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MOLECULAR DYNAMICS SIMULATION-BASED PHYTOCHEMICAL SCREENING OF BLINDING TREE *(EXOECARIA AGALLOCHA)* LEAF EXTRACT AGAINST CERVICAL CANCER BY TARGETING SIHA CELL LINE

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Abstract:

The anticancer fruit of the blinding tree, Exoecaria agallocha, is a member of the Euphorbiaceae family and is commonly found in India. It is used to treat paralysis, rheumatism, leprosy, ulcers, and epilepsy. When treating cervical cancer, exoecaria agallocha can be utilised as a comprehensive treatment. The precise chemical mechanism underlying blindness trees' anticancer action is still unknown. The (SiHa HPV 16+) cell line's crystal structure was obtained from the Protein Data Bank. When the active ingredients of exoecaria agallocha (bergenin, rutin, and afzelin) were molecularly docked with cervical cancer protein, the proliferation of cervical cancer cells was significantly reduced and altered. Exoecaria agallocha has the ability to prevent cervical cancer, as demonstrated by this experiment. The study's conclusions provide important new information.

Keywords*: Exoecaria agallocha, SiHa cell line, Bergenin, Rutin, Afzelin.*

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INTRODUCTION:

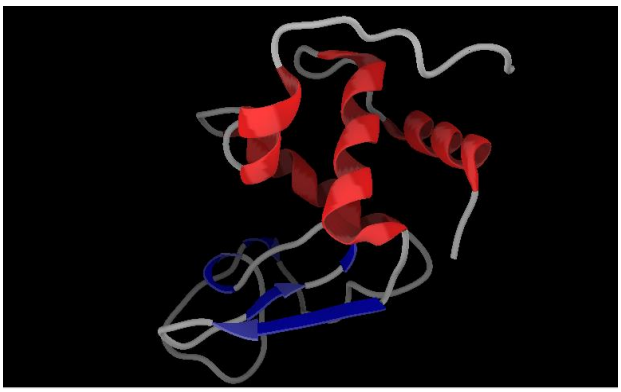
Approximately 85% of people on the planet use herbal or traditional medicine to treat health-related issues. Consequently, there has been significant advancement in the field of research in recent years that focuses on the scientific evaluation of herbal medicines derived from plants. Unchecked cell proliferation, or cancer, is one of the leading causes of death worldwide. About 7,900,000 persons died from it globally in 2007 (about 13% of all deaths) [1]. Cervical cancer is currently the most prevalent type of malignant cancer reported by Indian women, and its occurrence is rising at an alarming rate in many other nations. It is crucial to develop a unique strategy to cervical cancer therapy that spares healthy normal cells while overcoming the drawbacks of current treatments. Mangroves are a major source of medicinally valuable plants.. Therefore, the purpose of this study was to evaluate the anticancer potential of mangrove Excoecaria agallocha L. leaf extracts on the SiHa HPV 16+ human cervical cancer cell line. We also sought to characterise the bioactive compounds that contributed to the anticancer potential and investigate the likely mechanism of action of the purified plant extract [2] [3]. *One such mangrove plant, Excoecaria agallocha,* has long been utilised by Sundarbans residents in folklore medicine; however, not much research has been done to separate and purify the bioactive component that gives it its biological activity. There are 37 species of trees in the genus Excoecaria, which is a member of the Euphorbiaceae family. These trees can be found in Australia, Asia, and Africa's tropical regions. But in the mangroves, only *E. agallocha* and *E. indica* are found growing. *E. agallocha* is a common species in the Sundarbans of India. Its anti-diabetic, antimicrobial, anti-larvicidal, anti-nociceptive, and anti-cancerous qualities have been demonstrated by studies . A few intriguing results have been found in recent studies on the anticancer activity of E. agallocha extracts from various plant parts. According to recent research, E. agallocha's methanolic leaf extract has been shown to have cytotoxic effects on a variety of cell lines, including Miapaca-2, BxPC-3, PANC-1, and Capan-1, with IC50 values > 0.11 µg/mL. Based on chromatographic results, researchers have concluded that phytochemicals like phenolic derivatives, glycosides, and saponin that are present in the extracts of E. agallocha may be responsible for the cytotoxic effect displayed by the stem extracts. Molecular docking is one of the main computer-assisted drug design techniques for assessing the affinity of recently discovered drugs with the targeted enzymes, which is ligand – protein, in order to evaluate the effect of excoecaria agallocha against cervical cancer) [4]

In-silico docking studies:

Collection of materials

Protein

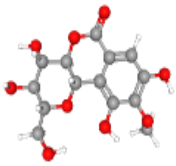
(SiHa HPV 16+) cell line collected from protein data bank.



