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IN SILICO MOLECULAR DOCKING STUDIES OF CYCLOARTOMUNIN COMPOUND AGAINST CANCER PROTEINS

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ABSTRACT

Cycloartomunin is a Pyranoflavonoids compound present in root and bark of *Artocarpus* family plants such *Artocarpus hirsutus,Artocarpus altilis*, *Artocarpus communis* and *Artocarpus heterophyllus*. Flavonoids are having antioxidant and anti-inflammatory effects that help control inflammation, kill cancer cells, and prevent heart disease. In this study, cycloartomunin was docked against pass analysis predicted activity proteins (HIFIA, NOS2, P65, TP53, AR, MMP9) and cancer protein such (CEA, C-Reactive protein, TNFalpha, BCL2, IL9, SOX2, ERBB2, APC, JUN, PTEN. PIK3CA, KRAS, EGFR, BRCA1, MITF, MYC). From the Insilico research completed, we can infer that Cycloartomunin show great efficacy in finding against cancer and can be used in cancer care. In Pharmacokinetic properties high gastrointestinal absorption and CYP2C9 inhibitor activity and Toxicity Prediction it is safety for consumption.

KEYWORDS: Insilico study, Molecular docking, *Artocarpus*, *Artocarpus hirsutus*, Pass analysis, ProTox-II, Cancer protein, Pharmacology.

Cancer is one of the most common diseases in the world and which is a major cause of death. According to WHO estimates, persons die from cancer in every year from lung, stomach, liver, colon, and breast cancer are increasing up globally.[1] The development of cancer is largely influenced by environmental and genetic factors. Factors such as radiation, sunlight exposure, tobacco use, smoking, x-rays, gamma rays, asbestos, diseases, fried or barbecued meat, obesity, lack of exercise, and caffeine are the main causes of cancer in humans.[2] According to the International Cancer Research Agency, red meat, including beef, lamb, and

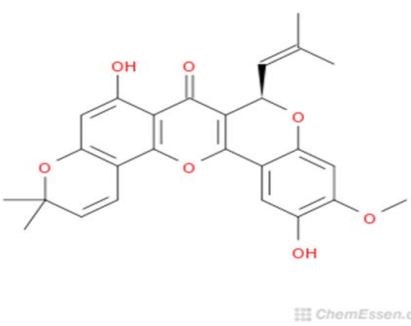
hog, carries the highest risk of developing cancer.[3] The flavonoid family comprises more than ten thousand structurally different molecules.[4]

Flavonoids are a significant group of natural products. Specifically, they are a type of secondary metabolites from plants that have a polyphenolic structure and are commonly present in fruits, vegetables, and some drinks. Their diverse beneficial biochemical and antioxidant properties are linked to a number of illnesses, including cancer, Alzheimer's disease (AD), atherosclerosis, etc.[5] Flavonoids are associated with a broad spectrum of health-promoting effects and are an indispensable component in a variety of nutraceutical, pharmaceutical, medicinal and cosmetic applications. Numerous food plants and herbs, such as citrus fruits, oregano, green tea, parsley, cacao, grapes, eggplants, and many more, are rich sources of flavonoids.[6] Numerous anti-cancer activities have been reported for flavonoids. Flavonoids were discovered to have both preventative and therapeutic properties for cancer. Extracted from plants, flavonoids are substances that have potent anti-inflammatory and anti-cancer effects. They are primarily secondary metabolites. The most common uses for flavonoids and their analogs are in the treatment of pancreatic, prostate, ovarian, breast, and cervical cancers.[7]

Experimental findings demonstrated how flavones affect signal transduction pathways in the development of cancer. It has been discovered that flavones regulate the angiogenesis, oxidative stress, cell cycle progression, and metastasis in addition to certain molecular pathways that eventually stop the diseases from progressing.[8]

Cycloartomunin are Flavonoids which are under the subclass Pyranoflavonoids. A type of flavonoids with a pyran group is called pyranoflavonoids. Which has been founded in root of *Artocarpus hirsutus,*[9]*Artocarpus altilis* ,*Artocarpus communis* and *Artocarpus heterophyllus*.[10] There is no previous study about the cycloartomunin compound in cancer.

