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RESEARCH ARTICLE

IN SILICO SCREENING AND *IN VITRO* ANALYSIS OF CERVICAL CANCER CELLS IN HELA CELL LINE VIA *CHRYSOPHYLLUMCANITO* LEAF

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ABSTRACT

In silico screening is a study of systems biology commences by computer along with proteins and chemical compounds. Diverse types of cancer responsible proteins were analyzed under schrodinger suite such as PTEN, NKCR, BRCT7 and BRCT8, Chek1, BRCA, Rad51D, BRCA1, HSP27 and HSP70. Proteins study revealed the maximum anti-cancer effects of cervical cancers. *Chrysophyllumcanito* plant chemical compounds roles were annotated by means of systems biology with strong evidence. *In vivo* analysis was obviously demonstrated the heat shock protein 70 kilo dalton role in cervical cancer via the HeLa cell line.

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INTRODUCTION

*In silico*screening is a computer based study precisely express the virtual reality via systems biology. (Sharmila *et al*., 2013) *in silico* drug design have been successful due to the artificially generating an environmental like biomolecules, proteins and chemical compounds towards in a computer. (Sangeetha *et al*., 2015) It's potential to accelerate the rate of invention by the powerful bioinformatics sagacity. (Preeti Srivastava *et al*., 2013) Predicting the chemical compounds role in living organisms is tough and arduous due to polyfunctionality of the molecules. (Guy Bouchoux *et al*., 2007; Bao *et al*., 2003) These types of systems based, methodical way can make obviously comprehend about biomolecules. (Sheila Tang *et al*., 2011) Integrated systems biology approach in a biological research field can massively emerge the role of components like enzymes, chemical compounds and molecules. (Siva Kishore Nandikolla *et al*., 2011) *In vitro* analysis is a type of biological study to create a context for living organisms. This method has been used for the past hundreds of years to invent the efficiency of viability of cell and microorganisms. (Ustunsoy *et al*., 2016) Actually, this is the commencement for drug invention at the same time colorimetric assay also processed like MTT assay and MTS assay. (Carrie Lovitt *et al*., 2014)Using chemical drugs for cancer is precarious due to side effects and tolerances. Identifying chemical compounds in medicinal plants may reveal the new roles against cancer cells. (Stefania Nobili *et al*., 2009) Medicinal plants have excellent chemical compounds,while isolating the anti-cancer liable compound in medicinal plants can only expose the natural property. (Rajandeep Kaur *et al*., 2011) Present days' research focused on medicinal plants due to the invention of anti-cancer liable compound. (SirimalGopi Krishna *et al*., 2015) *In silico* analysis also processed to many plants like vinca (*Catharanthusroseus)*, cape gooseberry *(Physalisperuviana)*, red water lily (*Nymphaea pubescens*) and amla (*Emblicaofficinalis*) (Subashini *et al*., 2015; Ayik Rosita Puspaningtyas *et al*., 2014; Kiran Kumar Angadi *et al*., 2013; Amaravani *et al*., 2012) Identifyinganti-cancer responsible compound in star apple (*Chrysophyllumcanito*) is a novel. One of the most universally threatening disease is cancer due to tumor formation, uncontrollable and promptly rearing feasibility. Indeed, there are more than one hundred types of cancer can potentially affect the normal, healthy tissues and functioning organs by dint of metastasis. (Siljaheilmann *et al*., 2015; Kata juhasz *et al*., 2014) Its utterly making fierce of cancer, actually disseminating the cancer cells in all body parts can imminently maket umorformation. (Flavio*et al*., 2008) Incessant, cancer cell rearing in many body parts leads to death.

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Some types of viral-infections, free radicals, radiation can undeniably take a person vicinity to cancer. (Chien-Jen Chen *et al*., 2014) Commencing stage of cancer slightly emerge with few types of symptoms like prolonged cough, unreasonable weight loss and abnormal bleeding. (Saskia *et al*., 2007) Most important, thing for a cancer patient is routinely taking nutrients because the incessant state of mitosis and meiosis cell metabolism requires lot of nutrition. Hence, the first pivotal part is better food consumption. (Xiao-Jing Du *et al*., 2015) Taking foods in Initial stage do not expose anorexia and cachexia. (Claire L. Donohoe *et al*., 2011) Gradually rising malady conditions make the patients to appetite and aversion of food. Incessantly rearing cancer cells highly requires adenine triphosphate, minerals, vitamins and nutrients for growth. (Generoso Uomo *et al*., 2006) Consequently, occur several metabolisms are alike lipolysis, proteolysis and glycogenolysis. (Maryam Ebadi *et al*., 2014) *In silico*study is a drug invention process initiates in computer. (SinoshSkariyachan *et al*., 2010) In fact, the acumen of atoms and its nature of rule areinterpreted by researchers. Consensus studies of physics, chemistry are synchronized towards with systems biology, it has been massively revealing the compounds and protein interaction. (Jane Calvert *et al*., 2009) Integrated methods can beaccurately analyzed the connections between protein and ligand. (Deepak Yaraguppi *et al*., 2012) In this study absolutely concentrated on the diverse types of cancer protein. Maintaining the genetic material, consensus information together is the survival way for the cells. (Lucy *et al*., 2014) PTEN is a gene present in the human genome, that has been responsible for synthesis of phosphatase and tens in homolog protein. (Hui-Kuan Lin *et al*., 2004) Mutation of this liable gene leads to proliferation of diverse cancer. Especially, prostate cancer arises owing to the deletion of this gene in prostate gland. (Antje Krohn *et al*., 2012) This gene regulates the versatile process in the body cells alike cell angiogenesis, adhesion, cell movement and apoptosis and this PTEN protein has sturdy role in tumor suppression. (Kenneth M. Yamada *et al*., 2001) NKCR is a natural killer cells receptor produced by the innate immune system the role of this cell is imminent viral infected cell response. (Lewis L. Lanier *et al*., 2003) Specifically, the infected cells express major histocompatibility complex upon the cell surfaces.

It rapidly also recognizes the stressed cells nucleated and unnucleated cells like matured red blood copper cells in the body. Nk cells can be discriminated upon their functions of cytotoxicity receptors. (Regis Josien *et al*., 1997) Directly involves whether it binds with Fas ligand, that interaction implies infected cell due to the pathogen. Balance of this activation and inactivation depends on the temptation. Initial state of infected cells has the MHC complex I molecules upon them and it is destructed by NK cell via release of cytokine. (Julie Dam *et al*., 2006) Harmful cells lose the MHC complex I markers on the cell surface, albeit it hard to detect by NK cells. (John D. Schatzle *et al*., 1999) One of the main role for BRCT and BRCA is recognition of the phosphorylated protein binding sequence via phosphorylated modules. (Charles Chung Yun Leung *et al*., 2011) BRCT7 and BRCT8 are family of interconnected evolutionary protein domain. This protein is found in breast cancer for DNA overhaul and BRCT domain have identified as the tumor suppressor and it have myriad of role in DNA metabolism. (Qian Wu *et al*., 2015) Truncation and deletion of region reverts ovarian cancer and breast cancer. BRCT domain have made up of ~ 95 amino acid residues and its liable for diverse functions. (Derbyshire *et al*., 2002) It folds the globular domains with secondary protein structures well-ordered as βαββαβα. (Zi-Zhang Sheng *et al*., 2011)23 human genes have been identified as BRCT domain and it encodes the BRCT domain protein. (David J. Adams *et al*., 2005)CHEK1 gene is encoded the serine & threonine specific protein kinase referred to as check point kinase 1 or chek1. (Veronique *et al*., 2015)Chek1 synchronizes for DNA damage response, involves in cell cycle arrest and cell death. (Jan Benada *et al*., 2015)RAD51L3 gene is encodes the RAD51D homolog protein. It's a pivotal protein encompasses the DNA repair role and also interact to XRCC2 and RAD51C. (Paulikova M. Chmelarova *et al*., 2013) HSPB1 gene is constructs the heat shock protein 27 as per encoded sequence via specific amino acids assembles.

