In Silico Studies On Colon Cancer Against Hexadecane, Hexadecanoic Acid Methyl Ester And Quinoline, 1,2-Dihydro-2,2,4-Trimethyl Compounds From Brown Seaweed.

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ABSTRACT

The main objective of this was to evaluate the potential drug for treating colon cancer and compound must be extracted from natural sources, especially in seaweed. The above-mentioned compounds are taken from GC-MS studies of Dictyotabartayresiana. There is more number of compounds present in GC-MS analysis, but hexadecane, hexadecanoic acid methyl ester and quinoline, 1,2-dihydro-2,2,4-trimethyl compounds have shown best activities compared to others that proven in previous research. NIST and SDBS databases are helping to draw the compound structure, name, fragments, molecular weight, chemical formula, hydrogen bond donor and acceptor count. The protein were obtained from PDB (Research Collaboratory for Structural Bioinformatics, Protein Data Bank), which is a repository for 3D structural data of large biological macromolecules. Auto Dock tools were utilized to generate grids, calculate dock score and evaluate the conformers of activators bound in the active site of protein as targets. The compound hexadecanoic acid methyl ester (C\textsubscript{17}H\textsubscript{34}O\textsubscript{2}) has the binding affinity of -6.60 kcal/mol that similar to results obtained from Quinoline, 1,2-dihydro-2,2,4-trimethyl-(C\textsubscript{12}H\textsubscript{15}N) and Hexadecane (C\textsubscript{16}H\textsubscript{34}) shows the binding affinity of -6.20 kcal/mol which greater than standard drug 5-fluorouracil (C\textsubscript{4}H\textsubscript{3}FN\textsubscript{2}O\textsubscript{2}). In the present study, technically proven the anticancer property of Dictyotabartayresiana. To conclude, these resulting compounds might be an alternative to synthetic anticancer drugs available in the market.

INTRODUCTION

The marine environment has contained an enormous amount of valuable nutritive food additives, especially the seaweeds, are immensity source of various vitamins like A, E, C, D, B, B12, folic acid, niacin, riboflavin, pantothenic acid and minerals such as Calcium, Potassium, Sodium and Phosphorus (Subathraa and Poonguzhali, 2013). There are many numbers of bioactive substances are naturally present in marine flora which includes flavonoids, Terpenoids, Saponins, Phenols, oxygen heterocyclics, Carbohydrate, nitrogen heterocyclics, Proteins, Tannins, Oils, Resins and Aminoacids, Alkaloids, etc. These are the phytochemical compounds that are enhancing the antibacterial and anticancer properties of corresponding florals that would fight against various foreign agents and balance the internal environment (Krish and Das, 2014).
of Phaeophyceae and 39 species of Rhodophyceae has contained an antibacterial and antifungal activity that were experimentally proven by past decades (Salvador et al., 2007). The previous researches support the fact that an abundance of cytotoxicity capacity and insecticide activity arise in marine floral organisms. The following list of seaweeds is few instances for an above statement, such as Ulva fasciata, Sargassum lanceolatum, Dictyota hauckiana, Lyngaria stellata, Melanothamnus afaghusainii, Dictyota dichotoma var. velutricata, Dictyotaindica, Jolyanthalaminarioides, Sargassumulicifolium (Ayesha et al., 2010). Algal species are a good alternative for allopathic medicines and help in designing new drugs in pharmaceutical industries.

The present investigation was aimed to explore the anticancer potential of D. bartayresiana in silico analysis. Dictyotabartayresiana Lamour (D. bartayresiana) belongs to the family Dictyotaceae; class Phaeophyceae; order Dictyotales. They are predominantly inhabit tropical and subtropical regions which have contained extremely rich amount of secondary metabolites with enormous biological activity especially these metabolites helps to act as a biological agent for antioxidant, antiviral, antifungal activities and antimicrobial activities (Chew et al., 2008).