Structure of cycloartomunin



MATERIALS AND METHODS

Tools and database used

Pubchem, RCSPDB, molegro molecular viewer, Autodock 4.2.6, Swiss ADME, ProTox-II and PASS online tool.

In Silico PASS Prediction Study

The Prediction of Activity Spectra for Substances (PASS) online tool was utilized to assess the potential bioactivities of the cycloartomunin compound. This program can predict up to 3750 bioactivities of a molecule based on a chemical structural analysis.

The examination's findings were displayed as Pa (probable activity) and Pi (probable inactivity), with Pa and Pi's values ranging from 0.000 to 1.000.

We took into account Pa > Pi and Pa > 0.700 values to identify a molecule's bioactivity.[11]

Preparation of ligand and protein

2D structure of "cycloartomunin" was downloaded from the PubChem website https://pubchem.ncbi.nlm.nih.gov/ and the file was converted into PDB format by using molegro molecular viewer 3D structure of the protein was downloaded from Pdb (Protein Data Bank) website https://www.rcsb.org/ and repeat the same for all the proteins.

Docking of cycloartomunin against the selected proteins

Molegro molecular viewer was used to remove the ligand and water molecule from the protein and help us get the exact protein structure alone. Autodock software was used to read the molecule, adding the polar hydrogens and Kollman charges. Eventually, a grid box was made and properly adjusted to form an ideal grid box. Torsion was chosen after the protein and ligand were docked, and the output file in PDBQT format was chosen via MGL tools. Pymol software was used to view the output, and validity was assessed.[12-13]

Prediction of drug bioactivity score and ADMET analysis

Canonical smiles for cycloartomunin were copied from PubChemhttps://pubchem.ncbi.nlm.nih.gov/ and pasted in the Swiss ADME and run.

The online web tool Swiss ADME was used to evaluate the ADME parameters using Lipinski's rule of five.[14] Lipinski stated that a compound could display drug-like behavior if it does not fail more than one of the criteria such as; (i) MW not more than 500; (ii)

Hydrogen bond donors ≤ 5; (iii) Hydrogen bond acceptors ≤ 10; (iv) Lipophilicity < 5; and (v) molar refractivity between 40 and 130. Those compounds are considered ideal drug candidates which obey the Lipinski rule.[15]

In Silico Toxicity Prediction Study

Toxicity and Lethal Dose (LD50) predictions for identified compounds were carried out by using ProTox-II. Accessed on (1 Jan 2024).

RESULTS

PASS Prediction Study

Prediction of biological activity of cycloartomunin

The PASS investigation identified potential targets and biological functions of the cycloartomunin. We looked at the biological functions based on Pa > Pi and Pa > 7.

In cycloartomunin most of the biological activity is anticancer activity such as Antineoplastic, Chemopreventive, Apoptosis agonist, Free radical scavenger.

And which also contain cancer-related expression inhibitors and enhancers activity such as HIF1A expression inhibitor, NOS2 expression inhibitor, TP53 expression enhancer, AR expression inhibitor, kinase inhibitors, MMP-9 inhibitors, RELA expression inhibitor (P65).

pa	pi	Biological Activity
0,930	0,004	HIF1A expression inhibitor
0,903	0,001	NOS2 expression inhibitor
0,856	0,004	Antiosteoporotic
0,844	0,007	Antineoplastic
0,827	0,007	Membrane permeability inhibitor
0,811	0,004	Bone diseases treatment
0,803	0,010	TP53 expression enhancer
0,790	0,003	AR expression inhibitor (NR3C4)
0,785	0,004	Chemopreventive
0,776	0,003	Estrogen agonist
0,773	0,001	Estrogen beta receptor agonist
0,777	0,009	Apoptosis agonist
0,770	0,003	Free radical scavenger
0,749	0,009	Kinase inhibitor
0,762	0,027	Chlordecone reductase inhibitor
0,718	0,002	RELA expression inhibitor (P65) or (NFKB3)
0,720	0,005	Antineoplastic (breast cancer)
0,714	0,002	Skin whitener
0,711	0,006	MMP9 expression inhibitor

