positions. For ligand CID 605775, it was important to establish many hydrogen and hydrophobic links. At LYS337, SER287, PHE286 and GLU345, hydrogen bonds were seen to form. For more information about the types of bonds, see Fig. S4 and Table S3. The figure showed that hydrophobic bonds were formed at LYS337, SER287, PHE286 and GLU345 and at PRO335 and PRO285. Hydrogen bonds were identified at GLN801 and LEU756 of the compound CID 608814, constituting this molecule as the third most potent ligand for the desire protein. Fig. S5 and Table 2 revealed that the required compounds, comprising LYS747, ARG797, PRO927, PRO928, and LEU800 in residual locations, formed a hydrophobic bond.

Table 1
Docking scores of nineteen compounds documented from the sea urchin (*Diadema savigny*).

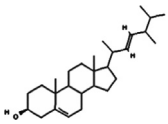
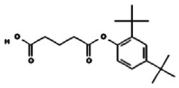
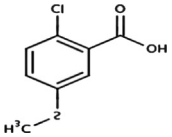
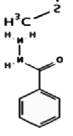
SI No.	PubChem ID	Chemical name	Molecular formula	Chemical structure	Molecular weight	Binding affinity (Kcal/mol)
1	CID 6432458	Ergosta-5,22-dien-3.beta.-ol	$C_{28}H_{46}O$		398.7 g/mol	−8.6
2	CID605775	Pentanedioic acid, (2,4-di-t-butylphenyl) mono-ester	$C_{19}H_{28}O_4$		320.4 g/mol	−8.2
3	CID 608814	2-Chloro-5-(methylthio)benzoic acid	$C_8H_7ClO_2S$		202.66 g/mol	−7.8
4	CID 11955	Benzoic hydrazide	$C_7H_8N_2O$		136.15 g/mol	−7.1

Table 2
List of the chosen compounds having pharmacokinetic features.

Endpoint	Target	CID 6432458	CID 608814	CID 11955	CID 605775
Tissue harmfulness	Hepatotoxicity	No	No	Yes	No
Toxicity endpoints	Carcinogenicity	No	No	Yes	No
	Immunotoxicity	Yes	No	Yes	No
	Mutagenicity	No	No	No	No
	Cytotoxicity	No	No	No	No
	Toxicity (class)	4	4	3	4
Tox21-Nuclear receptor signaling pathways	Androgen Receptor (AR)	No	No	Yes	No
	Aryl hydrocarbon Receptor (AhR)	No	No	No	No
Tox21-Stress response pathway	Heat shock factor response element	No	No	No	No
Fathead minnow LC50 (96 h)	-Log10 (mol/L)	6.71	3.89	2.84	5.98
48-h Daphnia magna LC50	-Log10 (mg/L)	0.34	3.65	3.11	4.81
Developmental toxicity	value	0.99	0.5	0.41	0.95
Oral rat LD50	mg/kg	751.96	2164.92	537.45	2704.54
Bioaccumulation factor	Log10	2.85	5.23	0.54	1.75

Compound CID 11955 was demonstrated to interact with the target protein Importin-11 through hydrogen bonding with many amino acids, including ASN455 and ARG406. However, desirable compounds such as ARG494, ARG495, LEU376, TRP384, GLU410, and TRP498 were discovered to establish the hydrophobic bond as well (Fig. S6). Bonding with importin-11 was investigated with four chemicals obtained from a marine organism (sea urchin). The table included residues, distances, classes, and kinds of compounds.

3.6. ADME properties of four selected compounds

The major causes of drug development failure include inadequate absorption, distribution, metabolism, and excretion (ADME) qualities, as well as toxicities (Tox). Evaluation of compounds potential as drugs at an early stage is crucial to boosting R&D productivity. However, the existing methods for assessing ADME characteristics need substantial animal research, which is both time-consuming and costly. As a result, ADME prediction using computational modeling approaches has replaced traditional methods as a benchmark in preclinical drug development. Easily predicting the hydrophobicity, solubility, permeability, etc. of substances and applying predictive models for blood–brain barrier (BBB) permeability, medication bioavailability, cytochrome P450, hERG toxicity, and more are possible using Creative Biostructure. To help lower the failure rate of drug development, we assist in the starting phases of drug detection by evaluating the ADME characteristics of compounds so that those with undesired ADME properties may be removed. The pharmacokinetic appearances of the selected compound are documented in Table S4.

