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Research Article

INVESTIGATION OF GCMS AND *IN SILICO* MOLECULAR DOCKING STUDY OF BROWN SEAWEED (*TURBINARIA ORNATA*) COLLECTED FROM GULF OF MANNAR, TAMILNADU, INDIA

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ABSTRACT

Turbinaria ornata is a species of marine brown seaweed and in the family sargassaceae. They were freshly collected from Mandapam Coastal Area, Rameswaram Tamilnadu, India and rinsed in seawater and packed in sterile bags for further proceedings to laboratory. Seaweeds are possible renewable resources in the marine environment. It has been used as antioxidant and antimutagen. Ethanol extract was prepared for further analysis. GCMS analysis of ethanol extract of *Turbinaria ornata* was performed using a Shimadzu 2010 plus comprising an AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer. The GCMS analysis reveals that many bioactive compounds such as 6-octadecenoic acid, 9,12-octadecadienoic acid, Bis (2-ethylhexyl) phthalate, oleic acid presents in the extract of *Turbinaria ornata*. The above bioactive compounds were taken for molecular docking studies to improve the reliability, accuracy of biological test and show the possible interactions between molecules and their target receptors. So, in this present study *in silico* molecules docking was carried out to analyze the binding properties of 6-octadecenoic acid, 9,12-octadecadienoic acid, Bis (2-ethylhexyl) phthalate, oleic acid against standard liver cancer drug such as Doxorubicin to target protein β-catenin (1JPW). The results show the better binding interactions and suppression activity of bioactive compounds.

Keywords: Brown Seaweed, *In silico* study, GCMS, *Turbinaria ornata,* Molecular docking, β catenin (1JPW).

1. INTRODUCTION

Seaweeds are possible renewable resources in the marine environment. It generates immense number of bioactive compounds with enormous medicinal potential. Nowadays, the uses of antibiotics have increased due to infections. Marine algae are the remarkable natural resources in the marine ecosystem which have been used as a source of food, feed and medicine. Seaweeds can be biosynthesizing secondary metabolites that can mediate a broad range of intra and inter specific ecological interactions between organisms including chemical defenses [1].

The components reported to be found are sterols (some are fucosterol), different molecules containing vinyl and ethyl cholesterol types, cyclohexane, and some sulfated polysaccharides fucoidan, neutral glucan and guluronic and mannuronic acid residues containing alginic acid providing a medicinal value for the brown and red algae [2]. Previous studies in animal models and cell culture have suggested that seaweed phytochemicals have the potential to inhibit progression of carcinoma formation [3].

Computer-Aided Drug Design (CADD) has been emerged as an efficient means of identifying potential lead compounds and for aiding the developments of possible drugs for a wide range of diseases. Today, several computational approaches are being used to identify potential lead molecules from huge compound libraries. Different *in vitro*, *in vivo,* and computational methods were employed to assess the antioxidant potential of drugs or chemicals. Among these methods, docking has been used widely in drug designing for free radical mediated diseases including cancer, diabetic etc. [4, 5]. Molecular docking and modeling studies improve the reliability, accuracy of biological test and show possible interactions between molecules and their target receptors.

Cancers are a group of diseases characterized by uncontrolled cell growth and spread. Cancer is one of the leading causes of death worldwide despite

chemotherapy, combination of drugs and many more for treatment are under research. Among cancer, Hepatocellular cancer (HCC) is a major health burden worldwide. It is the fifth most common cancer in men, and its annual incidence reaches more than half a million worldwide. There is a need for an improved treatment since less than 50% patients survive more than a year [6]. Various molecular pathways are implicated in the HCC pathogenesis including β-catenin, p53, EGF, HGF, TGFβ and others [7]. The β-catenin pathway implicated in hepatic tumorigenesis, also plays indispensable roles in hepatic development and regeneration. Aberrant activation of β-catenin signaling allows β-catenin to resist degradation and enter the nucleus where it acts as a cofactor for the T cell factor (TCF) family of transcription factors to regulate the expression of several genes relevant to cell proliferation and apoptosis, including *c-myc, cyclin-D1*, and *surviving* [8]. So, in present study 6-octadecenoic acid, 9,12-octadecadienoic acid, Bis (2-ethylhexyl) phthalate, oleic acid and Doxorubicin molecular docking was performed with cancer protein as β-catenin (1JPW).

