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Molecular Interaction Analysis of Mutant H-Ras P21 against *Phyllanthus emblica*

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KEYWORDS

Malignant melanoma, H-Ras p21, Molecular docking, MAP Kinase

A B S T R A C T

Phyllanthus emblica Linn is widely used for medicinal purpose, usually from the plant, fresh (or) dried fruits are used. There are many bioactive compounds including apigenin, gallic acid, ellagic acid, chebulinic acid, quercetin, chebulagic acid, corilagin, isostrictiniin, methylgallate, luteolin, emblicanin A, emblicanin B, phyllaemblicin B, punigluconin and pedunculagin are tannins in *Phyllanthus emblica*. *P.emblica* possess several biological effects such as anticancer, antifungal, antiulcerogenic, antioxidant, and antidepressant and so on. The activation of Ras/Mitogen Activated Protein (MAP) kinase in turn deactivates MEK, a member of the MAPK signalling cascade. The GTP-bound mutant form H-Ras (Harvey-Ras) protein are found in 30% of human tumors and caused due to point mutation at position 12, 13, 59 and/or 61 codon. Mutant forms of H-Ras protein is continuously involved in signal transduction for cell growth and proliferation through interaction of downstream regulated protein Raf. The analysis of mutated H-Ras p21 with nine compounds resulted in enormous interactions with many hydrogen bonds and least binding energy.

Introduction

Emblica officinalis is a medicinal plant commonly called as Amla (or) Indian gooseberry belongs to Euphorbiace ae family. Emblica officinalis gaertn *(Phyllanthus emblica linn*. Amla, Indian gooseberry), is widely used for medicinal purpose, usually from this plant, fresh, dried fruits are used. The *P.emblica* fruits are the rich source of ascorbic acid (vitamin-c) which itself has well documented nutritional and medicinal properties (Krishnaveni and Mirunalini., 2010). It indicate a strong cytotoxic action of *P.emblica* against cancer cell). Amla is rich in fibre, carbohydrate, iron and is reported as the richest sourse of vitamin-c (Singh *et al.*, 2011).The raw fruits of *P.emblica* is used for a wide variety of human ailments including cancer. *P.emblica* possesses several biological effects such as antibacterial activity, antifungal activity,

antioxidant and free scavenging activity, insecticidal activity, larvicidal and mosquitocidal activity, anticancer and anti – proliferative activity, hepato-protective activity, antimutagenic and wound healing activity, anti ulcerogenic activity, anti depressant activity, immuno-modulatory activity, anti-inflammatory activity, anti-diabetic and hypoglycemic activity, hypolipidemic activity radioprotective activity and many other activity (Ngamkitidechakul *et al*., 2010). Many herbal and patent drugs have been formulated by the constituents of this plant (Rai *et al* 2012). E.officinalis primarily contains tannins, flavonoids, phenolic compounds, saponins, terpenoids, ascorbic acids, carbohydrates and many other compounds (Khan, 2009). Supplements of fresh amla fruit is very favorable to individual suffering from anemia (Kumar *et al.,* 2012b). This herb has many bioactive compounds including apigenin,gallic acid, ellagic acid, chebulini acid, quercetin, chebulagic acid, corilagin,isostrictiniin, methylgallate, luteolin, emblicanin A, emblicanin B, phyllaemblicin B, punigluconin and pednculagin are tannins present in emblica officinalis(Kumar *et al*., 2012a).

In the post-genomic era, our understanding of the molecular biology of melanoma has also increased dramatically. Melanoma is a form of skin cancer that emanates from melanocytes, which are highly specialized in the formation and transfer of melanin pigment. One growth factor pathway that has garnered considerable attention in the last few year has been the RAS-BRAF-MAPK-ERK signaling cascade. Much of the attention surrounding this pathway in human melanoma focuses on the fact that in virtually all cases, there is an alteration at some level in the RAS signaling cascade (Haluska *et al.,* 2006). The rat sarcoma (RAS) virus homologue was the first oncogene to be described in human cancer (Der *et al.,* 1982). In cancer, the most commonly mutated member of the RAS superfamily include HRAS, KRAS, and NRAS. The Ras protein are small (21 kilodalton) G-protein that are active with bound GTP and inactive with bound GDP. Although the GTP can self-hydrolyze, there is a class of enzyme termed GAPs (GTPase activating protein) that facilitate this hydrolysis and terminate RAS activity. Two decades later,the RAS pathway still remains one of the most investigated pathways in human cancer (solit *et al.,* 2006), including melanoma, and our current understanding suggest that several possible mutation along this cascade lead to tumor-promoting physiology. These protein bind GDP/GTP and possess intrinsic GTPase activity allowing inactivation following signal transduction in the normal cellular environment (Khosravi-f *et al.,* 1994). Activation of point mutations in the Ras is one of the most frequent genetic alterations associated with human cancers (Spandidos, *et al.,* 1984). This mutation change a single amino acid in the H-Ras protein. Specifically, the mutation replaces the amino acid glycine with the amino acid valine at position 12 (RasG12V) (oxford, G 2003).The amino acid residues at position 45,46,48 , 49,50,52 and 54, near the previously identified effector region, differ in the Ras (Kitayama *et al* .,1989). The amino acid position which corresponds to effector region on the H-Ras is 32-40 (Ahmadian *et al*., 1997).The selection of this effector region as a binding site will act as potential site for docking studies. Ras is know to induce activation of c-RAF-1 and MAP Kinase (or) extracellular signal regulates kinase (ERK) (De vries-smits *et al*., 1992). Such signal transducing activities are abolished by presence of mutations in the effector regionTYR32-TYR40 (Sigal *et*