3.7. Predictions of toxicity from the selected compounds

Toxicology testing is crucial because it may reveal whether or not a molecule has any unfavorable effects on people, animals, or the environment before it is used as a medicine or drug candidate. As a result, toxicity prediction is regarded as the first stage in the process of choosing any molecule as medicine. Before the advent of in silico methods, toxicity analysis necessitated the use of several costly and time-consuming animal models. Because of this, it is possible to employ computer-based toxicity tests instead of costly and time-consuming traditional approaches without the requirement for an animal model. Four compounds were selected using the popular web-based servers admetSAR 2.0 and ProToxII. Hepatic toxicity and cytotoxicity were all anticipated to be high in an in computational toxicity test using rats as a mark model (Table 2 and Table S5).

3.8. MD simulation analysis

The binding stability of the sea urchin small molecules and the importin-11 receptor was observed through the MD simulation. It is a computerized complex biological system that shows how atoms move, interact, and change over time by using a default force field. To verify the intermolecular interaction, a 100 ns run was performed on impotin-11 and the sea urchin compound.

3.8.1. RMSD analysis

The difference between the initial structural conformation of the sea urchin compounds and the final position of the importin-11 backbones was documented. It helps to characterize the average atomic distance from the receptor and ranges between 1 and 3 Å or 0.1–0.3 nm. Importin-11 structural residues (Cα) and sea urchins were analyzed based on a 100 ns period. The projected Root mean square deviation (RMSD) from (Cα) atoms revealed a large variation among the four ligands from sea urchin that fit with importin-11. The display line chart showed that the compound CID 605775 was fluctuated from 10 ns to 65 ns and then remained stable until 90 ns. On the other hand, compound CID 608814 was stable over time. For the compound CID11955, it was stable from the beginning but fluctuated at 90 ns. Finally, the compound CID6432458 remained stable until 100 ns and showed the optimum fluctuation >3.0 Å for choosing the best compound (Fig. 2).

3.8.2. RMSF analysis

The root mean squared variation (RMSF) of an importin-11 residue quantifies the distance from its reference value over time. For the PubChem compounds with the identifiers four compounds, an RMSF estimate of the complex protein containing sea urchin compounds is shown in Fig. 3. Initially, the compound CID11955 showed the fluctuation over the periods where compound CID 605775 was stable from 280AA to 350AA. On the other hand, Compound CID 608814 was unstable for a long time except residues GLU340. Finally, compound CID643258 remained more stable than other compounds (Fig. 3).

3.8.3. Receptor- ligand contact mapping

A 100 ns MD simulation trajectory was used to analyse the contact map between a receptor and its ligand. Water bridges made contact with the vast majority of (A) CID 11955′s hydrogen bonds. As compared to the other amino acid residues, ARG494 was more noticeable. In addition, (B) CID605755 showed a stronger ionic connection than (C) CID608114. In addition, GLN768 in compound CID608114 was shown to interact with hydrogen and water bonds. Fig. 4 shows that compound (D) CID 6432458 had interacted