0,700	0,008	Histidine kinase inhibitor
0,724	0,050	Membrane integrity agonist

Pa = Possibility of activity; Pi = Possibility of inactivity.

Molecular Docking Studies

The molecular docking is carried out by activity predicted by pass analysis protein and cancer protein.

Table No. 1: Result for the molecular docking activity of predicted by pass analysis protein.

PROTEIN	ID	BINDING ENERGY	HYDROGEN BOND INTERACTION	STERIC BOND INTERACTION
HIF1A	8HE0	-8.38	LYS459, VAL414	MET463, HIS418
NOS2	4JS9	-8.95	GLU38, GLY273	GLU387, GLU38, GLY273, ILE271, TYR383, GLN31 ARY382
P65	2RAM	-5.55	ASP259, PRO69	LEU269, TYR257, ARG267
TP53	8SWJ	-7.89	ARG1490	GLY1601, G599, PRO1537, ASP153, LEU1534
AR	IT7T	-7.53	THR250	TYR247, LYS244, ILE246
MMP-9	4H1Q	-9.45	THR139	GLN118, HIS121

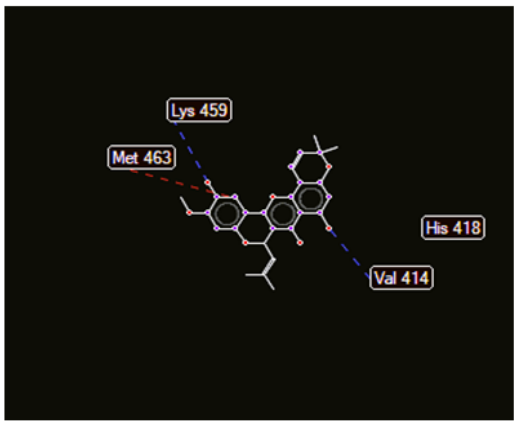


Fig. no. 1: HIFIA.

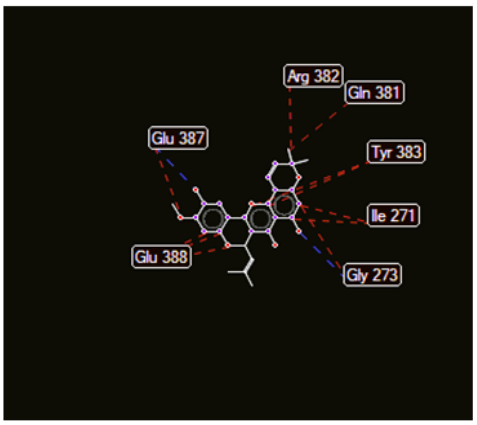


Fig. no. 2: NOS2.

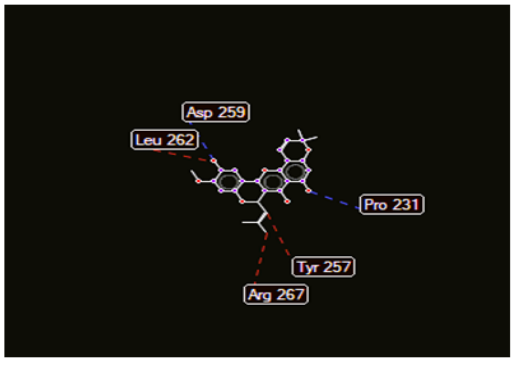


Fig. no. 3: P65.