Morphological features

D. bartayresiana has 8-20 cm thalli, which are attached by many patches of minute rhizoids, straps length measured at 10-15 cm long, 5.7-7.3 mm wide, branching angle noted in 70-80°, straw-colored confirms the species belongs to brown algae group. Strap surfaces have four sporangia on both sides, but gametangial and sterile cells are not known. Hair tufts are a common structure in water inhabitant plants and surface proliferation absent, but margins are smooth.

The current research paved the way to design drugs from D. bartayresiana lamour with the help of computational methods. The designed compounds have involves the following reactions such as assimilation, delivery, anabolism, catabolism, emission and toxic effect. These processes are essential pathways for a typical drug that can be easily understood by computational methods. The main target of the computational method is to establish the desired property of drugs and avoiding following unwanted things like toxic content, a sudden inactive state in response to the physical environment, the reaction on valuable compounds and so on. Molecular modeling studies are considered a good alternative for laboratory testing, which consumes less time and produces significant results. The molecular docking simulation effect transfers 10% of pharmaceutical R & D expenditure into 20% by 2016 (van de Waterbeemd and Gifford, 2003).

The importance of Molecular studies and molecular modeling helps to give strong evidence over biological experiments and understanding the interactions between target receptors and designed molecules. The present study involves Hexadecane, Hexadecanoic acid methyl ester and Quinoline, 1,2-dihydro-2,2,4-trimethyl) identified by GC-MS study were subjected to molecular docking for better recognition of their interaction with Vascular endothelial growth factor receptor 1 (VEGFR 1) and compared with standard as 5-fluorouracil.

**METHODOLODY**

**Ligand**

Ligands for our study included Hexadecane, Hexadecanoic acid methyl ester, Quinoline, 1,2-dihydro-2,2,4-trimethyl) and 5-fluorouracil used as standard. The structures and physicochemical properties of these compounds were retrieved from the PubChem database (www.ncbi.nlm.gov/pubchem), NIST and SDBS databases, which makes database search for a broad range of properties including compound structure, name, fragments, molecular weight, chemical formula, hydrogen bond donor and acceptor count. It has its own online editor with smiles (Simplified Molecular Input Line Entry Specification) format and our compounds are converted to pdf format using this converter. Lipinski’s properties, such as molecular weight and number of hydrogen bond donors and acceptors, were taken from the PubChem. Tables 1 and 2 represent the details of target ligands and protein. Figure 1 shows the structure of the ligands.

**Protein**

The RCSB PDB (Research Collaboratory for Structural Bioinformatics, Protein Data Bank) is a repository for the 3D structural data of large biological macromolecules such as proteins and nucleic acids. It provides simple and advanced searches based on annotations related to sequence, structure and function. The three-dimensional protein structure of the vascular endothelial growth factor receptor one was given in Figure 2. The crystal structure of the drug target Vascular endothelial growth factor receptor one was downloaded from the Protein Data Bank (PDB ID: 3HNG) for Colon cancer (http://www.pdb.org).

**In Silico Docking Studies**

The following two databases are repository units of storing an enormous amount of Protein and lig-
Table 1: Target Ligands

| S. No. | Ligands                                      | PubChem ID |
|--------|---------------------------------------------|------------|
| 1      | 5-fluorouracil*                             | 3385       |
| 2      | Hexadecane                                  | 11006      |
| 3      | Hexadecanoic acid, methyl ester             | 8181       |
| 4      | Quinoline, 1,2-dihydro-2,2,4-trimethyl-      | 8981       |

*Standard inhibitor for colon cancer

Table 2: Target Protein

| Molecule                                      | PDB ID | Chains | Sequence Length | Organism          |
|-----------------------------------------------|--------|--------|-----------------|-------------------|
| Vascular endothelial growth factor receptor 1 | 3HNG   | A      | 360             | Homo sapiens      |