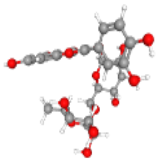
HPV Protein structure

Ligand

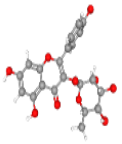
Bergenin, Rutin, afzelin chemical was obtained from pubchem.



BERGENIN



RUTIN



AFZELIN

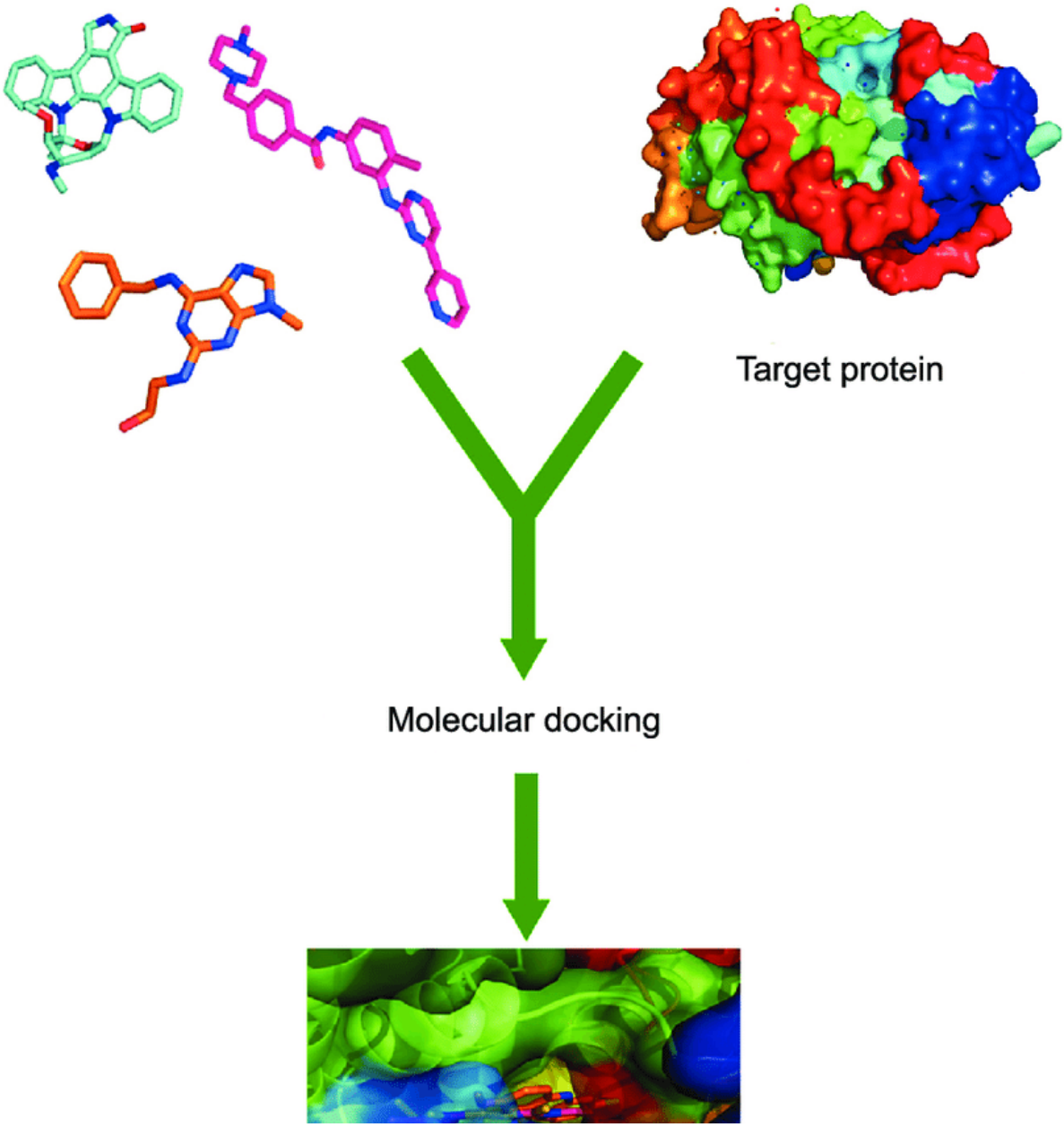


Fig 1: Representation of protein - ligand docking

The compound selected for the docking studies was determined by a review of the literature and the activity of the compounds. To find the binding affinity for the docking studies, ligands with this chemical structure were chosen and used to bind with targets. The online docking website seamdock was used to study the compounds that were identified. Three molecules—Bergenin, Rutin, and Afzelin—make up the protein sequence of the (SiHa HPV 16+) cell line.

Disease profile:

An infection with the human papillomavirus causes cervical cancer. A persistent infection with high-risk human papillomavirus, particularly type 16, can result in cancer of the cervix, vulva, vagina, anus, penis, and oropharynx. The majority of human papillomavirus infections are benign and resolve on their own. The virus only infects epithelium, and it only generates new viral particles in epithelial cells that have reached full maturity. The human papillomavirus interferes with the regular regulation of the cell cycle, which leads to unchecked cell