HSP 27 is involved in apoptosis inhibition and cell differentiation in cells. (Concannon *et al*., 2003)HSP 70 is a heat shock protein with 70 kilodalton this protein expressed ubiquitously during the stress and it has several functions. (Harm H. Kampinga *et al*., 2010) Protein folding requires heat shock protein to construct freedom of rotation. Essentially, C_α position in φ & ψ bond have freedom of 180° rotation ribosomes involves in protein folding. HSP important for ameliorating protein folding. (AdiPrayitno *et al*., 2013) HSP will over expressed in skin cancers.HSP 70 has major role in cervical cancer and it is a type of cancer commences in cervix due to human papilloma viral infection, occurring metastasis in cervix also initiate protuberance. (Badowska-Kozakiewicz *et al*., 2012) Premature stage does not exhibit symptoms and later emerge the pelvic pain and abnormal blood bleeding. Supporting risk factor leads to instantly peril,like smoking, liquor and taking more pills without doctor's prescription. (GoodarzDanaei *et al*., 2005) Recent days' cervical cancer *in vitro* evaluation is possible via a HeLa cell line.MTT assay is feasible to view viable cells via microscope and to evaluate the living cell concentration under spectrophotometer. The biological motivation for this assay is to assess the cell metabolic activity. (Mehdi *et al*., 2011)MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide salts revert the purple color in cytoplasm owing to the contact of nicotinamide adenine dinucleotide phosphate-oxidase. This oxidoreductase enzyme is unerringly being responsible for the conversion of MTT 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide to MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylformazan in mitochondria. Novel of the present studycommenced from *in silico* screening of *Chrysophyllumcanito* plant chemical compounds.

This is the first report provides *Chrysophyllumcanito* leaf containing anti-cancer compounds for diverse cancer such as PTEN, NKCR, BRCT7, BRCT8, Chek, BRCA, Rad51D, BRCA, HSP27 and HSP70. *In vitro* cervical cancer studies delivered the accurate anti-cancer liable compound in *Chrysophyllumcanito* leaf. This study elicited the highly energetic effects of cervical cancer via *in silico* and *in vitro* studies.

Experimental section

Plant collection

Chrysophyllumcanito leaf was collected in Anaikatti, Coimbatore. Authenticated by Tamilnadu Agriculture University.

Sample preparation for MTT assay

Fresh leaves were dried and pulverized, ethanol extracts were soaked in *Chrysophyllumcanito* powder via soxhilation. After that ameliorated *Chrysophyllumcanito* powder was detached for evaluation of MTT assay in cervical cancer from HeLa cell line.

Source of structures of protein and ligand

Protein Data bank was used to find out the structure of protein.(Saskia C.C.M. Teunissen, *et al*., 2007) Three parameters such as <4 A resolution of crystal studied protein, monomer from the complex and exclusion of complex ligand was used to select the protein from PDB hits. Isolated Compounds from ethanol extract of *Chrysophyllumcanito* was drawn using Chemsketch (http://www.acdlabs.com/download) and optimized in 3-dimensional way to view and import into the Schrodinger Suite.

Docking of targeted protein and Ligand

Preparation of Target protein

Protein preparation wizard

The typical structure file from the PDB is not suitable for immediate use in molecular modeling calculations. A typical PDB structure file consists only of heavy atoms and may include a co-crystallized ligand, water molecules, metal ions, and cofactors. PDB structures have missing information on connectivity, which must be assigned, along with bond orders and formal charges. Schrodinger has therefore assembled a set of tools to prepare proteins in a form that is suitable for modeling calculations.

Protein Preparation Process steps

The preparation of a protein involves a number of steps, which are outlined below. The procedure assumes that the initial protein structure is in a PDB-format file does not include explicit hydrogen. The result is refined, hydrogenated structures of the ligand and the ligand-receptor complex, suitable for use with other Schrödinger products.

- Importing modeled protein typically from Prime Module into Maestro.
- Located any waters keep, and then delete all others. These waters are identified by the oxygen atom, and usually do not have hydrogen attached. Generally, all waters (except those coordinated to metals) are deleted, but waters that bridge between the ligand and the protein are sometimes retained.
- Simplified multi-meric complexes.
- Determined whether the protein-ligand complex is a dimer or other multimer containing duplicate binding sites and duplicate chains that are redundant.
- Fixed any serious errors in the protein. Incomplete residues are the most common errors, but are relatively harmless if they are distant from the active site. Structures that are missing residues near the active site are repaired.
- Checked the protein structure for metal ions and cofactors.
- Set charges and correct atom types for any metal atoms, as needed.
- Set bond orders and formal charges for any cofactors, as needed.
- Fixed the orientation of any mis-oriented groups (such as amide groups of Asn and Gln).
- Adjusted the ligand bond orders and formal charges.
- Adjusted the ionization and tautomerization state of protein and ligand, if necessary.
- Refined the structure.
- Examined the refined ligand/protein/water structure for correct formal charges and protonation states and make final adjustments as needed.
- Check the orientation of water molecules and other groups, such as hydroxyls, amides, and so on.

Grid box generation

The receptor grid can be set up and generated from the Receptor Grid Generation panel. The options in each tab of this panel allow us to define the receptor structure by excluding any co-crystallized ligand that may be present, determine the position and size of the active site as it will be represented by receptor grids, set up Glide constraints, and set up flexible hydroxyl groups. Ligand docking jobs cannot be performed until the receptor grids have been generated. Receptor grid generation requires a "prepared" structure: an all-atom structure with appropriate bond orders and formal charges. In short, screening potential ligand to interact with proteins was performed using Glide Dock from flexible docking procedure. At first protein and ligand were prepared, to dock with protein, moiety grid has to set, finally prepared ligand was subjected to dock with prepared target proteins.

Flexible Docking using Glide Module - Schrodinger Suite

The prepared ligand molecules were docked with the selected target proteins using Glide Module to study whether the ligand molecules interact with the active binding sites of target proteins studied previously through protein-protein interaction.

Glide Dock

Glide uses a hierarchical series of filters to search for possible locations of the ligand in the active-site region of the receptor. The shape and properties of the receptor are represented on a grid by several different sets of fields that provide progressively more accurate scoring of the ligand poses. Conformational flexibility is handled in Glide by an extensive conformational search, augmented by a heuristic screen that rapidly eliminates unsuitable conformations, such as conformations that have long-range internal hydrogen bonds.

Glide is designed to assist you in screening of potential ligands based on binding mode and affinity for a given receptor molecule. Ligand scores can be compared with those of other test ligands or ligand geometries with those of a reference ligand. Additionally, Glide can be used to generate one or more plausible binding modes for a newly designed ligand. Once favorable structures or bonding conformations is located with Glide, Liaison or Q-Site can be used to obtain binding energies for ligand-receptor pairs.Glide searches for favorable interactions between one or more ligand molecules and a receptor molecule, usually a protein. Each ligand must be a single molecule, while the receptor may include more than one molecule, e.g., a protein and a cofactor. Glide can be run in rigid or exible docking modes; the latter automatically generates conformations for each input ligand. The combination of position and orientation of a ligand relative to the receptor, along with its conformation in exible docking, is referred to as a ligand pose. The ligand poses that Glide generates pass through a series of hierarchical filters that evaluate the ligand's interaction with the receptor. The initial filters test the spatial fit of the ligand to the defined active site, and examine the complementarily of ligand-receptor interactions using a grid-based method patterned after the empirical Chem Score function.Poses that pass these initial screens enter the final stage of the algorithm, which involves evaluation and minimization of a grid approximation to the OPLS-AA non-bonded ligand-receptor interaction energy. Final scoring is then carried out on the energy-minimized poses. By default, Schrodinger's proprietary Glide Score multi-ligand scoring function is used to score the poses. If Glide Score was selected as the scoring function, a composite E model score is then used to rank the poses of each ligand and to select the poses to be reported to the user. E model combines Glide Score, the non-bonded interaction energy, and, for exible docking, the excess internal energy of the generated ligand conformation.

Interaction of Targeted Protein and Isolated Ligand

Corrected Lewis structure was generated for ligand using Glide ligand docking jobs. Glide uses a hierarchical series of filters to search for possible locations of the ligand in the active-site region of the receptor. The shape and properties of the receptor are represented on a grid by several different sets of fields that provide progressively more accurate scoring of the ligand poses. Conformational flexibility is handled in Glide by an extensive conformational search, augmented by a heuristic screen that rapidly eliminates unsuitable conformations, such as conformations that have long-range internal hydrogen bonds. Each rotamer group is attached to the core by a rotatable bond, but does not contain additional rotatable bonds. The core is what remains when each terminus of the ligand is severed at the "last" rotatable bond. Carbon and nitrogen end groups terminated with hydrogen (—CH3, —NH2, —NH3+) are not considered rotatable because their conformational variation is of little significance. Schrodinger's proprietary Glide Score multi-ligand scoring function is used to score the poses.