Figure 1: Structure of target ligands
| Compound (s) name | Amino acids binding sites | Secondary Structure binding segment |
|------------------|--------------------------|-----------------------------------|
| 5-fluorouracil    | VAL 892, LEU 882, GLU 878, LEU 833, TYR 914, GLY 915, LEU 1029, VAL 841, LYS 913, TYR 911, CYS 912, VAL 860, ALA 859, PHE 1041, GLY 1042, ASP 1040, CYS 1039, ILE 1038, LYS 861, VAL 907, ILE 908, VAL 909, GLU 910. | α Helix, Sheet and Loop |
| Hexadecane       | PHE 1041, CYS 1039, ASP 1040, ILE 1038, HIS 1020, CYS 1018, LEU 1013, ILE 885, ILE 881, LEU 882, GLU 878, ARG 1026, ARG 1045, VAL 841, VAL 891, VAL 833, GLY 915, ASN 916, LEU 1029, LYS 861, VAL 860, ALA 859, LYS 913, CYS 912, TYR 911, GLU 910, VAL 909, ILE 908, VAL 907. | α Helix, Sheet and Loop |
| Hexadecanoic acid, methyl ester | GLU 910, ALA 859, VAL 909, VAL 892, VAL 891, ILE 1038, TYR 911, CYS 912, ARG 835, VAL 841, LEU 833, GLY 834, ASN 919, ILE 885, LEU 1013, CYS 1018, LEU 1029, ASN 916, GLY 915, ARG 1026, GLU 811, GLU 808, ASP 807, LYS 873, ALA 874, LEU 875, THR 877, LYS 861, GLU 878, ILE 881, LEU 882, ILE 1019, ARG 1021, ASP 1046, ARG 1045, HIS 1020, CYS 1039, ASP 1040, PHE 1041, LEU 1043, ALA 1044, GLY 1042. | α Helix, Sheet and Loop |
| Quinoline, 1,2-dihydro-2,2,4-trimethyl- | LEU 1013, VAL 891, VAL 892, ILE 885, LEU 882, ILE 881, HIS 1020, ARG 1021, ILE 1019, CYS 1018, ASP 807, GLU 878, THR 877, LEU 875, ALA 874, LEU 1043, GLY 1042, PHE 1041, ASP 1040, CYS 1039, ILE 1038, LYS 861, VAL 841, ALA 859, SER 832, LEU 833, GLY 834, ASN 1034, TYR 1002, ASP 998, TYR 920, LYS 924, ILE 994, PRO 993, GLU 992, LYS 991, ASN 916, GLY 915, TYR 914, LYS 913, CYS 912, TYR 911, LEU 1029, LEU 1030, SER 1031. | α Helix, Sheet and Loop |
Table 4: Docking results of plant derived compounds against 3HNG protein

| S. No. | Ligands                                              | Molecular formula      | Molecular Weight [g/mol] | Hydrogen donor | Hydrogen acceptor | Binding Affinity (kcal/mol) |
|--------|------------------------------------------------------|------------------------|--------------------------|----------------|------------------|----------------------------|
| 1      | 5-fluorouracil                                       | C4H3FN2O2              | 130.08                   | 2              | 3                | -5.1                       |
| 2      | Hexadecane                                           | C16H34                 | 226.44                   | 0              | 0                | -6.2                       |
| 3      | Hexadecanoic acid methyl ester                      | C17H34O2               | 270.5                    | 0              | 2                | -6.6                       |
| 4      | Quinoline, 1,2-dihydro-2,2,4-trimethyl-              | C12H15N                | 173.25                   | 1              | 1                | -6.6                       |

Figure 2: Protein (Vascular endothelial growth factor receptor 1 (PDB: 3HNG)) 3D Cartoon view

Plate 1: 5-fluorouracil as ligand and 3HNG as protein interaction
Plate 2: Hexadecane as ligand and 3HNG as protein interaction