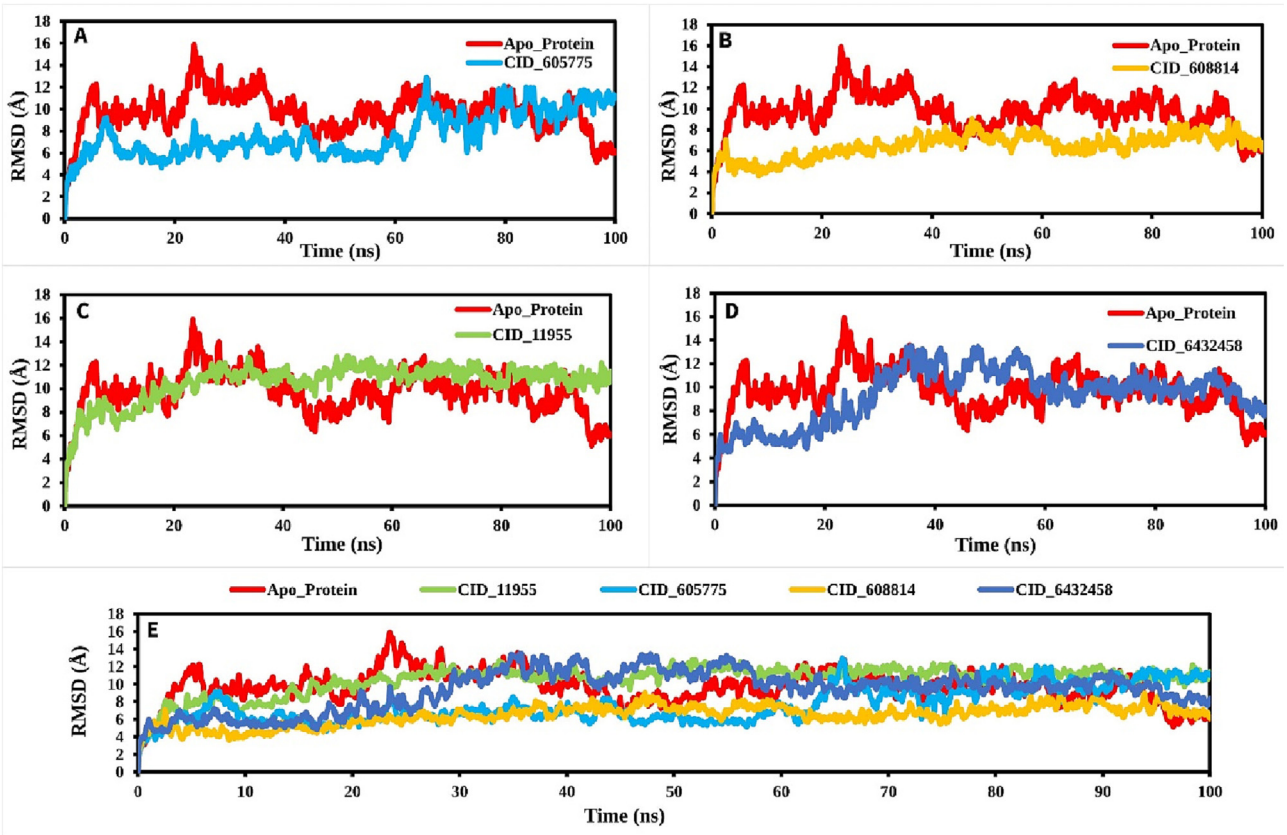
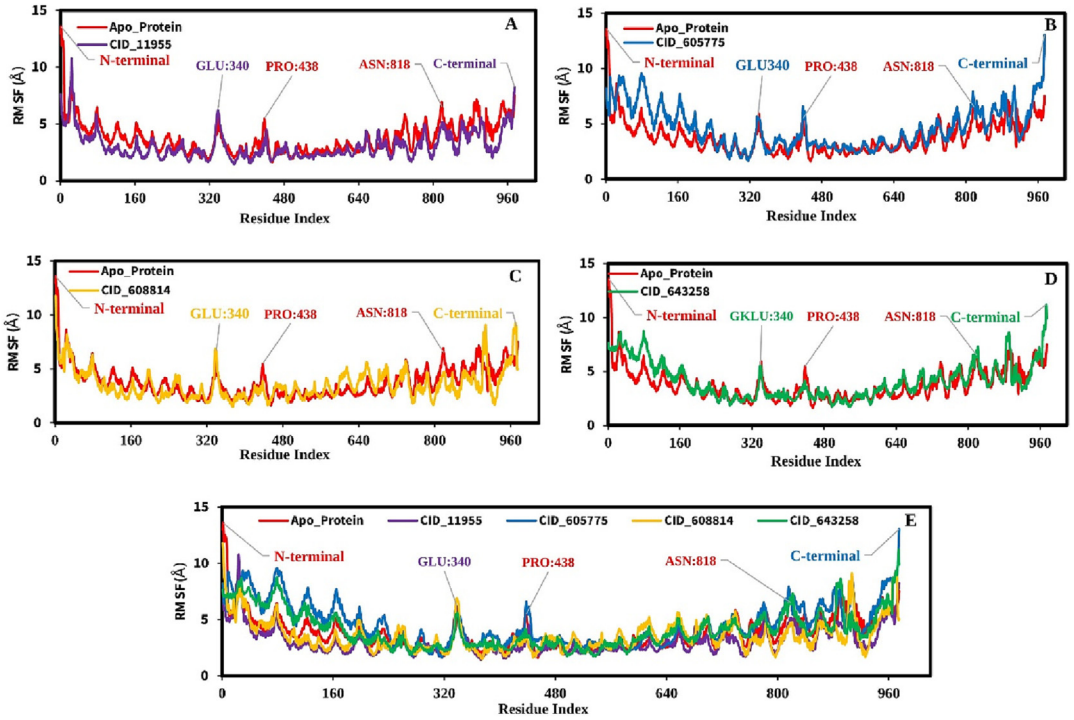


Fig. 2. RMSD values calculated from four selected compounds with Apoprotein.