division and the build-up of genetic damage. As a primary preventive measure, two effective prophylactic vaccines made of human papillomavirus types 16 and 18, as well as virus-like particles of these types, have been introduced in many developed countries. Testing for the human papillomavirus is clinically useful as a test of cure following treatment and for secondary prevention in the triage of low-grade cytology. Primary screening by human papillomavirus testing is more sensitive than cytology, and it may allow screening intervals to be increased. Thousands of lives could be saved in developing nations if these preventive measures could be put into practice.

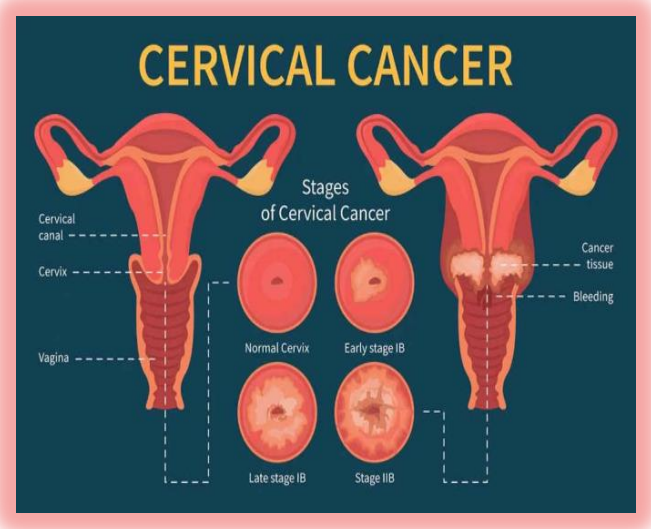


Figure 2: Represtation of stages involved in cervical cancer

HUMAN PAPILLOMA VIRUS

- An infection with the human papillomavirus causes cervical cancer. Cervical cancer can result from a persistent infection with high-risk human papillomavirus, particularly type 16, although the majority of papillomavirus infections are benign and resolve on their own.
- The human papillomavirus interferes with the regular regulation of the cell cycle, leading to unchecked cell division and the build-up of genetic damage.

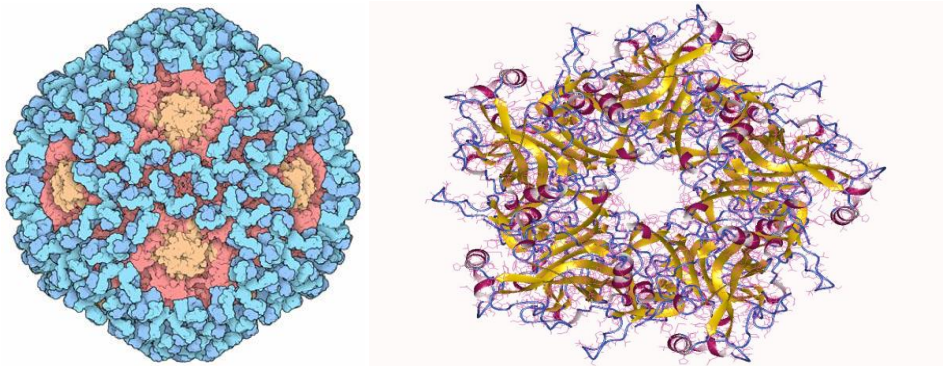


Figure 3 : Represtation of Human Palilloma Virus (HPV).