Plate 3: Hexadecanoic acid methylester as ligand and 3HNG as protein interaction

Plate 4: Quinoline, 1,2-dihydro-2,2,4-trimethyl as ligand and 3HNG as protein interaction
and structures in an available format such as protein data bank (PDB) database and Pubchem. Docking studies have involved a basic fundamental process including docking score calculation, framing of the grids and binding of activators into targeted proteins, which were done by using the autodock tool. The ChemDraw software helps to evaluate the energy minimization capacity of the resulting compound. Energy minimization is a vital step for understanding the potentiality of ligand to conquers the targeted protein. To get the purpose, the hetero atoms consisting of water molecules and other extra atoms were removed from the proteins. A Lamarckian genetic algorithm method, implemented in the program Auto Dock 4.1, was employed. The Autodock software plays an important role in the determination of energy during the contact of the ligand pose with the protein under minimum energy concentration. As per the genetic algorithm, all the torsions were allowed to rotate during docking. In the protein center, the place has a grid map which formulated by Auto Grid. The Lamarckian genetic algorithm and the pseudo-Solis and Wets methods were implemented for minimization, using default parameters (Ghose and Crippen, 1987; Shruthi et al., 2013). Complex structures were modeled using modeling software’s Pymol (2.2 version, Delano Scientific LLC, San Carlos, CA, USA), Pyrx virtual screening software 0.8, Chimera (1.10.1 version UCsf Resources for biocomputing visualization and informatics, NIH, CA, USA) and Pose view (Olson and J, 2010) installed on a desktop equipped with Pentium (R) Dual-E6600 at 3.05 GHz 3.06 GHz processor (2 GB RAM Core CPU) running the Ubuntu 12.01 (LINUX) and Windows (Windows 8, 64bit) operating system.

RESULTS AND DISCUSSION

The ligand molecules were selected by their docking energy and good interaction with the active site residues and the results are shown in Tables 2, 3 and 4. Hydrogen bond was indicated by violating color lines in between atoms involved and the rest of the interactions were hydrophobic. The activation energy of Hexadecane was found to be -6.20 kcal/mol. The hexadecanoic acid methyl ester was found to be -6.60 Kcal/mol. Quinoline, 1,2-dihydro-2,2,4-trimethyl was found to be -6.60 Kcal/mol and 5-fluorouracil used as a standard was found be -5.10 Kcal/mol. The molecular docking of the hits showed the binding mode and interaction energy. The h-bond pattern was analyzed and confirmed the activation of target protein 3HNG to show the anti-colon cancer activity of Hexadecane, Hexadecanoic acid methyl ester, Quinoline, 1,2-dihydro-2,2,4-trimethyl. Among the various compounds, Hexadecanoic acid methyl ester and Quinoline, 1,2-dihydro-2,2,4-trimethyl has greater binding energy than standard. Plates 1, 2, 3 and 4 show the interaction of ligands and protein shows the cartoon, 2D view and 3D surface view.

The colon cancer (CC) is one of the widespread malignancies which severely cause mortality than other cancer-related cases that reported from WHO. The colon cancer has been taken off the people’s life averagely one million per year and the process of initiation, development, progression and invasion not clearly understood to date (Muzny et al., 2012). The prevention of colon cancer is the main target for saving people’s lives from the terrible disease. 5-fluorouracil used as standard chemotherapy for colon cancer suggested by the national cancer institute. In silico approaches are not only encouraging in disclosing the complex nature of changes in genetic activity during colon carcinogenesis but also help in the information database, which may be useful for identifying novel therapeutic targets.

Molecular Docking has to enhance the innovative research by depicting the binding affinity of targeted protein with ligands. The formation of the complex is the main expected outcome of docking studies and the complex has to maintain stability with the highest concentration (Sandeep et al., 2011). Recent researchers are using this technique for inventing new drugs for their drug discovery process and the computational method reveals the exact capacity of molecules interaction for further research (Koppren, 2009). The pharmaceutical industry encourages structure-based drug design by docking tools because of the reliable results of binding affinity and receptor binding sites (Cosconati et al., 2010; Seeigel and de Groot, 2010).