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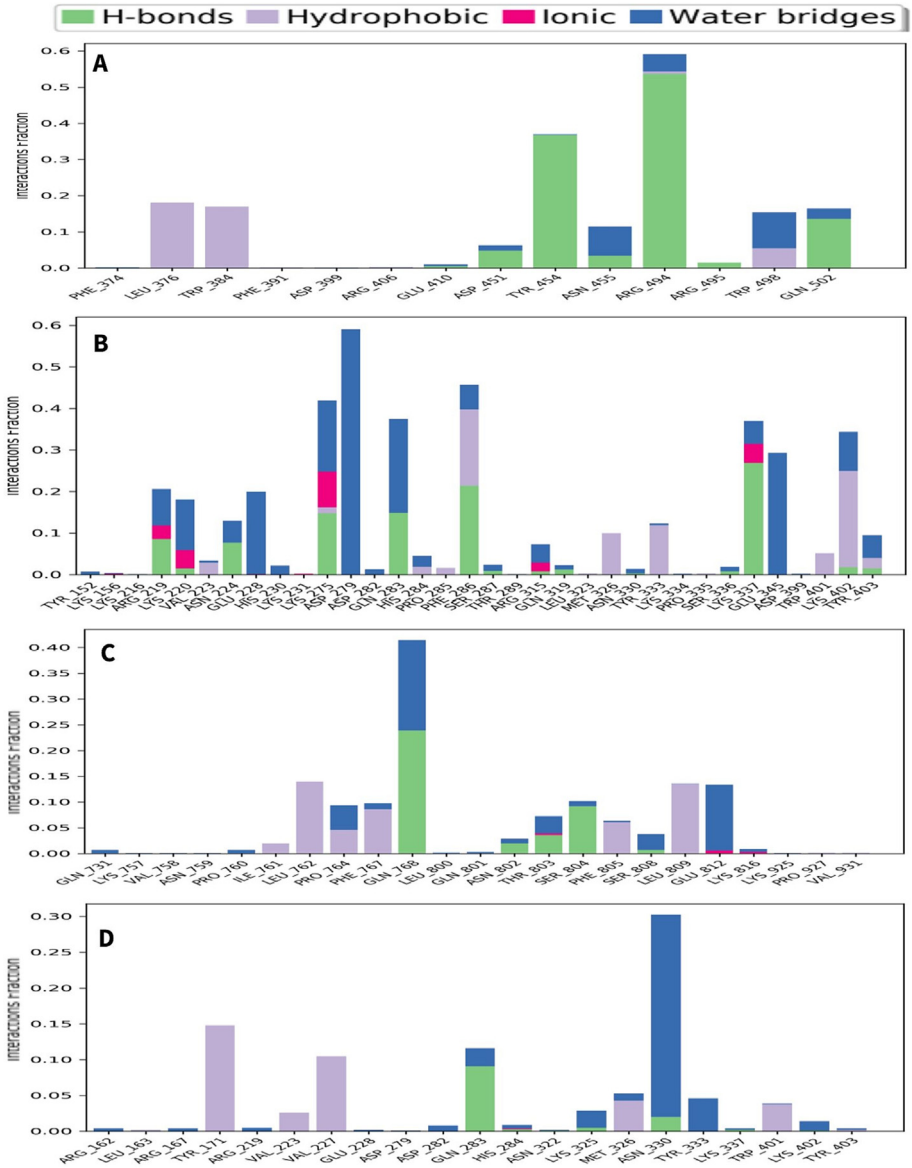

Fig. 4. The figure show the contact ratio of various ligands from the sea urchin with receptor.

through hydrogen bond and water bridge, in addition to certain hydrophobic ones.