Malignant transformation

The cell must go through terminal differentiation in order for the virus to finish its infectious lifecycle, which is a necessary step before virion assembly and release can occur. However, E6 and E7 are so effective at blocking cell cycle negative regulators for some high-risk papillomavirus infections that the infected cells never mature. The cells stop apoptosing and continue to actively participate in the progression of the cell cycle. Because of the ensuing genomic instability, genetic changes can accumulate and eventually cause a human papillomavirus-infected

cell to transform malignantly into an invasive cancer cell. Through well-established interactions with the products of tumour suppressor genes—retinoblastoma proteins for E7 and TP53 for E6—E6 and E7 initiate oncogenesis. Through the induction of cell-cycle arrest or forced apoptosis until errors in DNA replication can be corrected, TP53 plays a critical role in maintaining genomic integrity. By targeting TP53 for ubiquitin pathway-mediated degradation, E6 inhibits apoptosis and permits the proliferation of potentially transformed cells.30 E7 interacts with the so-called pocket proteins, RB1, RBL1, and RBL2, members of the retinoblastoma family, to contribute to oncogenesis. These proteins are bound by E7, which then directs their degradation.31, 32 This process leads to the release and activation of E2F transcription factors, which in turn accelerates cell-cycle entry and stimulates DNA synthesis by driving the expression of S-phase genes, including those encoding cyclins A and E. High-risk E5 promotes cellular proliferation in conjunction with E6 and E7, and it may be a minor contributor to the emergence of cancer.33 The human papillomavirus genome frequently co-occurs, often in the same cell, as both integrated and episomal copies. E6 and E7 expression may not significantly increase in this situation.

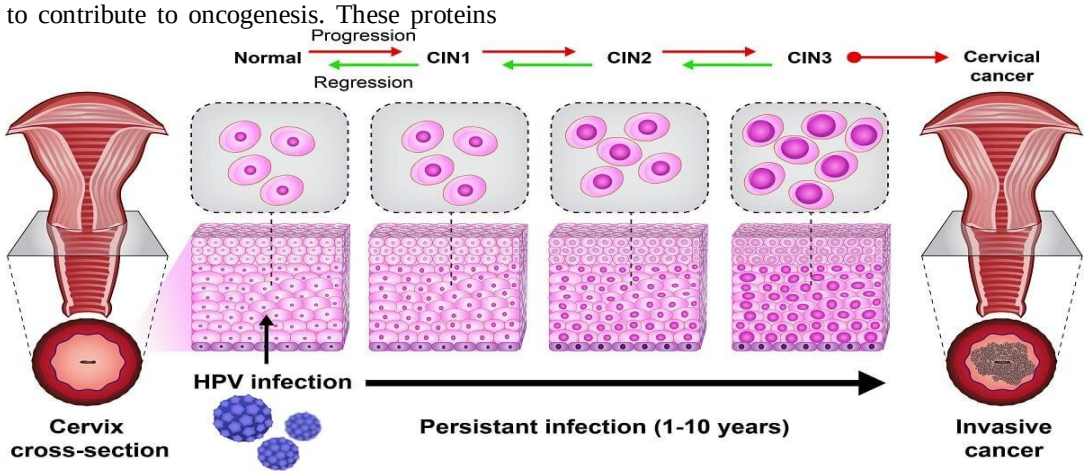


Figure 4 : Representation of malignant transfer of cancer cells.

Plant profile:

Botanical name	*EXOECARIA AGALLOCHA*
Synonym	*Exoecaria*
Family	Euphorbiaceae
English name	Blind tree
Phylum	Magnoliophyte
Class	Magnoliopsida
Order	Caryophyllales
Species	*Exoecaria Agallocha*

Figure 5 : *EXOECARIA AGALLOCHA*

Table 1 : Chemical constituents

Source	Active chemical constituents	Nature of extract
Leaves	Benzoate, Flavonoid, glycosides, Rutin, afzelin, Quercitrin, kaempferol-3-O-(2-O-acetyl-α-L-rhamnopyranoside, kaempferol 3-O-α-L-rhamnopyranoside ,berginin.	Methanolic extract, methanolic extracts, water, fraction.