Therefore, the present study was carried out to identify the bioactive compounds with the aid GC-MS technique and *insilico* molecular docking study of *Turbinaria ornata*.

2. MATERIAL AND METHODS

2.1. Collection of Seaweeds

Turbinaria ornata were collected from Gulf of Mannar, Rameswaram, Tamilnadu, India. The collected samples were cleaned well with sea water to remove all the extraneous matter such as epiphytes, sand particles, pebbles and shells and brought to the laboratory in sterile bags. Then the samples were washed with tap water and distilled water and spread in the dark room for drying, after which the dried samples were powdered and subsequently stored at 4°C.

2.2. Preparation of extract

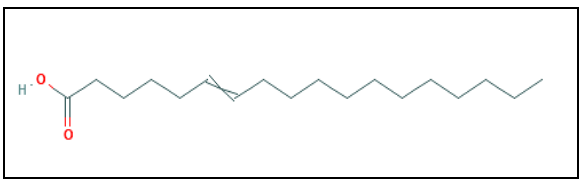
A dried sample of *Turbinaria ornata* was pulverized to powder in a mechanical grinder. 50g of dried seaweed powder was extracted with ethanol for 72h by maceration until the powder was fully immersed, incubated overnight and filtered through whatmann no.41 filter paper. The filtrate was then concentrated by bubbling nitrogen gas into the solution. The extract employed in GCMS for analysis of different bioactive compounds.

2.3. GC MS Instrument Program

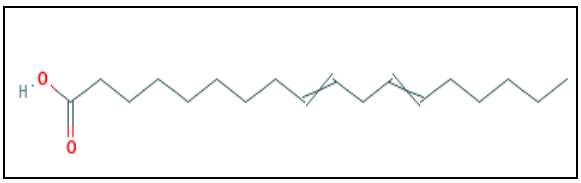
GC MS analysis was carried out on Shimadzu 2010 plus comprising a AOC-20i auto sampler and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions: column RTX 5Ms (Column diameter is 0..32mm, column length is 30m, column thickness 0.50µm), operating in electron impact mode at 70eV; Helium gas (99.999%) was used as carrier gas at a constant flow of 1.73 ml /min and an injection volume of 0.5 µl was employed (split ratio of 10:1) injector temperature 270°C; ion-source temperature 200°C. The oven temperature was programmed from 40°C (isothermal for 2 min), with an increase of 8°C/min, to 150°C, then 8°C/min to 250°C, ending with a 20min isothermal at 280°C. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time is 51.25min. The relative percentage amount of each component was calculated by comparing its averagepeak area to the total areas. Software adopted to handle mass spectra and chromatograms was a Turbo Mass Ver 5.2.0 [9].

2.4. Ligand and protein preparation

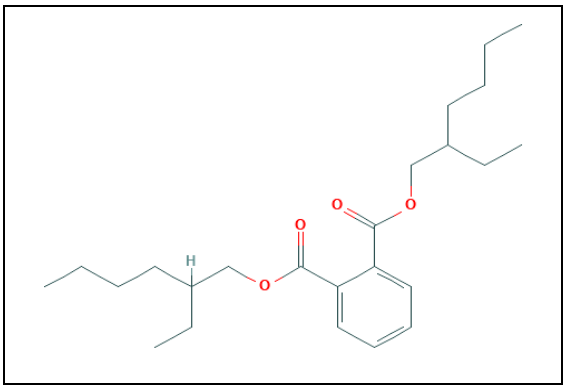
Ligands Bis(2-ethylhexyl) phthalate, 6-Octadecenoic acid, 9,12-Octadecadienoic acid, Oleic acid and Doxorubicin were obtained from Pubchem database, ligands were converted in to PDB format using Open bable software and Protein obtained from PDB database. β-catenin (1JPW) preparation was done to have a remove of all water molecules and any other Ligand molecules prior to docking [10]. The structure of individual ligands from extract of *Turbinaria ornate* shown in Fig.1 and Doxorubicin (Standard drug for Liver cancer) in Fig.2.