al., 1986).Mutation in the effector region affect neither guanine-nucleotide-binding nor GTPase activity, so the effector region is considered to be region that interacts with the target effectors of the Ras protein (Fujita-yoshigaki *et al.,* 1995). In second, experimental results of x-ray crystallographic and nuclear magnetic resonance (NMR) analyses have shown that the three-dimensional structure of the Ras protein changed upon GDP to GTP exchange (Yamasaki *et al* ., 1989). BRAF that promotes RAS-RAF-MEK-ERK pathway activation and melanoma proliferation. Docking is a process by which one can predict the significant orientation of one molecule to a second when bound to each other to form a stable complex. It is mostly used for finding the binding between the ligand and the protein. The information obtained from the docking technique can be used to suggest the binding energy, free energy and stability of complex. At present, docking technique is utilized to predict the tentative binding parameter of ligand – receptor complex beforehand, Molecular docking generates different possible adduct structures that are ranked and grouped together using scoring function in the software. Docking simulations predict optimized docked conformer based upon total energy of the system. In spite of all potential approaches, ligand chemistry (tautomerism and ionization), receptor flexibility (single conformation of rigid receptor) and scoring function (differentiate true binding mode) still remained the challenge. [Shafia Mir *et al.,* 2017].

Materials and Methods

Ligand structure preparation

The 2D structure of apigenin, gallic acid, ellagic acid, quercetin, corilagin, isostrictiniin, methylgallate, emblicanin A, phyllaemblicin B, pedunculagin are drawn in ACD/Chemsketch and then converted to 3D structure and saved as Mdl mol format. Then it is converted to Pdb format for further docking process using Open Babel.

Protein structure preparation

Uniprot is a freely accessible database of protein sequence and functional information may entires being derived from genome sequencing project. (http://www.uniprot.org/).The protein databank (PDB: http://www.rcsb.org/pdb/) is the single worldwide archive of structural data of biological macromolecules.

Today depositors to the pdb have varying expertise in the techniques of x-ray crystal structure determination, NMR, cryoelectron microscopy and theoretical modeling.

ACD/Chemsketch

ACD/Chemsketch is a molecular modeling program used to create and modify images of chemical structures. Also there is a software that allows molecules and molecular models displayed in two and three dimensions, to understand the structure of chemical bonds and the nature of the functional groups.

Openbabel

Open babel is a chemical tool box designed to speak the many languages of chemical data. The molecular format convert tool (open babel) is used to convert this file into the pdb format and is used during analysis.

SPDBV

Target and template proteins have been loaded in SPDBV—superimposed and

structurally aligned. The RMSD value found to be 0.90 Å and results.

Autodock

Auto dock is a suite of automated docking tools. The software is used for modeling flexible small molecule such as drug molecule binding to receptor proteins of known three dimensional structures.

It uses genetic algorithms for the conformational search and is a suitable method for the docking studies.

The technique combines simulated annealing for conformation searching with a rapid grid based method of energy evalution.

Auto dock tools are used to prepare, run and analyze the docking simulations.

Auto dock is the most cited docking software because it is very fast, it provides high quality prediction of ligand conformations and good corrections between inhibition constants and experiments ones (http ://autodock.scripps.edu/resources/tools)

Pymol

Pymol is computer software, a molecular visualization tool use in structural biology. Pymol can produce high-quality 3D images of small molecules and biological macromolecules such as proteins.

Results and Discussion

Sequence Retrieval

The sequence of H-Ras is retrieved from uniprot (http://www.uniprot.org/ uniprot/) database and sequence accession number is P01112 from the *Homosapiens* (Human)

Structure Retrieval

The three-dimensional structure of RAS was available in the PDB database. The PDB id is 2RGB and A chain . The 3D structure was visualized using the Rasmol Tool and shown in figure 1. RAS have the specific domain region. Alternates between an inactive form bound to GDP and an active form bound to GTP. Activated by a guanine nucleotide-exchange factor (GEF) and inactivated by a GTPase-activating protein (GAP).