MATERIALS AND METHODS:

Materials:

We used biological databases such as PDB (Protein Data Bank), PubChem, seamdock, and software such as autodock vina for our current study.

PDB (Protein Data Bank):

The only global database for structural data on biological macromolecules is the Protein Data Bank (PDB), which was established in 1971 at Brookhaven National Laboratories (BNL).

PubChem:

Details about chemicals and their biological roles can be found in the public database PubChem (https://pubchem.ncbi.nlm.nih.gov).

Pub Chem was first launched in 2004 as a component of the US National Institutes of Health (NIH) Molecular Libraries Roadmap Initiatives. Since then, it has grown to become a major repository of chemical data supporting scientific communities in cheminformatics, chemical biology, medicinal chemistry, and drug discovery, among other areas.

PubChem has grown over the previous eleven years into a sizable infrastructure that gives access to chemical information to the scientific research community. PubChem is made up of three interconnected databases: substance, compound, and bioassay.

Chemical data has been added to the Substance database by individual PubChem data contributors, and certain chemical structures have been extracted for the Compound database from the Substance database. Information about the biological activity of chemical compounds that have been investigated in assay tests can be found in the Bio Assay database.
This document provides an overview of the PubChem Substance and Compound databases, including information on their data sources, contents, organization, and standardization of chemical structures. It also includes web-based interfaces for text and non-text searches, as well as programmatic access.
It also gives a brief overview of PubChem RDF, a formatted version of PubChem data for use in data sharing, analysis, and integration with data from other databases, and PubChem3D, a resource built from theoretically accurate three-dimensional models of molecules in PubChem.

Autodocking:

Libraries of compounds can be screened against possible drug targets using AUTODOCK VINA, a virtual screening tool for computational drug discovery. Medicinal chemists can conduct virtual screening using Autodock Vina, which assists users with every stage of the process, from preparing data to viewing grid boxes. Although there aren't any magic buttons that can be used to find new drugs, the Autodock wizard's chemical spreads heat-like functionality and user-friendly interface make it a useful tool for rational drug design.
The application will install the Autodock Vina app.

SEAMDOCK:

Drug discovery pipelines now routinely include in silico evaluation of protein receptor interactions with small ligands, and a plethora of tools and protocols have been created to that end. The online SeamDock service unifies various docking tools into a unified framework, enabling both local and/or global docking of ligands as well as a hierarchical method that combines the two for simple interaction site identification. This service only requires a standard web browser to operate, and it doesn't require the installation of any additional software. The user can navigate the Seam Dock website easily and interactively by using the seamless library, which connects the RPBS calculation server to the user's webpage. A significant amount of work has gone into visualizing ligand, receptor, and docking poses in three dimensions as well as how they interact with the receptor. A user can share a docking session and all of its visualization states with an infinite number of collaborators thanks to the advanced visualization

features and the seamless library. SeamDock is therefore a free, straightforward, instructional, dynamic online docking tool that is most appropriate for teaching and training.

METHODOLOGY:

Docking procedure:

Step1: Preparation of receptor protein:

Protein Data Bank (PDB) provided the 3D crystallographic structure of the protein Human Epidermal Growth Factor Receptor (PDB ID:1M17). The protein's three-dimensional structure was found. Open the file and read the molecule.

- Molegro Molecular Viewer → Import file → Export molecule.
- Protein is only applied → Export → Save as pdb file.
- Save as prepared protein (PDB form).

Step2: Preparation of ligand:

Quercetin, Rutin, Vitexin, Isoorientin is taken as ligand molecule. They are downloaded from pubchem and then converted into PDB format.

- Molegro Molecular Viewer → Import → File → Export molecule.
- Save as (PDB form)

Step3: Docking:

AUTODOCK:

- File → Read molecule → Select the protein structure.
- Click on edit → Hydrogens → Add polar only.
- click on edit→ Charges → Add compute charges.
- Click on ligand → Choose ligand → Select the ligand structure.
- Click on edit →Charges → Add kollamen charges.
- Click on grid → Select the grid box → Select the dimensions of your grid box.
- Note your grid dimensions.