6-Octadecenoic acid



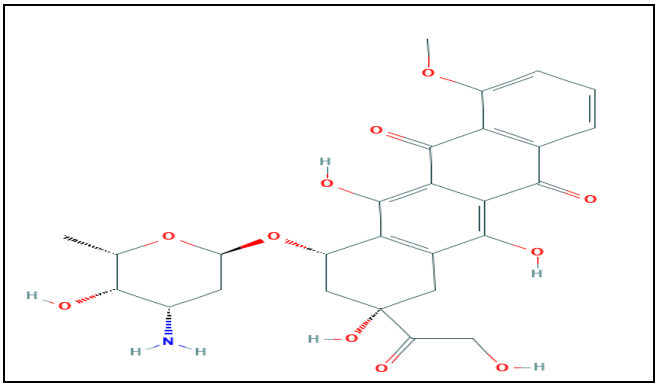
9,12-Octadecadienoic acid



Bis(2-ethylhexyl) phthalate

Oleic acid

Fig. 1: Structures of Ligands



Doxorubicin

Fig. 2: Standard drug for Liver cancer

3. RESULTS AND DISCUSSION

3.1. GC MS analysis

Interpretation on GCMS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The sample chromatogram of extract of *Turbinaria ornate is* shown in Fig.3. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight, and structure of the components of the test materials were ascertained [11]. The bioactive compounds present in the extract of *Turbinaria ornata* is shown in Table 1.