Cell line

The human cervical cancer cell lines (HeLa) was obtained from National Centre for Cell Science (NCCS), Pune and grown in Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). The cells were maintained at 37°C, 5% CO_2, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

Cell treatment procedure

The monolayer cells were detached with trypsin-ethylenediaminetetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium containing 5% FBS to give final density of $1x10^5$ cells/mL. One hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37°C, 5% CO_2, 95% air and 100% relative humidity. After 24 hours of the cells were treated with serial concentrations of the test samples. They were initially dissolved in neat dimethyl sulfoxide (DMSO) and an aliquot of the sample solution was diluted to twice the desired final maximum test concentration with serum free medium. Additional four serial dilutions were made to provide a total of five sample concentrations. Aliquots of 100 µL of these different sample dilutions were added to the appropriate wells already containing 100 µL of medium, resulting in the required final sample concentrations. Following sample addition, the plates were incubated for an additional 48 hours at 37°C, 5% CO_2, 95% air and 100% relative humidity. The medium containing without samples were served as control and triplicate was maintained for all concentrations.

MTT assay

3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore,the amount of formazan produced is directly proportional to the number of viable cells. After 48 hours of incubation, 15µL of MTT (5mg/mL) in phosphate buffered saline (PBS) was added to each well and incubated at 37°C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100µL of DMSO and then measured the absorbance at 570 nm using micro plate reader. The % cell inhibition was determined using the following formula. % Cell Inhibition = 100- Abs (sample)/Abs (control) x100. Nonlinear regression graph was plotted between % Cell inhibition and Log concentration and IC_{50} was determined using Graph Pad Prism software.

RESULTS AND DISCUSSION

Retrieval of protein for anticancer study

PDB is a repository for the 3-D structural data typically obtained by X-ray crystallography or NMR spectroscopy and submitted by biologists and biochemists from around the world, are released into the public domain, and can be accessed at no charge on the internet (Table 1 and Figure 2).

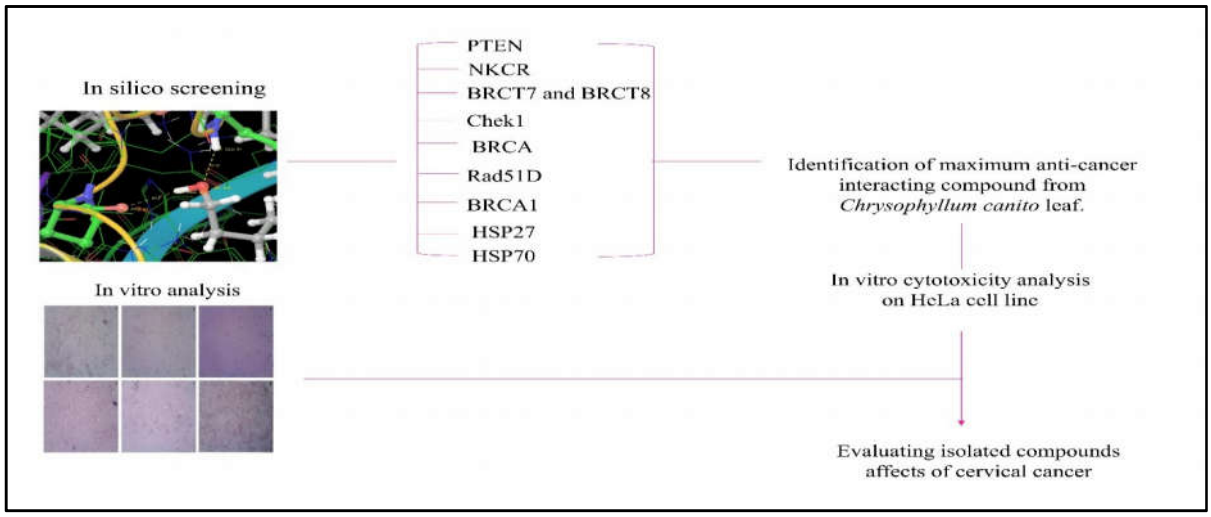


Fig.1. Experimental design

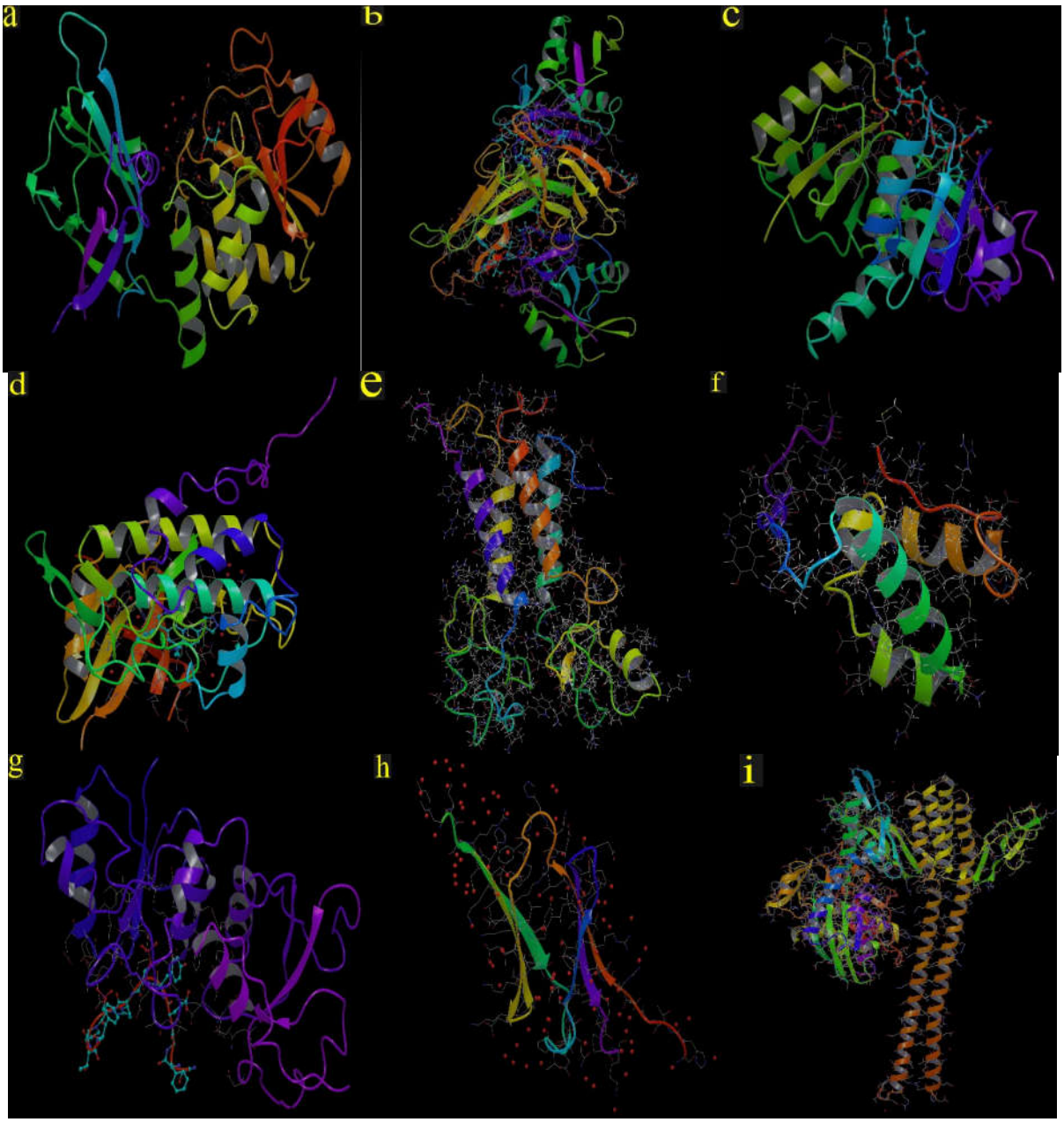


Fig. 2. Stereoview of the cancer proteins as ribbon representations. a) 1D5R-PTEN Crystal structure. b) 3FF7-NKCR Crystal structure. c) 3AL3-BRCT7 and BRCT8 Crystal structure. d) 2R0U-CHEK1 Crystal structure. e) 1JM7-BRCA Crystal structure. f) 2KZ3-RAD51D Crystal structure. g) 4JLU-BRCA1 Crystal structure. h) 3Q9P-HSP27 Crystal structure. i) 1DKG-HSP70 Crystal structure

For screening of anti-cancer properties, all the molecules obtained from the ethanol extract was interacted with various anticancer proteins as listed in Table. The docking features of the best molecule based interaction with various anti-cancer proteins resulted in revealing potent anti-cancer property of *Chrysophyllumcanito.*

Prepared tumor suppressor PTEN vs 1-docosanol

The binding conformation of 1-docosanol within the active site of Prepared tumor suppressor PTEN had a Glide score of -4.12. Ligand 1-docosanol formed two hydroxyl interactions with tumour suppressor PTEN protein therefore enhancing its suppressing potential. One hydrogen bond with H atom of the active site of the Prepared tumor suppressor PTEN at GLU91 residue (2.12Å) and another with the O atom of the active site of the Prepared tumor suppressor PTEN at PRO 95 residue (2.12Å) was observed (Fig 3).