Step 4: Docking:

- By using https;//bioserv.rpbs.univ-paris-diderot.fr/services/seamdock
- Click on run seamdock →Choose ligand (in the .PDB, .SDF form) → click on open.
- Click on choose protein →Choose protein (in the form) → click on open.
- Set the dimensions →Adujusting x,y,z → Launch Docking.

Finally 2D interactions are predicted from MOLEGRO molecular viewer.

RESULT AND DISCUSSION:

Docking result:

- The 3D structure of siHa HPV 16+ (PDB ID:6wx5) ,with a resolution of 1.30Å respectively is obtained from the **protein data bank (RCSB-PDB).**
- The structure of the berginin (CID_5316673),rutin (CID_5280805), afzelin (CID_5316673) was obtained from **Pubchem** .
- The energy minimization of the ligands was performed using **Autodock vina**.
- **Seamdock** is used to estimate the affinities and interactions of protein and ligand.

Table 2 : Binding affinities

Chemical Contituents	Protein target	Binding Affinity
Berginin	SIHa cell line (PDB ID:6wx5)	-6 kcal/mol
Rutin		-5.5 kcal/mol
Afzelin		-7.8 kcal/mol

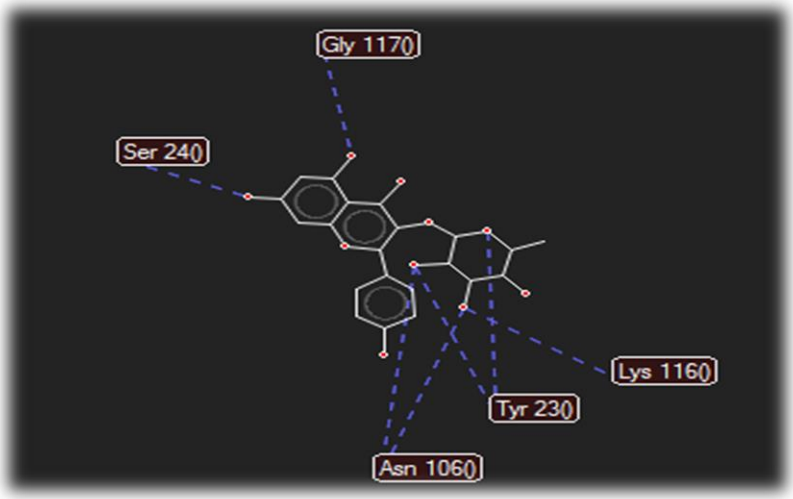


Figure 6 : 2D interaction of berginin with SiHa cell

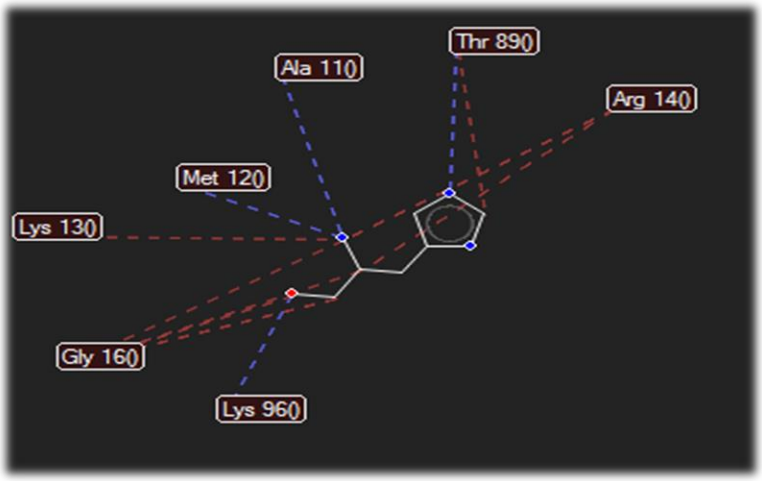


Figure 7 : 2D interaction of afzelin with SiHa cell

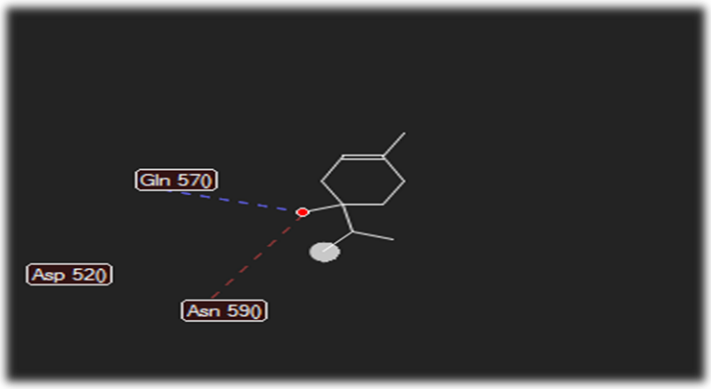


Figure 8: 2D interaction of rutin with SiHa cell

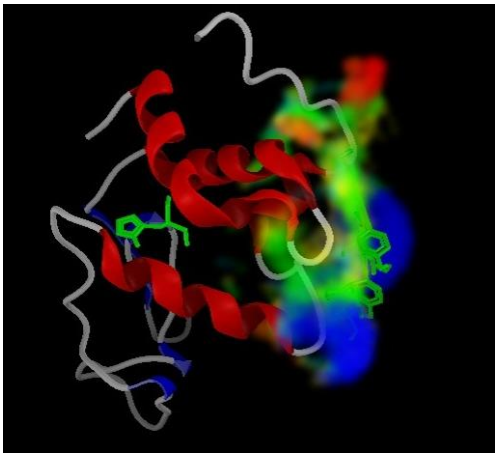


Figure 9: 3D representation with energy mapping of interaction of berginin with SiHa cell

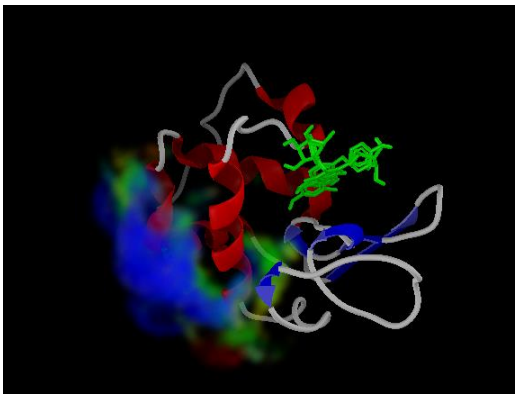


Figure 10: 3D representation with energy mapping of interaction of afzelin with SiHa cell

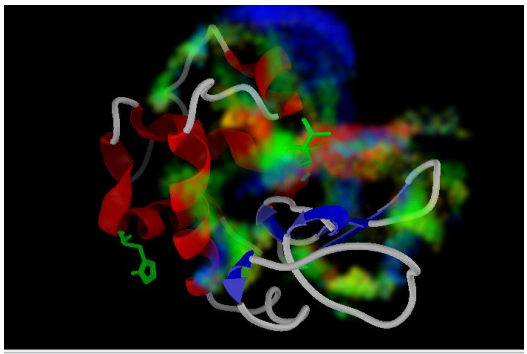


Figure 11: 3D representation with energy mapping of interaction of rutin with SiHa cell