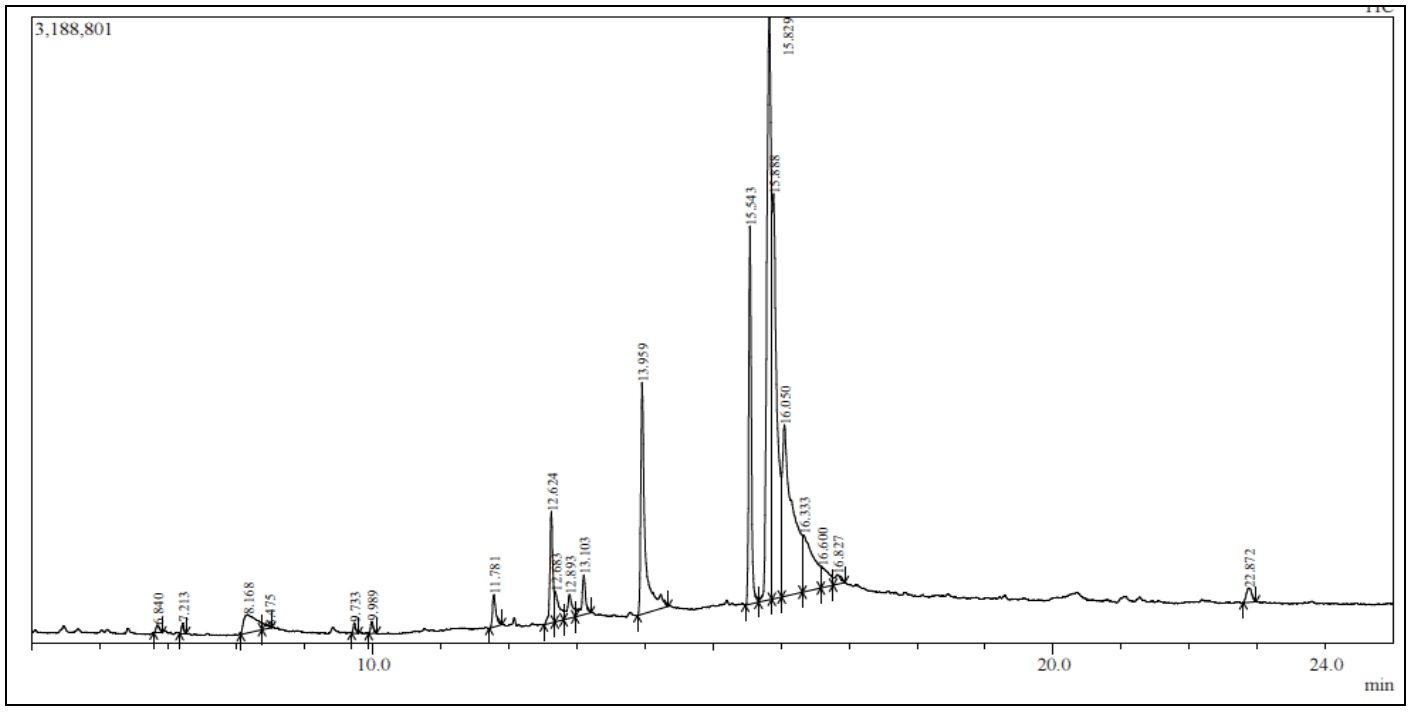


Fig. 3: GC-MS Chromatogram of *Turbinaria ornata* sample

Table 1: Bioactive compounds identified in *Turbinaria ornata* sample extract

Peak	Ret.Time	Name of the Compound	Biological Activity
1	6.840	1-Octanol, 2-butyl	Antimicrobial activity
2	9.733	9-Octadecene	Antibacterial, antifungal, and anti-larva activity
3	11.781	Pentadecanoic acid	Lubricants, Adhesive agents
4	12.624	2,6,10-Trimethyl,14-ethylene-14-P	Anti-proliferative activity
5	13.103	3-Eicosyne	Antimicrobial, Anti-inflammatory activity
6	13.959	9-Octadecenoic acid	Flavour, Cancer preventive, Anti-inflammatory activity
7	15.543	Phytol Isomer	Precursor for the manufacture of synthetic forms of Vitamin E & K1
8	15.829	9,12-Octadecadienoic acid	Anti-inflammatory, Nematicide, Insectifuge, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Antihistaminic, Antiacne, Antiarthritic, Antieczemic
9	16.050	Eicosanoic acid	Arachidic acid is used for the production of detergents, photographic materials and lubricants.
10	16.333	Heptadecanoic acid, ethyl ester	Antioxidant activity
11	16.600	Oleic acid, Propyl ester	Anti-inflammatory, Anti-androgenic Cancer preventive,Hypocholesterolemic, 5- Alpha reductase inhibitor
12	22.872	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	Antimicrobial, Anti-inflammatory anti-Cancer activity

3.2. Molecular Docking

Protein structures were obtained from the protein data bank (PDB) database and ligand was obtained as Pubchem. Auto Dock tools was utilized to generate grids, calculate dock score, and evaluate the conformers of activators bound in the active site of protein as targets. Energy minimization was done in ChemDraw. The minimized structures were then subjected to docking studies. To achieve the purpose, the hetero atoms consisting of water molecules and other additional atoms were removed from the proteins. A Lamarckian genetic algorithm method, implemented in the program Auto Dock 4.1, was employed. This software is used for the estimation of energy during the interaction and identifies the best flexible ligand pose with minimum energy. The scoring function is based on the intermolecular interaction of ligand and protein during docking. As per genetic algorithm, all the torsions were allowed to rotate during docking.

The grid map was centered at residues of the protein and was generated with grid dimension prepared (Center x = 106.13, center y = -4.25 and center z = 18.57). The Lamarckian genetic algorithm and the pseudo-Solis and Wets methods were applied for minimization, using default parameters [12-15]. Complex structures were modeled using modeling software's Pymol (1.1 version, Delano Scientific LLC, San Carlos, CA, USA), Chimera (1.10.1 version UCSF Resources for biocomputing visualization and informatics, NIH, CA, USA) and Pose view [16].

The docked ligand molecules were selected based on docking energy and good interaction with the active site residues and the results are shown in Table 2. Fig. 4 showed the 3D Cartoon view of prepared 1JPW protein. Fig 5 to 8 represent the docking of 6-octadecenoic acid, 9, 12-octadecadienoic acid, bis (2-ethylhexyl) phthalate, oleic acid, and Standard as Doxorubicin shown in Fig.9.