Prepared - NK cell receptor 3FF7 vs 4,4,7,7-tetramethyl deca-1,9-diene

The docking simulation of 4,4,7,7-tetramethyldeca-1,9-diene into binding site of Prepared NK cell receptor 3FF7 was analyzed. The Glide score (--5.136233 kcal/mol) was calculated. 4,4,7,7-tetramethyldeca-1,9-diene formed one hydrogen bonds with the active site of the Prepared NK cell receptor 3FF7 interacting with residues like ILE 38 (2.01 Å) (Fig 4).

Prepared stem cell line 3AL3 vs (1S,2R,5R)-5-methyl-2-propan-2-yl-1-cyclohexanol

The docking simulation of (1S,2R,5R)-5-methyl-2-propan-2-yl-1-cyclohexanol with prepared stem cell line 3AL3 had a glide score of -6.08. Upon the examination of docking features between 1S,2R,5R)-5-methyl-2-propan-2-yl-1-cyclohexanol with Prepared stem cell line 3AL3 one hydrogen bonds was observed. The hydrogen atom of the ligand molecule was nicely bonded with backbone oxygen atom of the residue of ARG 1314 (1.97 Å) (Fig 5).

Prepared breast cancer protein BRCA vs 3,4-dimethyl-1-cyclohexanol

The binding conformation of 3,4-dimethyl-1-cyclohexanol within the active site of prepared breast cancer protein BRCA had a Glide scores of -3.8. Ligand 3,4-dimethyl-1-cyclohexanol formed only one hydroxyl bonds with the active site of the prepared breast cancer protein BRCA at HIS 41 residue (2.08Å). Although no further interactions were observed the stability of the complex justified its potential use as an anti-breast cancer agent (Fig 6).

Prepared Breast cancer protein BRCA1 vs 3,4-dimethyl-1-cyclohexanol

The binding conformation of 3,4-dimethyl-1-cyclohexanol within the active site of Prepared Breast cancer protein BRCA1 had a Glide score of -5.659478. Ligand 3,4-dimethyl-1-cyclohexanol formed only one hydrogen bond with H atom of the active site of the Prepared Breast cancer protein BRCA1 at LEU1701 residue (2.18Å) (Fig 7).

Prepared Heat Shock protein 70 vs 3,7,11-trimethyl-3-dodecanol

The docking simulation of 3,7,11-trimethyl-3-dodecanol with Prepared Heat Shock protein 70 had a glide score of -6.27. Upon the examination of docking features between 3,7,11-trimethyl-3-dodecanol with Prepared Heat Shock protein 70, one hydrogen bond was formed between the 3,7,11-trimethyl-3-dodecanol with Prepared Heat Shock protein 70 with LYS 254 at 2.06A (Fig 8).Comparison among all the interactions observed, the binding simulation with Heat shock protein 70 as a cancer protein target shows a higher Glide score indicating the stability in interaction. The HSP70 family of proteins can be thought of as a potent buffering system for proteotoxic stress, cellular stress, either from extrinsic like physiological, viral and environmental or intrinsic like replicative stimuli. As such, this family very essential for survival functions in the cell. Remarkably, cancer cells absolutely rely on the buffering system for continued survival. (Elisa Zorzi *et al*., 2011) It has been acknowledged for many years that HSP70 is frequently over expressed in transformed or cancerous cells. Cancer cells over expressed few types of protein in cytoplasm, high levels of proteotoxic stress in tumors and subsequent activation of HSF1, which further contributes to the frequent over expression of the HSP70 gene in cancer cells. (Sandy *et al*., 2009)In habitually cytochrome c in mitochondria is liable for external and intrinsic stimuli for apoptosis. Mitochondria DNA is responsible for apoptosome release via cytochrome c. It releases the quaternary apoptosomeproteins to the cytoplasm, Apoptotic proteinase activating factor1 interconnected to apoptosome and then binds with deoxyadenosine triphosphate factor. Interacted entire factors can revert the inactivated pro caspase 9 to activate scaspase, and this event commences the cell shrinkage. (Luigi Ravagnan *et al*., 2001) Activated apoptosis lead to cell death (Fig. 9). Ribosomes synthesis the Heat shock protein 70 in the cytoplasm, it transports the proteins to mitochondria and retains the folded state of proteins. (Ntsiki M. Held *et al*., 2015) Mitochondria recognizes the proteotoxic stress in the cytoplasm via aneuploidy. Apoptosome mechanisms already annotated in (Fig.11). Heat shock protein highly expressed cancer, it inhibits the apaf1 factor in cytoplasm to retain the cell structure for survival. HSP transfer protein to mitochondria, blocks apaf1 factor in cytoplasm. (Hua Zou *et al*., 1997; Kim *et al*., 1999) Docking study revealed maximum anti-cancer property for 3,7,11-trimethyl-3-dodecanolofHSP70 in cervical cancer (Table 2). Incessant cancer cell stability requires HSP 70, 3,7,11-trimethyl-3-dodecanol, it suppresses the heat shock protein ultimately alters the cancer cell shrinkage and commits to cell death (Fig. 10).

MTT assay

Molecular docking (*Schrodinger software)* meticulously identified protein suppressing compound role against prepared heat shock protein 70 *(HSP 70)* cervical cancer.

Chrysophyllumcanito leaf various concentrations of sample were tested in HeLa cell line. Colorimetric assay exposed the presence of viable cellsin (Fig. 11).

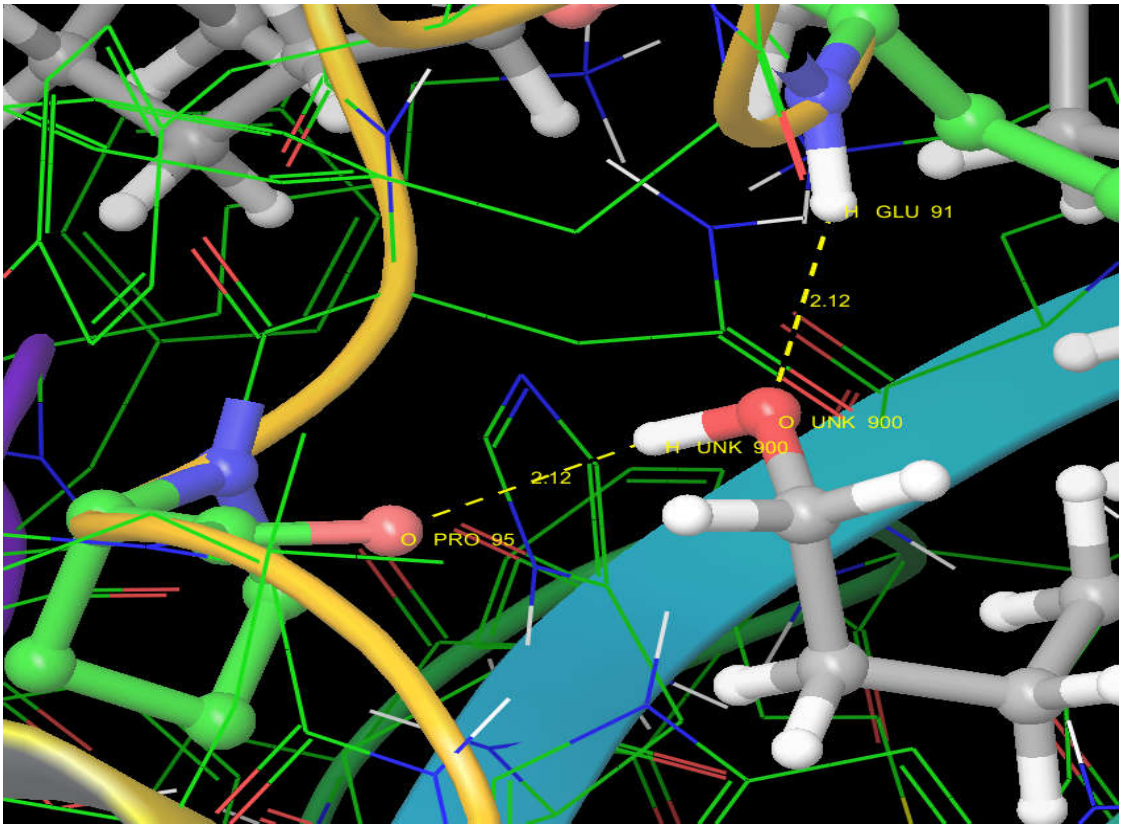


Fig. 3. Docking interaction of 1-docosanol within the active site of Prepared PTEN tumor suppressor PTEN