3.9. MM-GBSA analysis

The binding free energy of small molecules from sea urchin to importin-11 was often estimated using molecular energies in combination with the widespread Born and surface area continuum solvation approaches. It was stood between the extremes of empirical scoring and rigorous alchemical perturbation approaches (Fig. 5). The negative binding free energy values calculated from the four compounds were −45.8499 ± 6.03, −58.1706 ± 11.35, −57.0858 ± 9.89, and −92.4586 ± 4.86 kcal/mol for CID: 6432458, CID: 608814, CID: 11955, and CID: 605775, respectively, with importin-11 (Table S5).

3.10. Cell death analysis

Multiple concentrations of the cytotoxicity of our chosen drug (CID: 6432458) were used to identify half of the cell mortality. The IC50 for Er-gosta-5,22-dien-3.beta.-ol in HCT116 was determined to be 6.8854 μM. (Fig. 6). Compared to other concentrations, the highest inhibition (ZINC103239230: 58.50%) of cell death was observed at 20 μM (Fig. 6). The IC50 was calculated using Graph pad Prism version 9.0.

3.11. Cell death measurement by flow cytometry

Cell viability comprises analyzing phosphatidylserines in death cells and evaluating them based on the binding of an-nexin V-FITC to phosphatidylserines. In the untreated control group, 1.2% of HCT116 cells were deemed to have died. Using IC50 concentration after 48 h, the 20 μm dose increased early apoptosis (8.2.%), the 30 μm dose increased late apoptosis (11.3%), while early apoptosis remained at 7.7%. Comparing the 15 μm dose alone with IC50 concentration, the proportion of cells entering early apoptosis decreased to 4.4% (Fig. 7).

3.12. Cell cycle estimation

The application of cell cycle analysis revealed that small molecules (CID 6432458) had effectiveness against HCT116 cell line. It

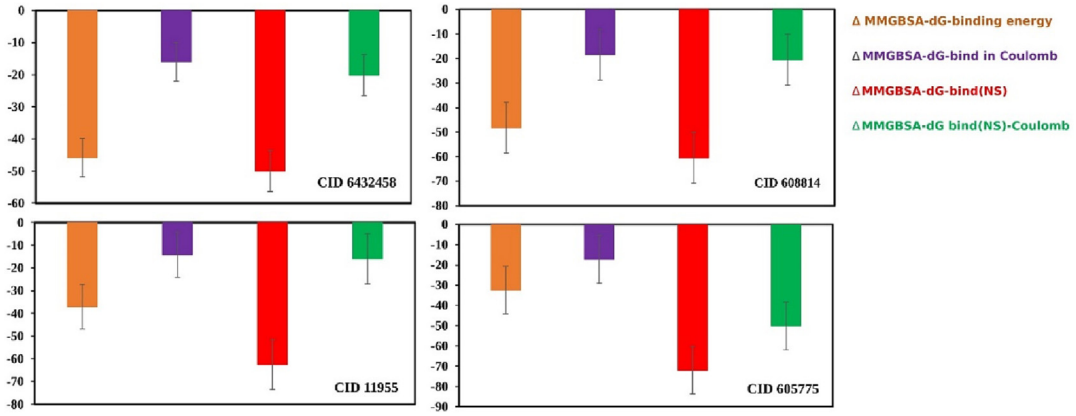

Fig. 5. MM/GBSA calculation from the selected four compounds.

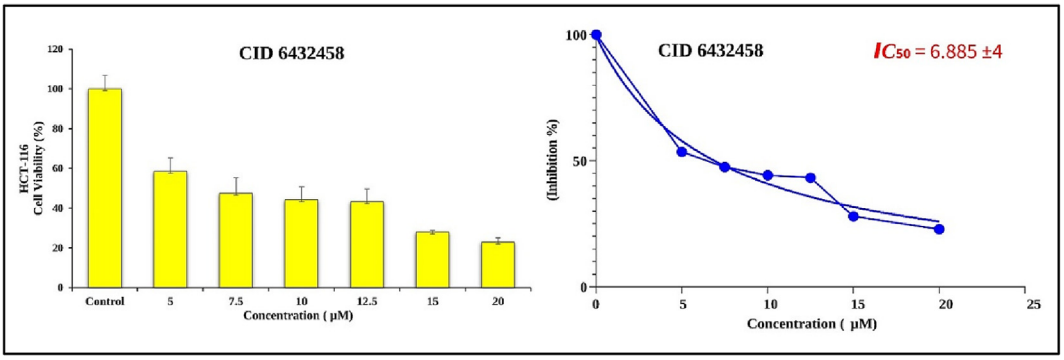

Fig. 6. The cell viability of the HCT116 cell line and cell death at various concentrations.