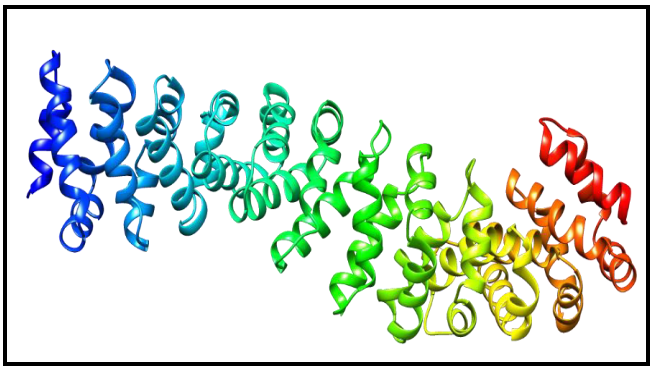
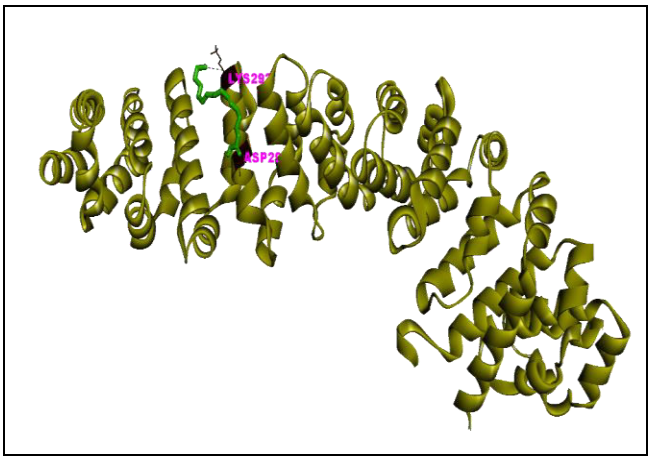
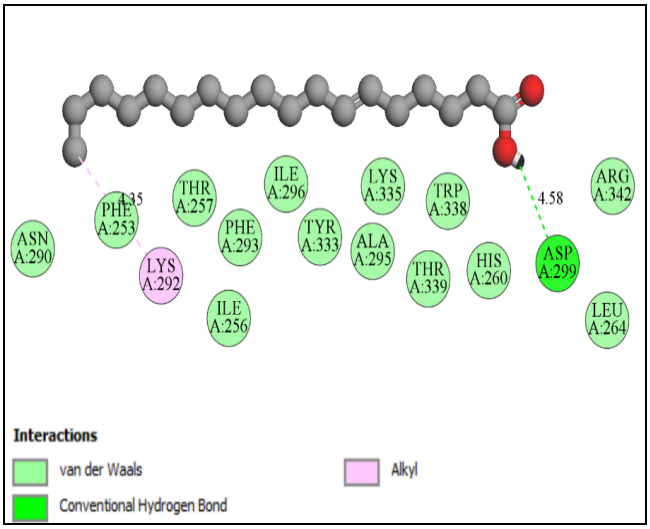


Fig. 4: 3D Cartoon View of 1 JPW (chain A) protein

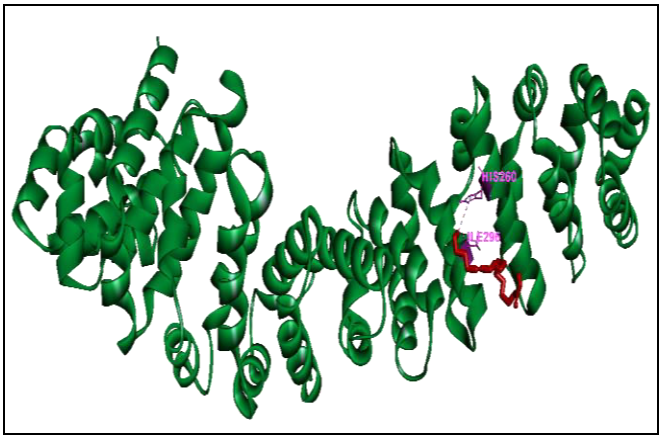


3D Cartoon view of 6-Octadecenoic acid ligand binding with 1JPW (chain A) protein