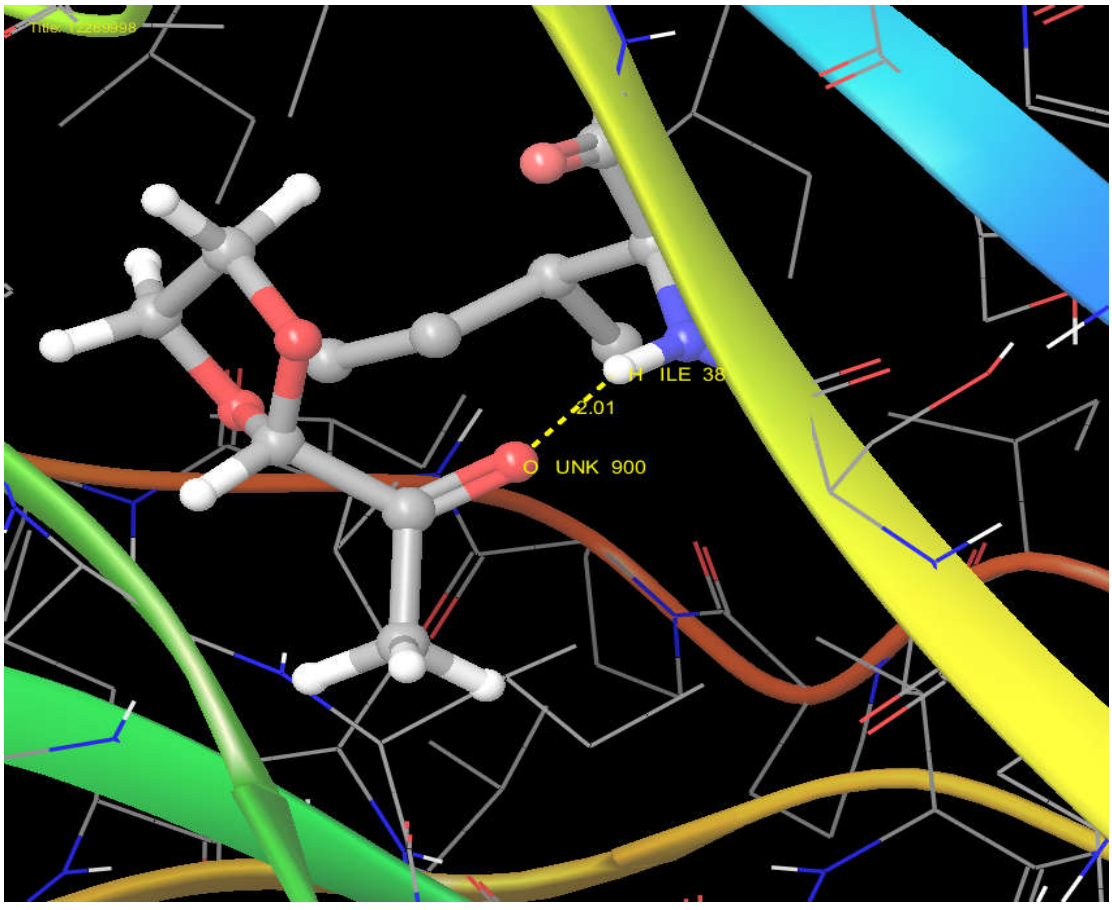


Fig. 4. Docking interaction of 4,4,7,7-tetramethyldeca-1,9-diene within the active site of Prepared - NK cell receptor 3FF7

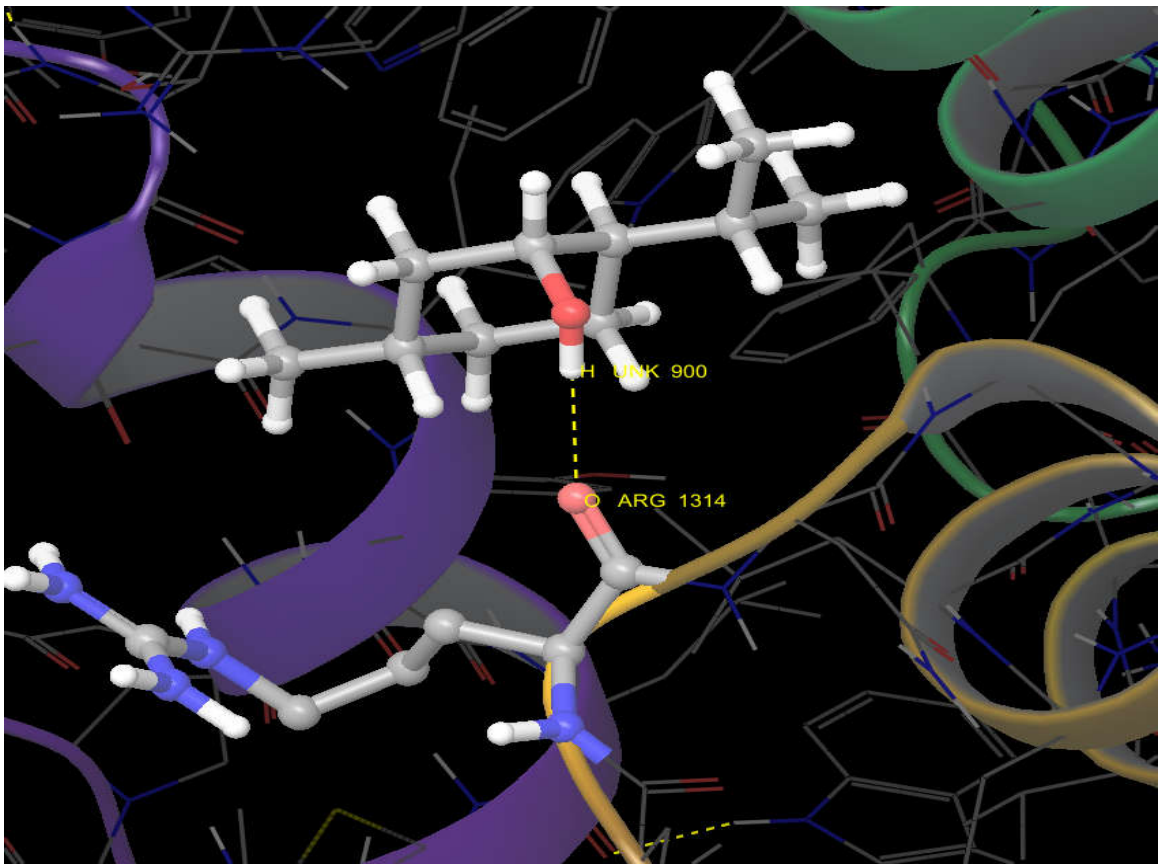


Fig. 5. Docking interaction of (1S,2R,5R)-5-methyl-2-propan-2-yl-1-cyclohexanol within the active site of Prepared stem cell line 3AL3