Cancer treatment involves either suppressing tumor growth or completely terminate the growth mass. The stimulation of natural cell death has a promising method for all cancers treatment and considered as non-invasive therapy. The advantage of eliciting apoptosis is not only specific to single cancer but also almost all cancers. Recent research supports the apoptosis process and the main target for anti-cancer therapy (M and T, 2018).

Phytochemicals are extensively and effectively used in the treatment of various diseases in conventional medicinal treatment. The treatment of chronic diseases like cancer with phytochemicals is critical in the research studies (Ali et al., 2016). Our in silico approach on phytochemicals Hexadecane, Hexadecanoic acid methyl ester, Quinoline, 1,2-dihydro-2,2,4-trimethyl against colon cancer target protein

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Vascular Endothelial Growth Factor 1 (3HNG) is carried out using virtual screening, molecular docking and ADMET methods. Virtual screening of Hexadecane, Hexadecanoic acid methyl ester, Quinoline, 1,2-dihydro-2,2,4-trimethyl and 5-fluorouracil used as standard compound showed the binding affinity towards target 3HNG. The compound was screened with binding affinity and the compound was selected as hits.

VEGF, commonly known as a vascular endothelial growth factor, is a key point formation of tumors and later become angiogenesis. In colon cancer consists of abundant malignant tumor which elicits from VEGF. The angiogenesis is a process that formed due to tumor growth and metastasis. Evidence from preclinical and clinical study indicates Vascular endothelial growth factor is the major angiogenic factor in human colon cancer that coupled with the arrangement of metastases and poor prognosis (Ellis, 2000). Several studies have implicated VEGF in human colon cancer angiogenesis (Brown et al., 1992). VEGF receptor-1 (VEGFR-1) is a high-affinity receptor for VEGF and is normally considered specific to colon cancer (Fan et al., 2005).

The activation energy of Hexadecane, Hexadecanoic acid methyl ester, Quinoline, 1,2-dihydro-2,2,4-trimethyl and 5-fluorouracil was found be -6.20 Kcal/mol, -6.60 Kcal/mol, -6.60 Kcal/mol and -5.10 Kcal/mol respectively. Among the various compounds, Hexadecanoic acid methyl ester and Quinoline, 1,2-dihydro-2,2,4-trimethyl has potential activity than standard. The docking studies of Hexadecane, Hexadecanoic acid methyl ester, and Quinoline, 1,2-dihydro-2,2,4-trimethyl confirmed the suppression of target protein Vascular endothelial growth factor receptor 1 (VEGFR 1) to show the anti-colon cancer activity.

CONCLUSIONS

The molecular docking studies are one of the most influential techniques to find out new ligand for known protein and facilitates in treating dreadful diseases. In this present work, we have carried out molecular docking to analyze the binding properties of the mediator called 3HNG with Hexadecane, Hexadecanoic acid methyl ester, Quinoline, 1,2-dihydro-2,2,4-trimethyl reported from Dicotyotobartayresiana and 5-fluorouracil used as standard. The wet analysis carried out by us showed the best result with 3HNG and proven anti-colon cancer property. Among the various compound, of Hexadecanoic acid methyl ester and Quinoline, 1,2-dihydro-2,2,4-trimethyl has higher binding energy than standard. So the present study may strongly conclude that anti-colon cancer property of the seaweed extract.

REFERENCES

Ali, S., Nasreen, S., Safeer, S., Andleeb, S., Ejaz, M., Bano, S., Shakir, H. A. 2016. Medicinal plants as therapeutic agents for cancer treatment. Punjab Univ J Zool, 31(2):305–305.