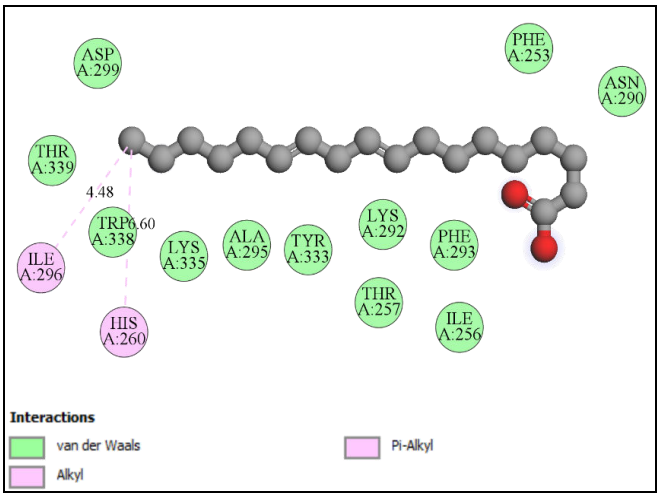


2D view of 6-Octadecenoic acid ligand interaction with 1JPW (chain A) protein

Fig. 5: 6-Octadecenoic acid ligand binding with 1JPW (chain A) protein

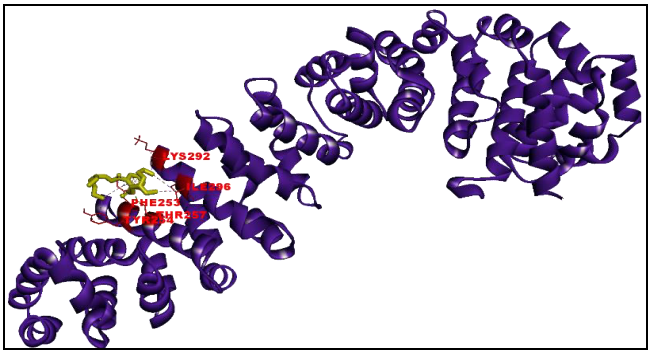


3D Cartoon view of 9, 12-Octadecadienoic acid ligand binding with 1JPW (chain A) protein

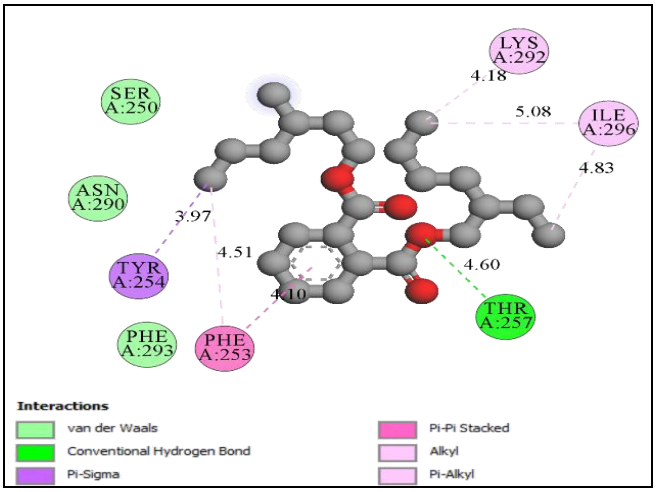


2D view of 9, 12-Octadecadienoic acid ligand interaction with 1JPW (chain A) protein

Fig. 6: 9, 12-Octadecadienoic acid ligand binding with 1JPW (chain A) protein

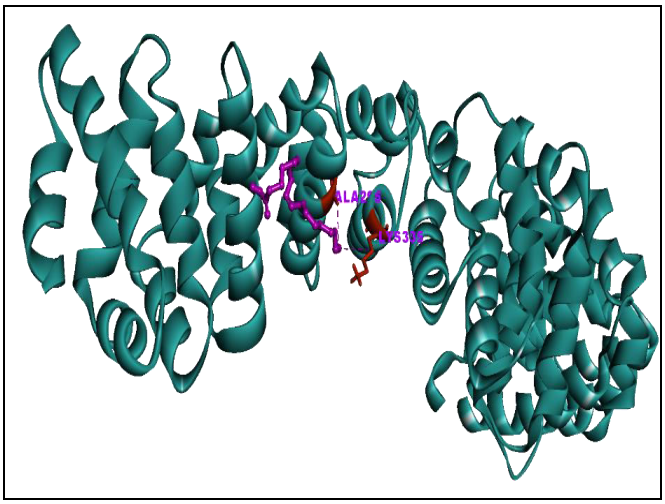


3D Cartoon view of Bis (2-ethylhexyl) phthalate ligand binding with 1JPW (chain A) protein