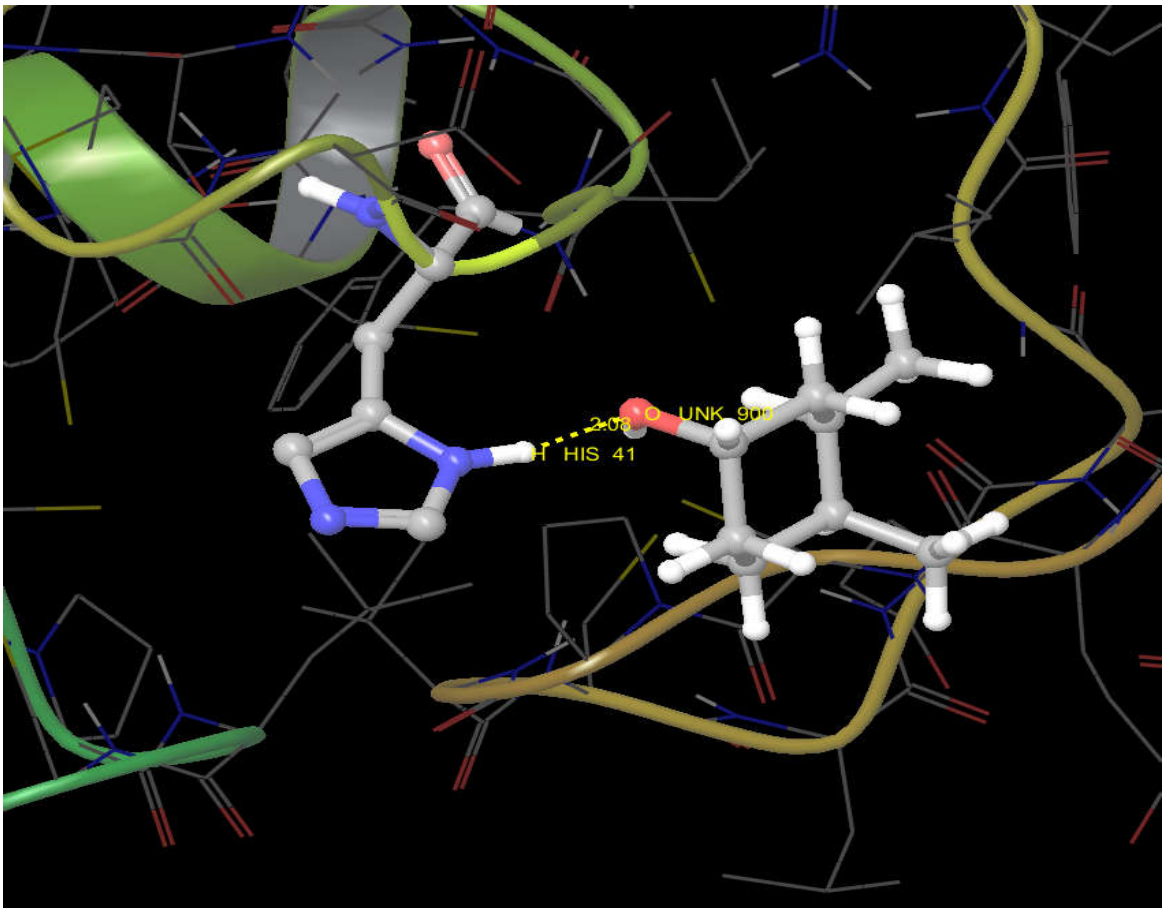


Fig. 6. Docking interaction of 3,4-dimethyl-1-cyclohexanol within the active site of prepared breast cancer protein BRCA

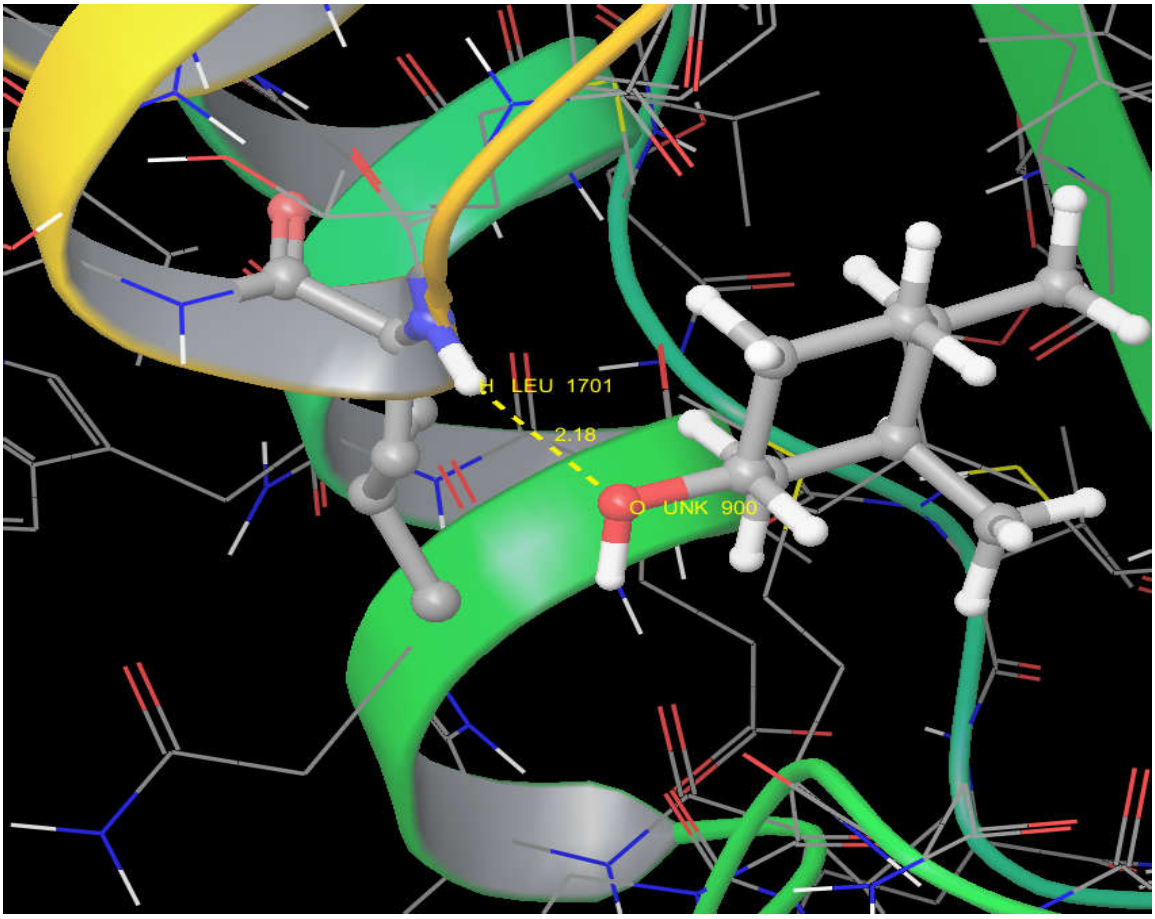


Fig. 7. Docking interaction of 3,4-dimethyl-1-cyclohexanol within the active site of Prepared Breast cancer protein BRCA1

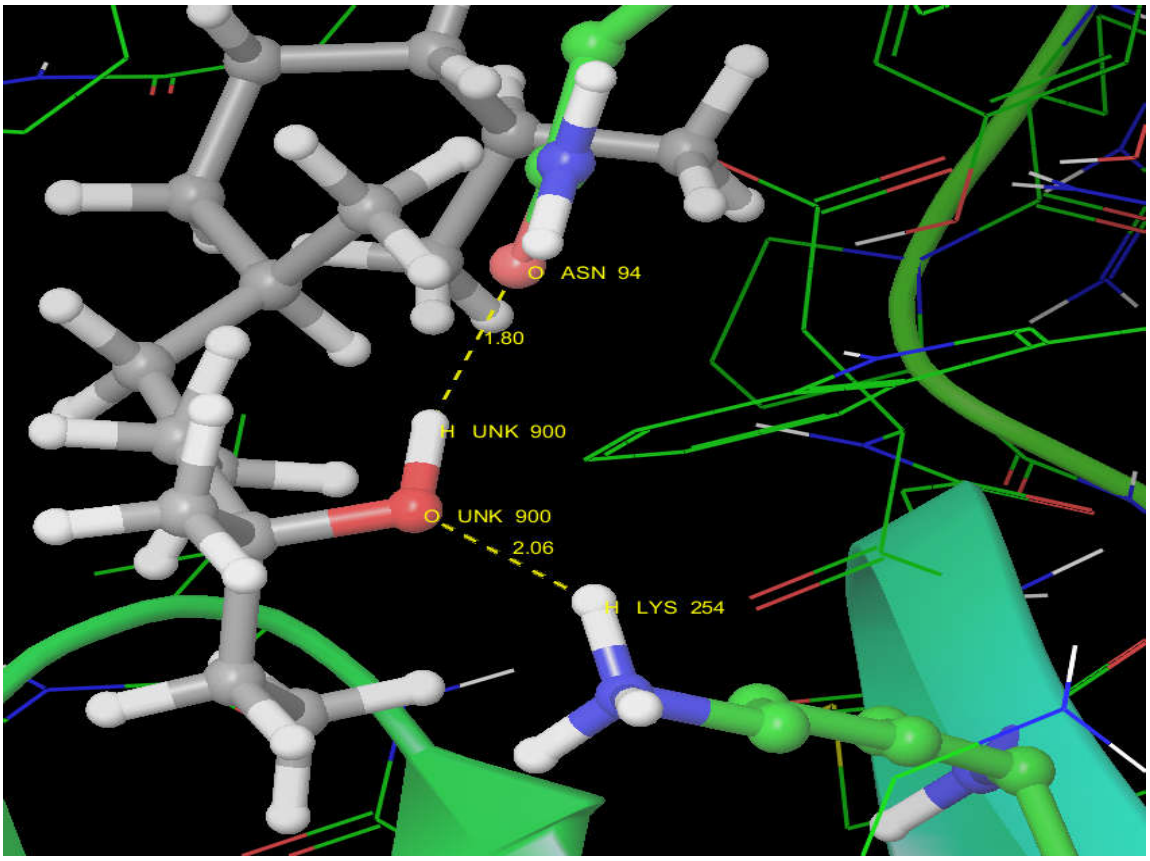


Fig. 8. Docking interaction of 3,7,11-trimethyl-3-dodecanol within the active site of Prepared Heat Shock protein 70