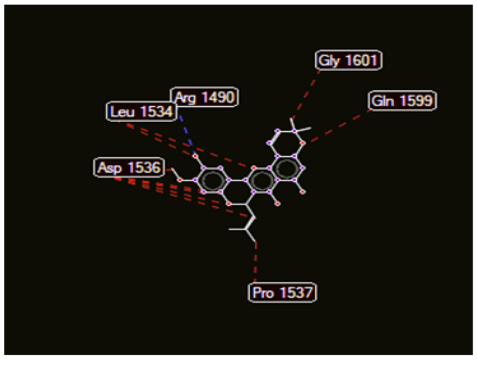


Fig. no. 4: TP53.

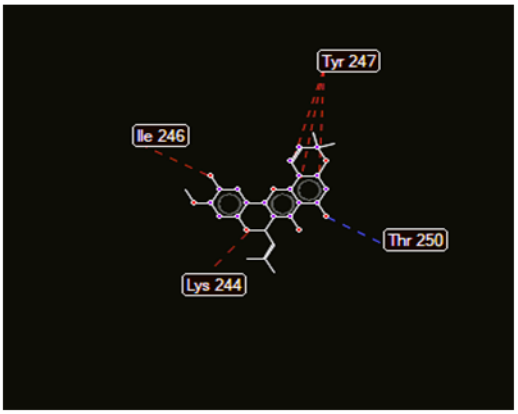


Fig. no. 5: AR.

Fig. no. 6: MMP-9.

Fig. no. 1-6: Image for the molecular docking activity of predicted by pass analysis protein.

The compounds were docked with the various protein which are predicted by the pass analysis and the binding efficiency of the compounds were studied. The compound had binding scores that were between -5.55 to -9.45 kcal/mol A negative score in the scoring represents the minimum binding energy required to form a complex between a protein complex and a ligand. The results are summarized. The results revealed that the cycloartomunin compound have higher binding energy towords the particular proteins.

The docking studies of the ligands to protein active sites were performed by an autodock software 1.5.7 for determining the binding affinities of the compounds. The MMP-9 protein has a higher binding efficiency (-9.45) and the P65 protein have lower binding efficacy (-5.55). The cycloartomunin are have (-8.35) binding energy towards the HIF1A protein.

Table no. 2: The result for molecular docking is carried out on the cancer proteins.

PROTEIN	ID	B.E	HYDROGEN BOND INTERACTION	STERIC BOND INTERACTION
CEA	2QSQ	-7.25	ARG43	TYR86, ASP82
C-REACTIVE PROTEIN	3PVN	-8.17	ASP155, GLU42	VAL153
TNF-alpha	2AZ5	-7.48	SER60	LEU120, TYR119, HIS15
BCL-2	6GL8	-8.01	GLN	ASP111, MET115, ALA149
IL-6	5FUC	-6.9	PRO139	ASN144
SOX-2	6T7B	-9.06	ALA104, ALA47	SER88, LEU51
ERBB2	2JWA	-6.28	LYS81, ARG77	GLN79, GLN80, ARG78
APC	1DEB	-5.65	----------	LEU16, LYS17
JUN	1JMS	-6.35	GLN304, ARG302	VAL305
PTEN	1D5R	-8.1	TYR176	TYR176, TYR180, ASP324, PHE279
PIK3CA	7R9V	-9.06	GLN1014,	ILE456, GLY1007, HIS450

			CYS604	
KRAS	8FMI	-9.44	GLY15, LYS16 ASP30	ASP30, GLY15, VAL14, SER17, TYR32, PHE28 LYS147, LYS117
EGFR	7U98	-9.73	ASP855, LYS745	ASP855, MET730 ASP837, GLY721
BRCA1	4IGK	-8.31	LYS1847LYS1759	CYS1847,LEU1764ARG1762
MITF	4ATH	-6.49	ASP13	----------
MYC	6G6J	-6.92	----------	GLU932, PHE922

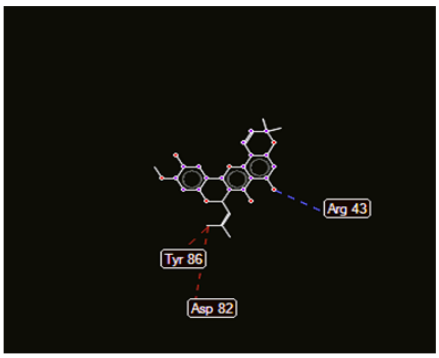


Fig. no. 7: CEA.