Ayesha, Hira, Sultana, V., Jehan, Ara 2010. In vitro cytotoxicity of seaweeds from Karachi coast on brine shrimp. Pakistan Journal of Botany, 42(5):3555–3560.

Brown, L. F., Yeo, K. T., Berse, B., Yeo, T. K., Senger, D. R., Dvorak, H. F., van de Water, L. 1992. Expression of vascular permeability factor (vascular endothelial growth factor) by epidermal keratinocytes during wound healing. The Journal of Experimental Medicine, 176(5):1375–1379.

Chew, Y. L., Lim, Y. Y., Omar, M., Khoo, K. S. 2008. Antioxidant activity of three edible seaweeds from two areas in South East Asia. LWT - Food Science and Technology, 41:1067–1072.

Cosconati, S., Forli, S., Perryman, A. L., Harris, R., Goodsell, D. S., Olson, A. J. 2010. Virtual screening with AutoDock: theory and practice. Expert Opinion on Drug Discovery, 5(6):597–607.

Ellis, L. M. 2000. Vascular Endothelial Growth Factor in Human Colon Cancer: Biology and Therapeutic Implications. The Oncologist, 5(90001):11–15.

Fan, F., Wey, J. S., McCarty, M. F., Belcheva, A., Liu, W., Bauer, T. W., Somcio, R. J., Wu, Y., Hooper, A., Hicklin, D. J., Ellis, L. M. 2005. Expression and function of vascular endothelial growth factor receptor-1 on human colorectal cancer cells. Oncogene, 24(16):2647–2653.

Ghose, A. K., Crippen, G. M. 1987. Atomic physicochemical parameters for three-dimensional-structure-directed quantitative structure-activity relationships. 2. Modeling dispersive and hydrophobic interactions. Journal of Chemical Information and Modeling, 27(1):21–35.

Koppen, H. 2009. Virtual screening - what does it give us? Curr Opin Drug Discov Devel, 12(3):397–407.

Krish, S., Das, A. 2014. In-vitro bioactivity of marine seaweed, Cladophora rupestris. Int. J. Pharm. Biol. Sci, 5:898–908.

M, P. C., T, S. A. 2018. Apoptosis: A Target for Anticancer Therapy. International Journal of Molecular Sciences, 19(2):448–448.

Muzny, D. M., Bainbridge, M. N., Chang, K., Dinh, H. H., Drummond, J. A., Fowler, G., Thomson, E.
2012. Comprehensive molecular characterization of human colon and rectal cancer. *Nature*, 487(7407):330–337.

Olson, O. T., J., A. 2010. AutoDockVina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. *J. Comput. Chem*, 31(2):455–461.

Salvador, N., Garreta, A. G., Lavelli, L., Ribera, M. A. 2007. Antimicrobial activity of Iberian macroalgae. *Scientia Marina*, 71(1):101–114.

Sandeep, G., Nagasree, K. P., Hanisha, M., Kumar, M. M. K. 2011. AUDocker LE: A GUI for virtual screening with AUTODOCK Vina. *BMC Research Notes*, 4(1):445–445.

Seeliger, D., de Groot, B. L. 2010. Ligand docking and binding site analysis with PyMOL and Autodock/Vina. *Journal of Computer-Aided Molecular Design*, 24(5):417–422.

Shruthi, S. D., Rai, S. P., Ramachandra, Y. L. 2013. Isolation, characterization, antibacterial, antihelminthic, and in silico studies of polyprenol from Kirganelia reticulata Baill. *Medicinal Chemistry Research*, 22(6):2938–2945.

Subathraa, K., Poonguzhali, T. V. 2013. Effect of different extracts of Chaetomorpha antennina and their phytochemical screening. *Int J Curr Sci*, 6:35–39.

van de Waterbeemd, H., Gifford, E. 2003. ADMET in silico modelling: towards prediction paradise? *Nature Reviews Drug Discovery*, 2(3):192–204.