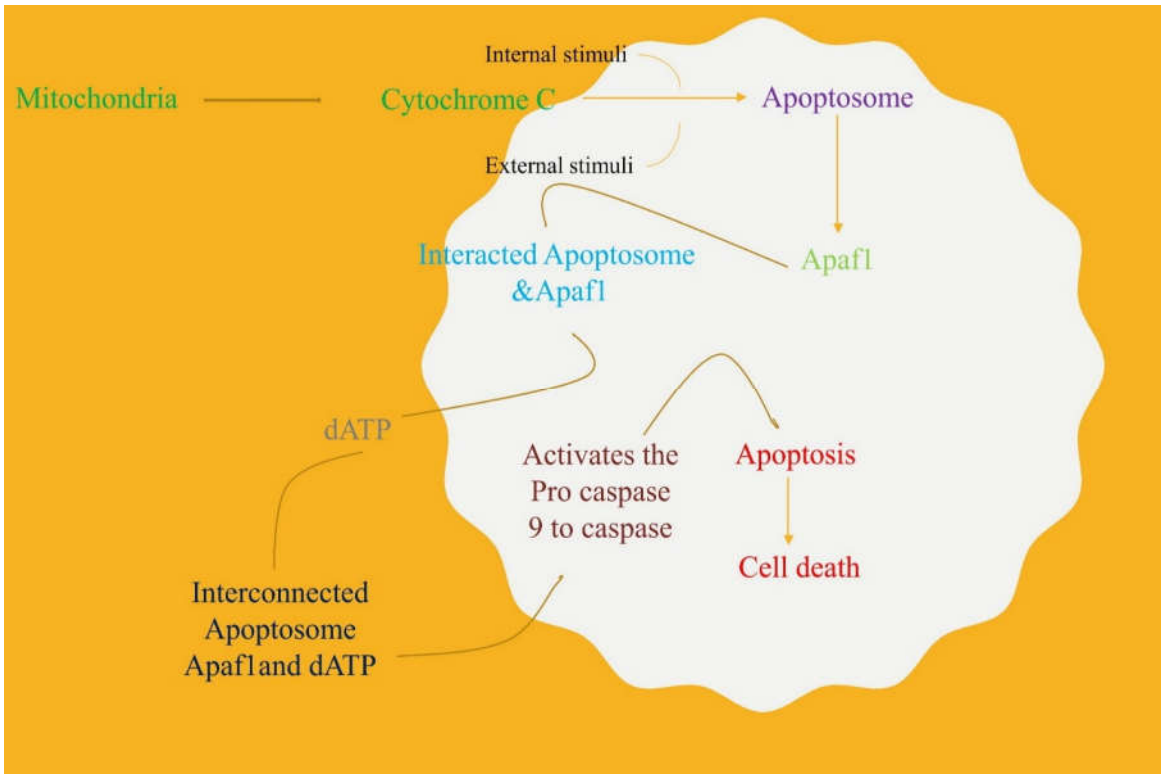


Fig. 9. Prevailing apoptosis mechanism in cancer cell

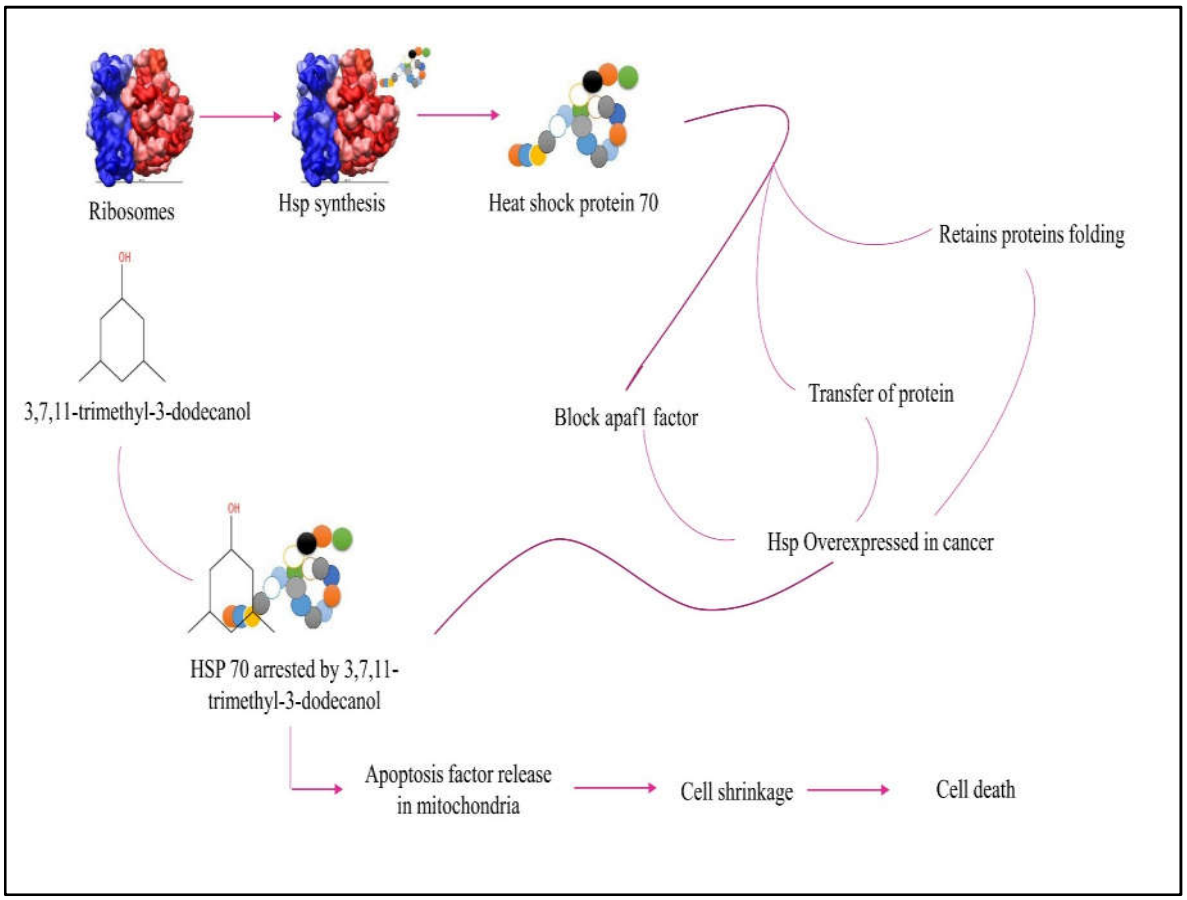


Fig. 10. Occurring3,7,11-trimethyl-3-dodecanol drug role in cervical cancer

Table 2. Evaluated anti-cancer effects for molecularly docked proteinsin *Chrysophyllumcanito*

PDB ID	Crystal Structure	Glide Score	Interacted compound
1D5R	PTEN	-4.12	1-docosanol
3FF7	NKCR	-5.13	4,4,7,7-tetramethyldeca-1,9-diene
3AL3	BRCT7 and BRCT8	-6.08	5-methyl-2-propan-2-yl-1-cyclohexanol
2R0U	Chek1	nil	nought
1JM7	BRCA	-3.08	3,4-dimethyl-1-cyclohexanol
2KZ3	Rad51D	nil	nought
4JLU	BRCA1	-5.65	3,4-dimethyl-1-cyclohexanol
3Q9P	HSP27	nil	nought
1DKG	HSP70	-6.27	3,7,11-trimethyl-3-dodecanol

Table 3. Summary of docking studies in diverse cancers

S.No	Docking Study	Target status	Clinical implications	Reference
1	Quercetin, Guggulsterone	Confirmed	Breast cancer	58
2	Abruquinones	Confirmed	Breast cancer	59
3	Protein Modeling	Confirmed	Ovarian cancer	60
4	Protein Modeling	Confirmed	Breast cancer	61
5	Heat shock protein 70	Confirmed	Cervical cancer	Present study