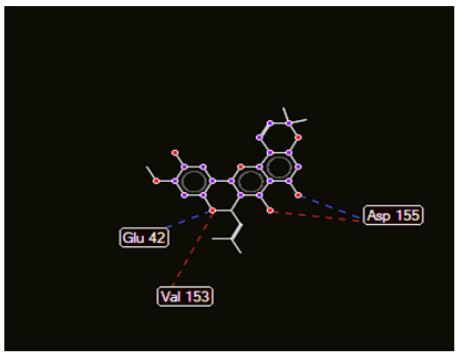


Fig. no. 8: C-reactive protein.

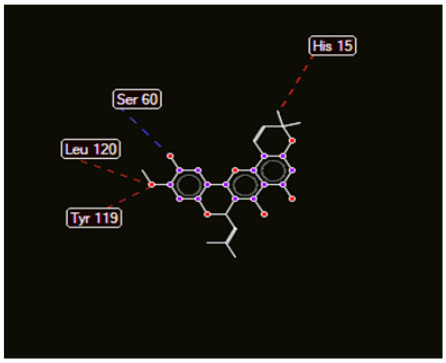


Fig. no. 9: TNF- alpha.

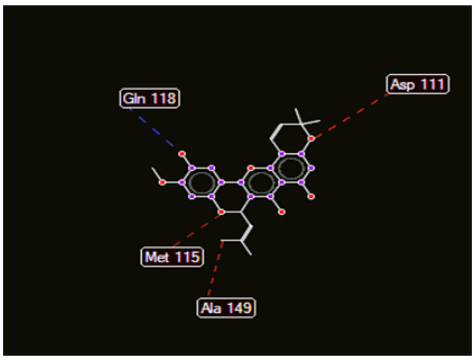


Fig. no. 10: BCL-2.

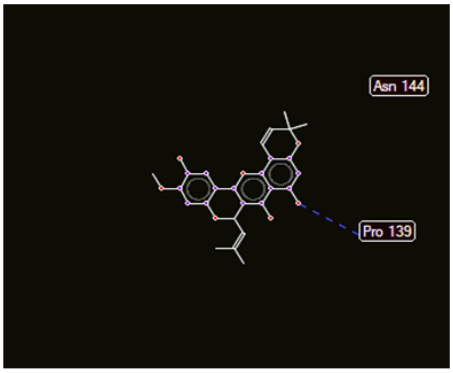


Fig. no. 11: IL-6.

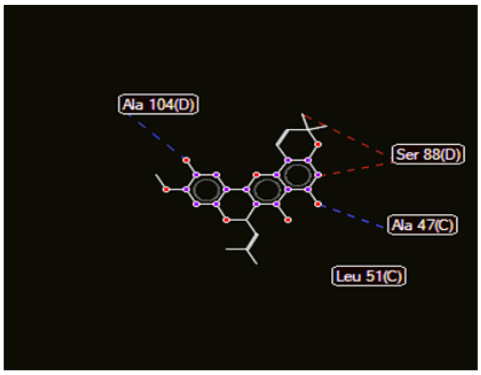


Fig. no. 12: SOX-2.

Fig. no. 13: ERBB2.