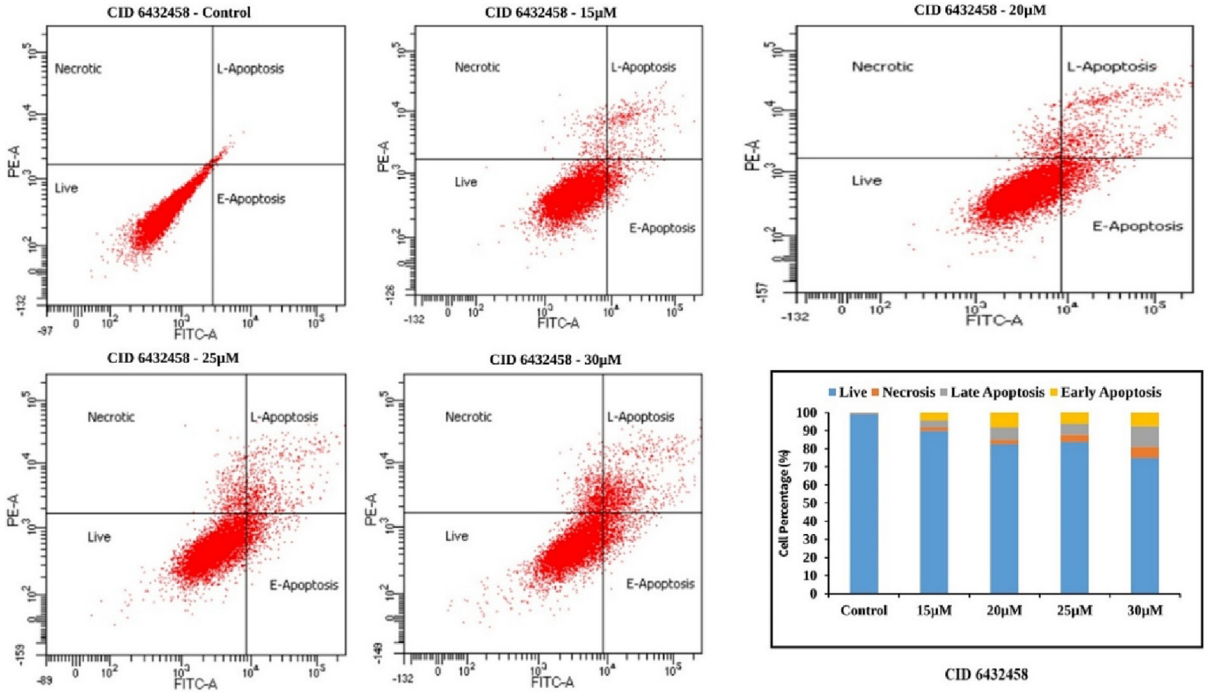

Fig. 7. Quantification of apoptosis-induced cell death by Flow Cytometry.