***In vitro* cytotoxicity in MTT assay at HeLa cell line**

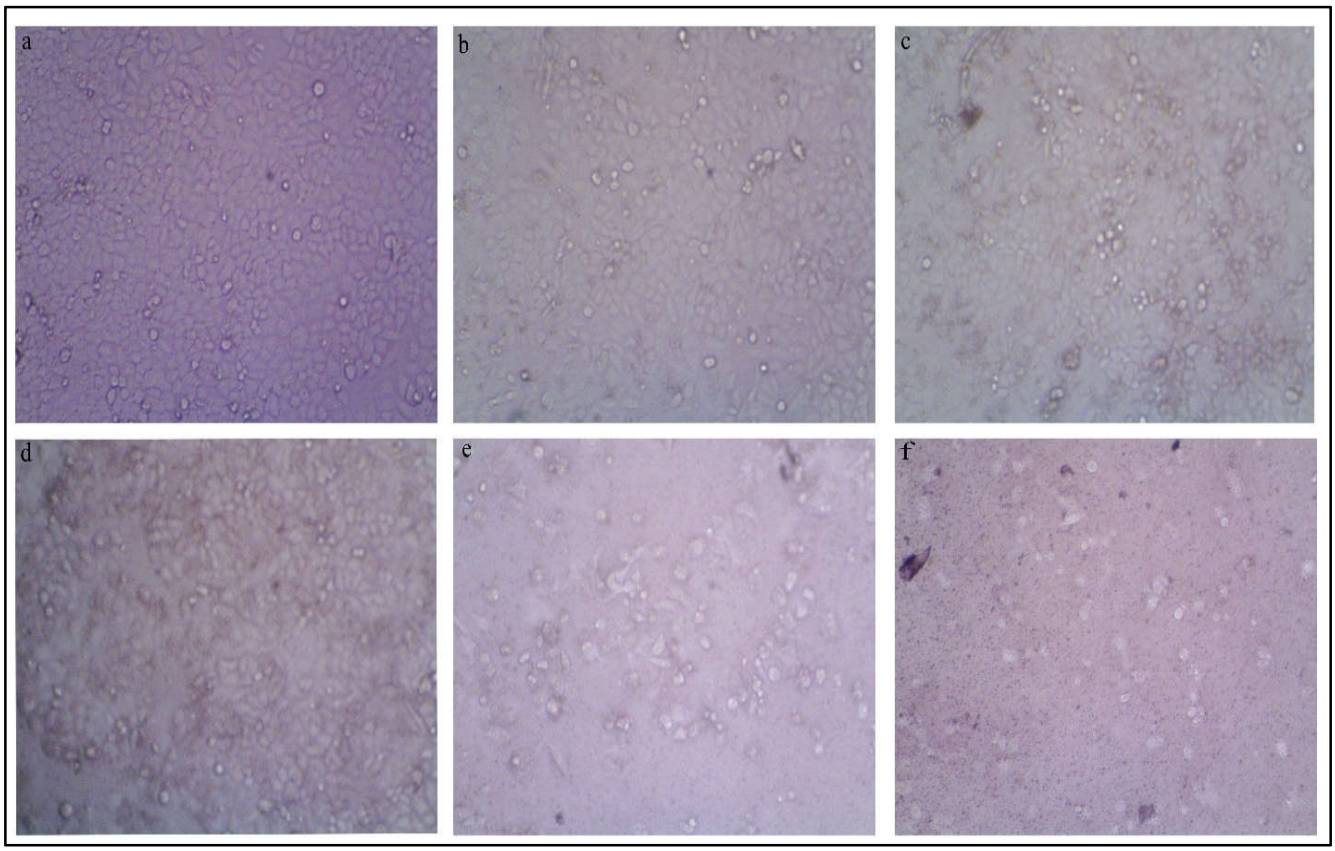


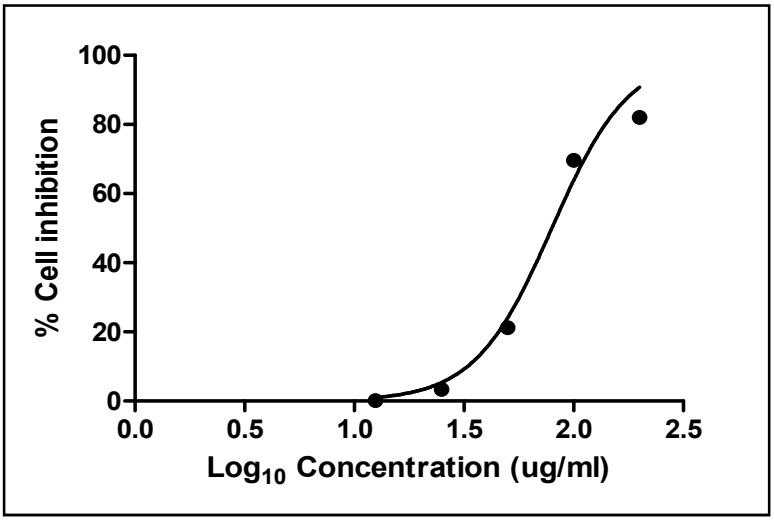
Fig. 11. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) MTT assay. Diverse concentrations of *Chrysophyllumcanito* leaf effects against cervical cancer in HeLa cell line a) control normal cells. b) 12.5 µg concentration of cancer cells. c) 25 µg concentration of cancer cells. d) 50 µg concentration of cancer cells. e) 100 µg concentration of cancer cells. f) 200 µg concentration of cancer cells

Table 4. Provides percentage of cell inhibition concentrations

Concentration (µg/ml)	% Cell inhibition
12.5	0.144509
25	3.395954
50	21.24277
100	69.65318

Table 5. Provides median value of cell inhibition concentrations

Concentrations	12.5 µg	25 µg	50 µg	100 µg	200 µg	control
Absorbance	0.465	0.439	0.355	0.133	0.069	0.451
	0.457	0.448	0.366	0.155	0.087	0.467
	0.46	0.45	0.369	0.132	0.093	0.466
Average	0.460667	0.445667	0.363333	0.14	0.083	0.461333



Graph 1. For the cytotoxicity assay

Table 6. The results of *Chrysophyllumcanito* holds good cytotoxicity effects

S.No	Cytotoxicity	Medicinal plant	IC_{50}	References
1	MTT assay	*Myxopyrumsmilacifolium*	98.75	62
2	MTT assay	*Cynodondactylon*	96.2	63
3	MTT assay	*Cucurbita maxima*	212.7	64
4	MTT assay	*Chrysophyllumcanito*	79.25	Present study

In this *in vitro* cytotoxicity study revealed the *Chrysophyllumcanito* leaf maximum anti-cancer property. *In silico* study accurately identified the liable compound role in ethanol extract of *Chrysophyllumcanito* leaf. Wet laboratory 3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide colorimetric assay is a metabolic study commences after the assessment of *Chrysophyllumcanito* leaf effects. Consequently, unfurl the viable cells via spectrophotometer between the wavelength of 500 to 600 nm. Human cervical cancer cell lines (HeLa) was obtained from National Centre for Cell Science (NCCS), Pune.Eventually, the calculated average values and various concentrations of cancer cell exact inhibition state. Y=% of inhibition, X concentration, C constant and M coefficient formula applied and was found to be IC_{50}79.56 µg/mL and $R^2$0. 9788. Obtained results undeniablysimilar, adjacently proved the heat shock protein 70 interaction role in docking. Hence, *in vitro* study unraveled the anti-cancer responsibility for *Chrysophyllumcanito*.

Conclusion

*In silico*study has provided the very beneficial information forresearchers about *Chrysophyllumcanito*. Molecular docking studies were explored indiverse cancer, supporting and anti-stress maintaining proteins. Present study fulsomely commenced with various proteins such as PTEN, NKCR, BRCT7, BRCT8, BRCA, BRCA1 and HSP70. Many of them, have disclosed the anti-cancer property, but the heat shock protein 70 has unveiled the extreme activity. Docking study was evaluated the protein interaction under the glide score, the BRCT7 and BRCT8 also emerged the excellent antagonizes factor, but heat shock protein 70 had interacted greatly than BRCT7 and BRCT8. Cervical cancerhighly relies and depends on HSP 70 protein to maintain and balance cellular stress. Present study has deduced the 3,7,11-trimethyl-3-dodecanol compound vital role in cervical cancer, it has admirably blocked the internal stress factor although it hinges on wet laboratory evidence. Albeit, MTT assay was investigated in HSP70 protein and it was revealed the 3,7,11-trimethyl-3-dodecanol compound anti-cancer property for *Chrysophyllumcanito*. Reconnoitered molecular docking study, MTT assay results synchronizestowards obviously, hence the natural behaviors of 3,7,11-trimethyl-3-dodecanol compound role in cervical cancer was deduced in *Chrysophyllumcanito*.

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