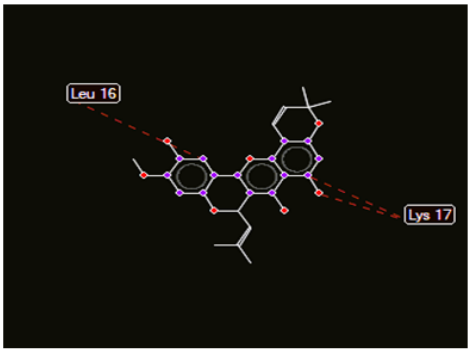


Fig. no. 14: APC.

Fig. no. 15: JUN.

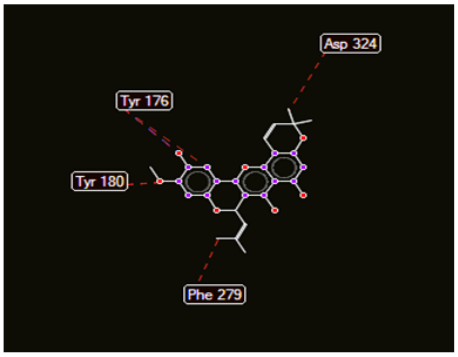


Fig. no. 16: PTEN.

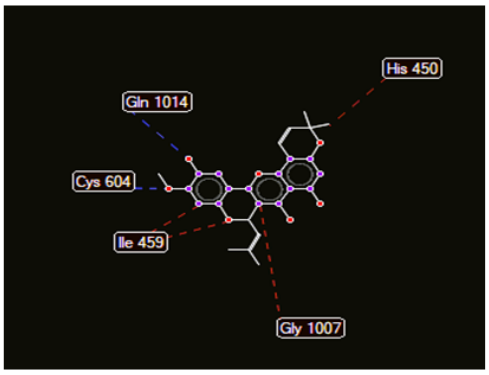


Fig. no. 17: PIK3CA.

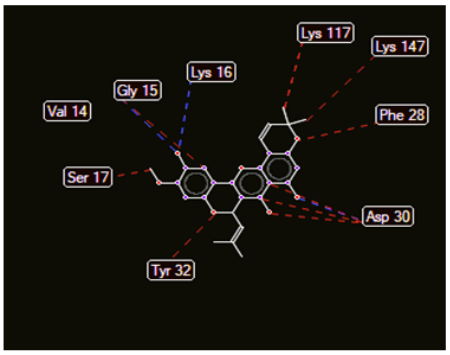


Fig. no. 18: KRAS.

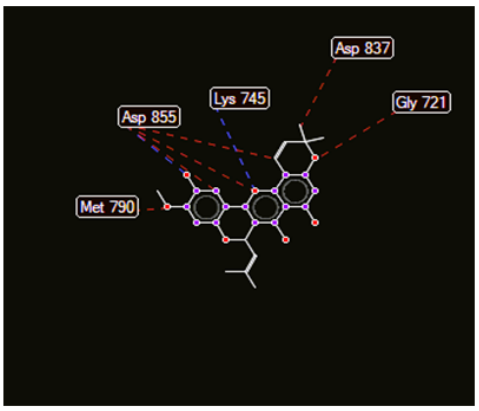


Fig. no. 19: EGFR.

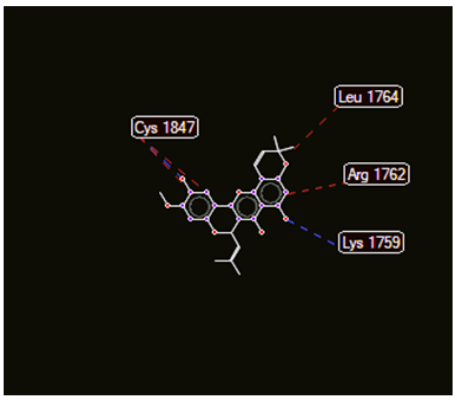


Fig. no. 20: BRCA1.