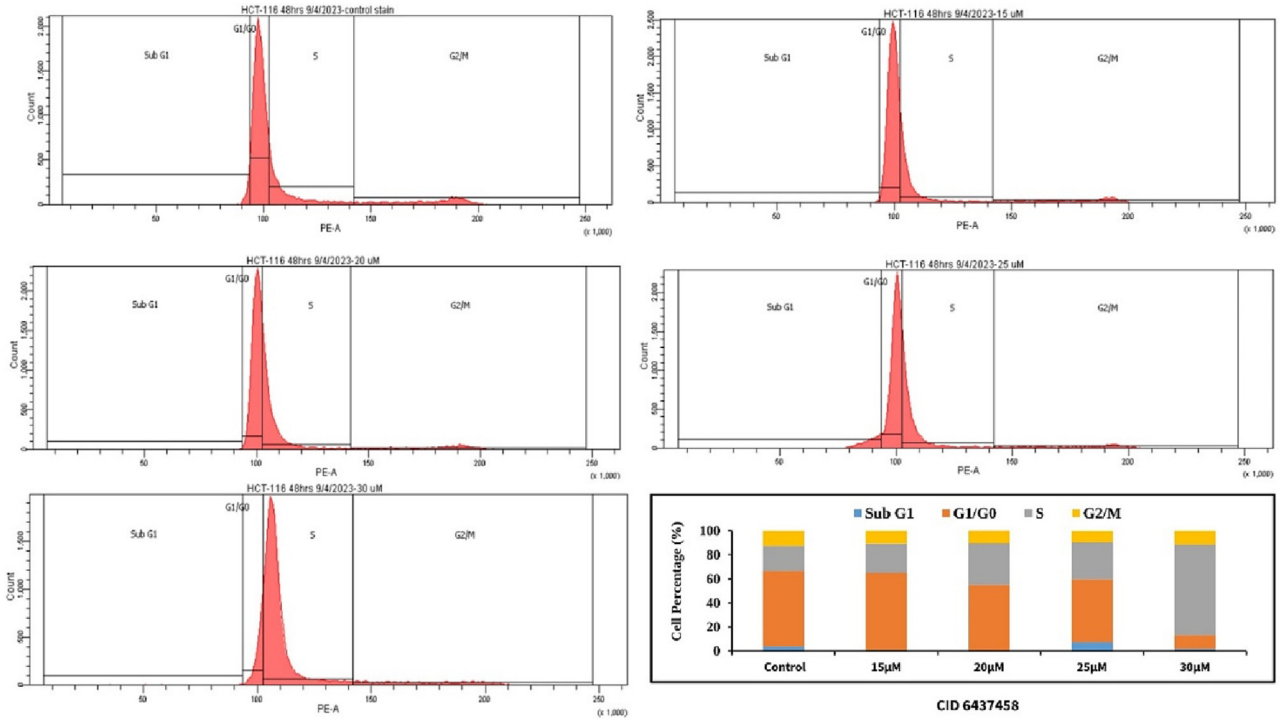

Fig. 8. CID 6432458 inhibited progression of the HCT116 cell line through the S and G0/G1 phases of the cell cycle.

detected cell death at several stages, including G0/G1, S, and G2/M. The SubG1 phase documented 7.30% more cell death at the 25 µm dose than at other doses. The G0/G1 phase was decreased gradually at different concentration of doses. The highest cell cycle apoptosis arrest rate of 75.1% at a 30 µm dose was in the S phase (Fig. 8).

3.13. Gene expression analysis

The gene expression was estimated through the qPCR analysis. It is provided in different expression depended on time. The expression of genes BAX, and BCL-2 showed the apoptotic cell death on 48, 72 and 96 h respectively. In the study, the minimum expression was documented from the IPO11. BAX was somewhat increased than BCL-2 gene. The IPO11 gene was shown to be linked to the activation of apoptosis in the HCT116 cell line in response to pharmacological treatment. The IPO11 expression was downregulated for 24 h activities, but marginally increased for both 48 h and 96 h. The chosen complex demonstrated that total apoptotic gene expression was high in the compound and also observed downregulation of IPO11 gene expression (Fig. 9).

4. Discussion

The Red Sea is the hub of medicinally related plants and animals, and sea urchins are one of the most abundant invertebrates, having various uses in the food and pharmaceutical sectors (Moreno-García et al., 2022). Sea urchins have anticancer activities that have already been proven and applied to different cancer cell lines (Romano et al., 2022). The bioactive compounds included steroids, sulphate, polysaccharides, and so on (Quitério et al., 2021). In our study, we isolated 19 small molecules from the sea urchin by using GC–MS analysis. The study of sea urchin (*Diadema setosum*) also revealed that several bioactive compounds were documented in the sea urchin through GC–MS (El-Sayed et al., 2020). It has been shown that compounds have antibacterial and anticancer properties (Sibiya et al., 2021).