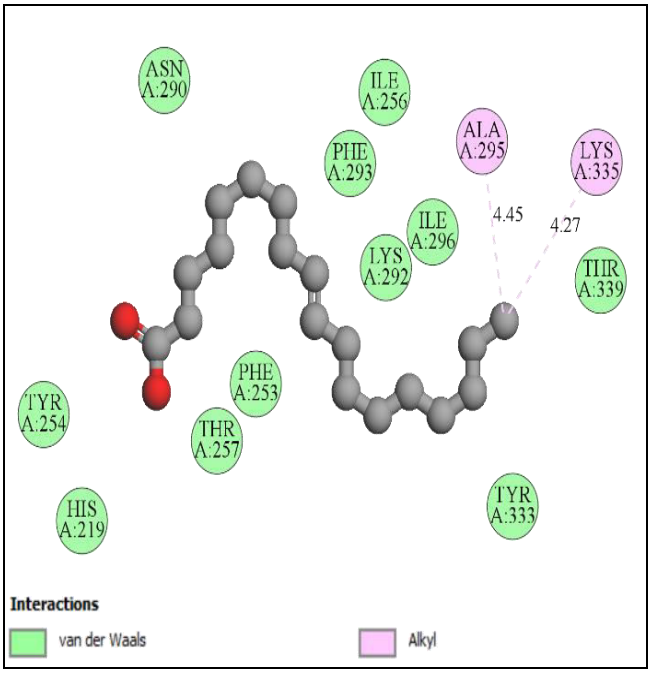


2D view of Bis (2-ethylhexyl) phthalate ligand interaction with 1JPW (chain A) protein

Fig. 7: Bis (2-ethylhexyl) phthalate ligand binding with 1JPW (chain A) protein



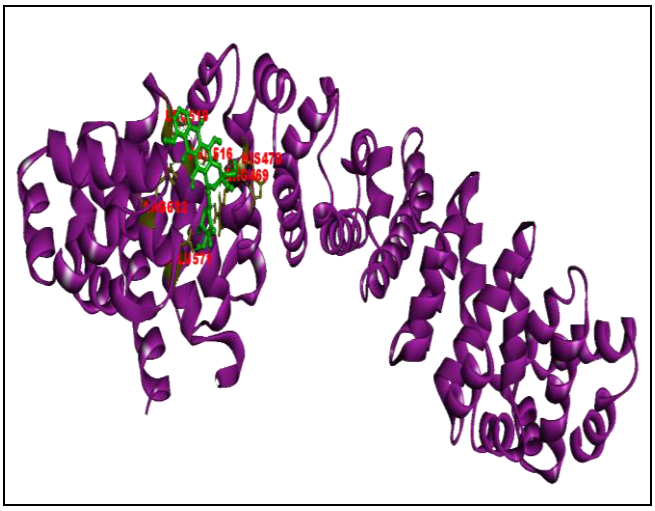
3D Cartoon view of Oleic acid ligand binding with 1JPW (chain A) protein



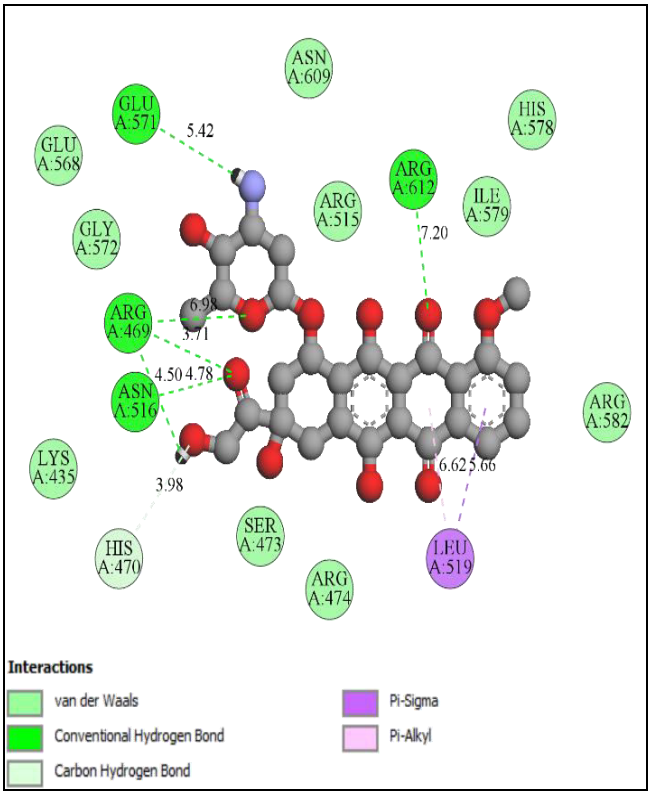
2D view of Oleic acid ligand interaction with 1JPW (chain A) protein