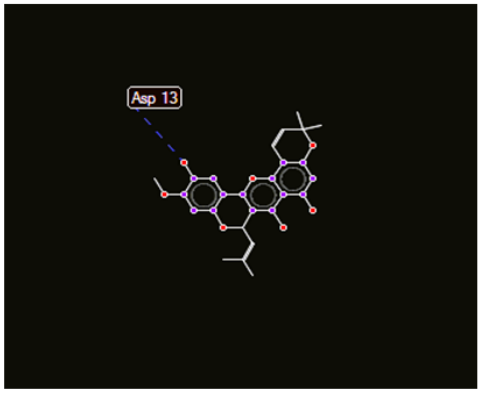


Fig. no. 21: MITF.

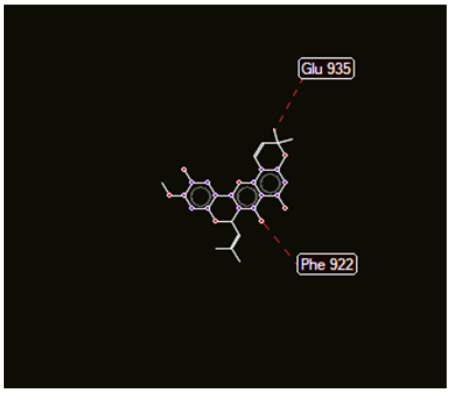


Fig. no. 22: MYC.

The compound binding efficiencies were examined after they were docked with different Cancer proteins. The binding scores of cycloartomunin towards each protein ranged from -5.65 to -9.73 kcal/m.

A negative score in the scoring represents the minimum binding energy required to form a complex between a protein complex and a ligand. A summary of the outcomes is provided. The findings showed that the cycloartomunin molecule had a greater affinity for cancer proteins.

ADME Analysis for the Cycloartomunin

The ADME properties of Cycloartomunin compound were studied using the online program SwissADME to further investigate their pharmacokinetics, drug-likeness, physiochemical properties. According to Lipinski's rule of five, the cycloartomunin are is violation in lipophilicity.

Cycloartomunin showed orally active drug-likeness properties. It is reported that compounds with, molecular weight, and hydrogen bond capacity.

Compound	Molecular weight	HB Acceptor	HB Donor	Lipophilicity	Molar refractivity	Rule of five
cycloartomunin	434.44	7	3	5.46	121.40	1

Molecular weight (acceptable range: <500). HB, Hydrogen bond acceptor (acceptable range: ≤10). HB, Hydrogen bond donor (acceptable range: ≤5). Lipophilicity (expressed as Log Po/w, acceptable range: < 5). Molar refractivity should be between 40 and 130.6 Rule of five: Number of violations of Lipinski's rule of five.

Pharmacokinetics properties

The cycloartomunin has a high gastrointestinal absorption and CYP2C9 inhibitor activity

compound	GI absorption	BBB permeant	P-gp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor
cycloartomunin	High	No	No	No	No	Yes	No

Toxicity Prediction class of best docked compounds by ProTox-II.

In ProTox-II, Cycloartomunin is predicted as Class 5. And the LD50 value is are expressed in table.

Compound	Predicted LD50, (mg/kg)	Predicted Toxicity Class
Cycloartomunin	5000	5

Class 1: deadly if consumed (LD50 ≤ 5); Class 2: deadly if consumed (5 < LD50 ≤ 50); Class 3: lethal if consumed (50 < LD50 ≤ 300); Class 4: harmful if consumed (300 < LD50 ≤ 2000); Class 5: maybe harmful if consumed (2000 < LD50 ≤ 5000); Class 6: non-lethal (LD50 > 5000).

DISCUSSION

Cancer is seen as an important health issue on a global level. The potential of phytochemicals derived from medicinal plants to cure a range of illnesses, including cancer, is becoming more widely acknowledged. About 60 percent of approved cancer medicines are derived from natural sources. Many Indian medicines have been studied and used for the treatment and prevention of many chronic illnesses, such as cancer and cardiovascular disease, after extensive use in folk medicine.