DISCUSSION:

- According to Ahammad et al. Briefings in Bioinformatics, 22(5) Pharmacoinformatics and molecular dynamics simulation-based phytochemical screening of herbal plants *against human cancer* by targeting protein was taken……
- According to Ahmed John Syed Batsa and Kumar Periyasamy anti-cancer activity of *E.Agallocha* leaf extract against cell line was taken for present docking studies.
- According to Sultana *et al.* siHa cell lines of HPV are the causatives for cervical cancer was taken for the present docking studies.
- The protein siHa cell line was docked with rutin, berginin, and afzelin using Seamdock in the current investigation.
- The anti-cancer protein ligands' binding scores were -5.5 kcal/mol, -7.8 kcal/mol, and -6 kcal/mol.
- As a result, the protein and ligand have a stronger binding affinity when the docking score is least negative. The binding energy of afzelin with the siHa cell line was found to be -7.8 kcal/mol.

CONCLUSION:

- The outcomes of the insilico research provided a useful deduction regarding the compounds' usage, demonstrating increased binding energy and affinity towards enzymes and being utilised as cervical cancer treatment aids..
- In the current investigation, the target protein siHa cell line in the Human Papilloma Virus was successfully docked with active constituents (Afzelin, Berginin, and Rutin). Afzelin's binding energy was recored at -7.8 kcal/mol.
- Because the protein and ligand have a stronger binding affinity when there is less negative binding score, this could potentially be a medication with anti-cancer activity.

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