Fig. 8: Oleic acid ligand binding with 1JPW (chain A) protein

The binding interactions of all compounds have shown strong hydrogen bonding and hydrophobic interactions with the target protein. All the ligands show that lower the binding energy higher is the stability of bound confirmation [17]. The docking score of 6-Octadecenoic acid was -5.10 (kcal/mol), 9, 12-Octadecadienoic acid was -4.90 (kcal/mol), Bis (2-ethylhexyl) phthalate was -5.10 (kcal/mol), Oleic acid was -5.00 (kcal/mol) and Doxorubicin was -7.20 (kcal/mol) against 1JPW protein [18]. The docking score was nearest to the standard. The molecular docking of the hits showed the binding mode and interaction energy nearest to the standard which shows that all these ligands can be used as potential drug candidates.



3D Cartoon view of Doxorubicin ligand binding with 1JPW (chain A) protein



2D view of Doxorubicin ligand interaction with 1JPW (chain A) protein

Fig. 9: Doxorubicin ligand binding with 1JPW (chain A) protein

Table 2: Molecular docking with 1JPW protein

Ligand	Molecular formula	M. weight (g/mol)	H-bond acceptors /donors	Binding Affinity (kcal/mol)	1JPW (chain A) Amino acids binding
6-Octadecenoic acid	$C_{18}H_{34}O_2$	282.50	2/1	-5.10	ASN 290, PHE 253, LYS 292, THR 257, PHE 293, ILE 256, ILE 296, TYR 333, ALA 295, LYS 335, THR 339, TRP 338, HIS 260, ASP 299, LEU 264, ARG 342.
9,12-Octadecadienoic acid	$C_{18}H_{32}O_2$	280.40	2/1	-4.90	ASP 299, THR 339, ILE 296, TRP 338, HIS 260, LYS 335, ALA 295, TYR 333, THR 257, LYS 292, PHE 293, ILE 256, ASN 290, PHE 253.
Bis(2-ethylhexyl) phthalate	$C_{24}H_{38}O_4$	390.60	4/0	-5.10	LYS 292, ILE 296, THR 257, PHE 253, PHE 293, TYR 254, ASN 290, SER 250.
Oleic acid	$C_{18}H_{34}O_2$	282.50	2/1	-5.00	ASN 290, PHE 293, ILE 256, LYS 292, ILE 296, ALA 295, LYS 335, THR 339, TYR 333, PHE 253, THR 257, HIS 219, TYR 254.
Doxorubicin (Standard Drug for liver Cancer)	$C_{27}H_{29}NO_{11}$	543.50	12/6	-7.20	GLU 568, GLU 571, ASN 609, ARG 515, ARG 612, ILE 579, HIS 578, ARG 582, LEU 519, ARG 474, SER 473, HIS 470, LYS 435, ASN 516, ARG 469, GLY 572.

4. CONCLUSION

In silico molecular docking is one of the most powerful techniques to discover novel ligand for proteins of known structure and thus play key role in structure-based drug design. Hence in this present work *in silico* molecular docking was carried out to analyze the binding properties of 6-octadecenoic acid, 9,12-octadecadienoic acid, Bis (2-ethylhexyl) phthalate, oleic acid and Doxorubicin to target protein β-catenin (1JPW). The docking studies confirmed the suppressive activity of 6-octadecenoic acid, 9, 12-octadecadienoic acid, Bis (2-ethylhexyl) phthalate, oleic acid and standard as Doxorubicin and thereby suppression of target protein β-catenin (1JPW) through the binding interactions. So the present study might act as supportive evidence for *in vivo* anticancer activity of plant extract *Turbinaria Ornata* on liver cancer which surely help these molecules in reaching the market as commercial drug.

5. ACKNOWLEDGEMENT

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Conflict of interest

The authors declare that there are no conflicts of interest. The research received no specific grant from any funding agency in the public, community, or non-for-profit sectors.

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