Natural phytochemicals can slow the growth of tumors by a number of mechanisms, such as genotoxic effects, anti-inflammatory and antioxidant effects, decreased cell proliferation, and preservation of intracellular communication to affect apoptosis and signal transduction pathways.

Docking results tabulated between various cancer protein and pass analysis resulted protein with the structure of cyclomorusin which is obtained from *Artocarpus* family plants.

In pass analysis which has predicted various activity of compound most of the biological activity is anticancer activity such as Antineoplastic, Chemopreventive, Apoptosis agonist, Free radical scavenger.

Additionally, it contains expression inhibitors linked to cancer, including those for HIF1A, NOS2, AR, kinase, MMP-9, kinase inhibitors and RELA expression inhibitor (P65). And enhancer activity, like the enhancer of TP53 expression.

The compounds' binding efficiencies were examined after they were docked with different proteins that the pass analysis had predicted. The binding scores of compound ranged from -5.55 to -9.45 kcal/mol. The smallest binding energy needed to create a complex between a ligand and a protein complex is represented by a negative score in the scoring system. A summary of the outcomes is provided. The findings showed that the cycloartomunin molecule had a greater affinity for certain specific proteins.

An autodock software 4.2.6 was used to perform the docking studies of the ligands to protein active sites in order to determine the compounds' binding affinities. P65 has a lower binding efficacy (-5.55) while the MMP-9 protein has a higher binding efficiency (-9.45).

A class of medications known as MMP-9 inhibitors is used to block the action of matrix metalloproteinase-9 (MMP-9), an enzyme linked to the development and progression of cancer and essential to the remodeling of the extracellular matrix (ECM).

The binding energy of cycloartomunin to the HIF1A protein is (-8.35). which control the expression of genes involved in tumor angiogenesis, glucose metabolism, and oxidative stress resistance. They are crucial mediators of the cellular response to hypoxia. Solid tumors frequently exhibit hypoxia, which is caused by the tumor's out-of-control growth that exceeds the oxygen supply and by the tumor's unusual blood vessel development that restricts blood flow. Tumor hypoxia has been shown to: increase tumor survival; decrease anti-tumor immunity; impair the therapeutic response; and stimulate angiogenesis, which increases invasiveness and the risk of metastasis.

NOS2 protein has a higher binding efficiency (-8.95). For malignant tumors, blocking NOS-2 overexpression and the inflammatory milieu around the tumor along with returning sGC/cGMP signaling to normal may be a good substitute for radiation and chemotherapy.

After the compounds were docked with several cancer proteins, their binding efficiencies were investigated. Cycloartomunin's binding scores to each protein varied, ranging from -5.65 to -9.73 kcal/m. The results demonstrated a higher affinity of the cycloartomunin

molecule for cancer proteins. The SOX-2 protein have higher binding energy -9.02. The role of SOX2 in cancer stemness.

Using the online tool SwissADME, the ADME characteristics of the molecule cycloartomunin were examined in order to learn more about its pharmacokinetics, drug-likeness, and physiochemical characteristics.

The cycloartomunin is a lipophilicity violation, as per Lipinski's rule of five. Cycloartomunin demonstrated characteristics of an oral active medication. Compounds with molecular weight and hydrogen bond capability have been reported. And it will have high gastrointestinal absorption and CYP2C9 inhibitor activity.

Cycloartomunin is predicted to be Class 5 in ProTox-II. The LD50 value is 5000 (mg/kg).

CONCLUSION

From the in-silico research completed, we can infer that cycloartomunin show great efficacy in finding against cancer and can be used in cancer care. Since cycloartomunin is a natural Pyranoflavonoids that is easily found *Artocarpus hirsutus, Artocarpus altilis, Artocarpus communis* and *Artocarpus heterophyllus* in root can help people fight cancer more effectively. It will help the developers produce a successful outcome of a drug and give a triumphant result in Clinical trials.

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Conflicts of Interest

Nil.

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Nil.

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