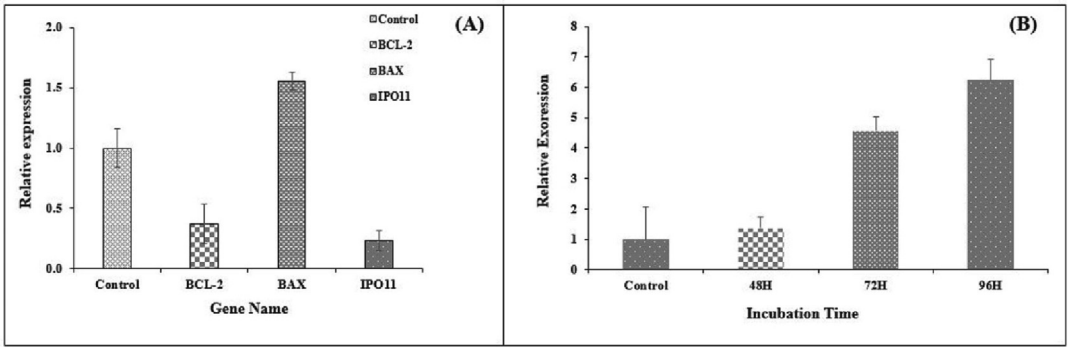

Fig. 9. The qPCR analysis of apoptotic genes (Bcl-2, Bax, and IPO11) in HCT116 cells. The HCT116 cells were treated with a sea urchin compound for 48, 72, and 96 h and identified the expression of Bcl-2, Bax, and IPO11 (A). GAPDH was used as a control for the relative expression of other genes (B).

Furthermore, these compounds were utilized for a variety of anticancer activities through the combined use of CADD techniques and *in vitro* trials. Moreover, the recent advancement of technology can reduce the time and cost of the discovery of new drugs. Computer-aided drug design can predict specific small molecules for various infectious diseases (Sliwoski et al., 2014). In our study, we utilized molecular docking methods for screening compounds from the sea urchin based on their binding affinities. Similarly, the molecular study on breast cancer showed that natural products targeted overexpressed receptors (Taghizadeh et al., 2022). We utilized ADME-Tox software to determine the compound bioavailability, solubility, and other properties. ADME property analysis proved that the selected four compounds have the ability to be used as drug candidates against the HCT116 cell line. The study of colorectal cancer found that the ADME result predicted the toxicity and pharmacokinetic properties of Emodin and Chrysophanol (Ahmad et al., 2022). In our study, we selected four compounds that had no toxic substance for humans as drug candidates through the ProToxII software, where several studies reported the same result (Banerjee et al., 2018). Moreover, an in silico molecular dynamic simulation was performed to determine the protein–ligand interaction between four compounds and importin-11. It is proven that four compounds have different types of interactions with the importin-11 receptor. To estimate the free energy of binding of a tiny sea urchin compounds to the Importin-11 receptor, MM/GBSA techniques, which use the energies from molecular mechanics combined with the Poisson-Boltzmann or generalised Born and surface area continuum solvation, are often used. Negative binding free energies were documented from the sea urchin compounds such as −45.8499 ± 6.03, −58.1706 ± 11.3 5, −57.0858 ± 9.89, and −92.4586 ± 4.86 kcal/mol against importin-11.

Colorectal cancer is frequently initiated by APC gene mutations. It is not only Wnt-ligand-independent but also accumulates βcatenin in the nuclear. The previous study suggested that IPO11 is a trigger for βcatenin nuclear import, and IPO11 knockout reduced the cell growth and proliferation of the CRC line (Mis et al., 2020). In our study, we utilized sea urchin compounds to observe IPO11 and other apoptotic gene expression. The IPO11 gene was downregulated on the HCT116 cell line in 48 h of treatment and then sharply increased by 72 h and 96 h. It is proven that our sea urchin compound is effective for the downregulation of IPO11, which is mainly responsible for APC mutants in colorectal cancer, although more experiments are needed for further validation. The anticancer activity of the four selected compounds against colorectal cancer can be observed. Cell death was higher at concentrations of 25 and 30 μM and it was about 58.50% on the HCT116 cell line. As a result, the selected compounds will not only have effectiveness on the HCT116 cell line but also assist the progress of cancer treatment on different cancer cell lines.

5. Conclusion

Small molecules from the sea urchin have anti-tumour effectiveness and have previously been reported in many studies, but our study defines the particular compounds having the effectiveness of colorectal cancer. The study utilized CADD techniques to find out the specific candidate against the colorectal cancer cell line that is responsible for downregulation of IPO11 gene expression. As a result, it was validated by lab-based experiment. In the MTT experiment, concentrations were associated with cell death; however, flow cytometry analysis using the IC50 value showed that the chosen chemical triggered greater apoptosis in HCT116. Finally, in the cell cycle, the S phase for the 30 μm dose showed 75.1% apoptosis, which is also more than the other doses used alone. Moreover, an *in vivo* trial is required for further validation.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2023.103748.

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