Docking Analysis of bioactive compounds present in *Phyllanthus emblica*against mutant H-Ras and H-Ras

The bioactive compounds including apigenin, gallic acid, ellagic acid, quercetin, corilagin, isostrictiniin, methylgallate, emblicanin A, and pednculagin docked against mutant H-Ras. The graphical user interface program "Auto-Dock Tools" was used to prepare, run, and analyze the docking simulations. Kollman united atom charges, solvation parameters and polar hydrogens were added into the PDB file for the preparation of protein in docking simulation. Autodock (Goodsell *et al.,* 1996) requires precalculated grid maps, one for each atom type present in the flexible molecules being docked and its stores the energy arising from the interaction with rigid macromolecules. The grid box size was set at 126, 126, and 126 A^0 (x, y, and z) to include all the amino acid residues that present in rigid macro molecules. Auto grid 4 program, supplied with Autodock 4 was used to produce grid maps (Rarey *et al*., 1996). The Lamarckian Genetic Algorithm (LGA) (Morris *et al*., 1998) was chosen search for the best conformers. The best ligand-receptor structure from the docked structure was chosen based on the lowest energy and minimal solvent accessibility of

the ligand (Jones *et al*., 1997). The bioactivecompounds (namely apigenin, gallic acid, ellagic acid, quercetin, corilagin, isostrictiniin, methylgallate, emblicanin A, pednculagin) and mutant H-Ras binding energy are shown in Figures 1(a)-9(a), final conformation from auto dock is shown in Figures 1(b)-9(b), and the hydrogen bond were interactions visualized using PyMol were shown in Figures 1(c)-9(c).

Mutant H-RAS and Isostrictiniin

Docking of mutant H-ras against isostrictiniin produced six clusters of conformers using RMSD tolerance of 2.0 Å out of 10 docking runs. Cluster Rank 1 with binding energy -11.64 kcal/mol at eighth run has formed five hydrogen bond with active binding sites of mutant H-ras shown in the figure1. Docking conformation between the isostrictiniin and mutant H-ras the is shown in Figure 1(a), docking score is shown in Figure 1(b). The interactions between atoms of isostrictiniin and atoms of aminoacids of mutant H-ras is shown in Figure 1(c). Hydrogen bond distance between the donor and acceptor atoms was shown in table-2.

Mutant H-RAS and Quercetin

Docking of mutant H-ras against quercetin produced three clusters of conformers using RMSD tolerance of 2.0 Å out of 10 docking runs. Cluster Rank 1 with binding energy -9.67 kcal/mol at first run has formed six hydrogen bond with active binding sites of mutant H-ras shown in the figure2. Docking conformation between the quercetin and mutant H-ras the is shown in Figure 2(a), docking score is shown in Figure 2(b). The interactions between atoms of quercetin and atoms of aminoacids of mutant H-ras is shown in Figure 2(c). Hydrogen bond distance between the donor and acceptor atoms was shown in table-3.

Mutant H-RAS and Gallic Acid

Docking of mutant H-ras against gallic acid produced four clusters of conformers using RMSD tolerance of 2.0 Å out of 10 docking runs. Cluster Rank 1 with binding energy -8.48 kcal/mol at fifth run has formed eight hydrogen bond with active binding sites of mutant H-ras shown in the figure3. Docking conformation between the gallic acid and mutant H-ras the is shown in Figure 3(a), docking score is shown in Figure 3(b).

The interactions between atoms of gallic acid and atoms of aminoacids of mutant H-ras is shown in Figure 3(c). Hydrogen bond distance between the donor and acceptor atoms was shown in table-4.

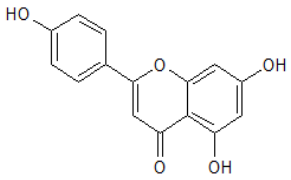
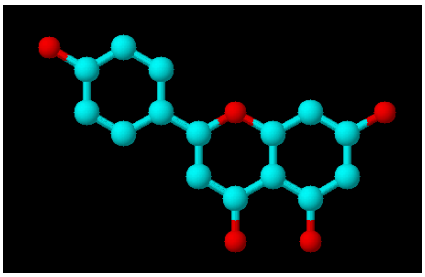
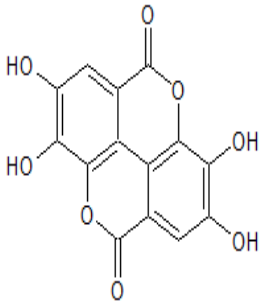
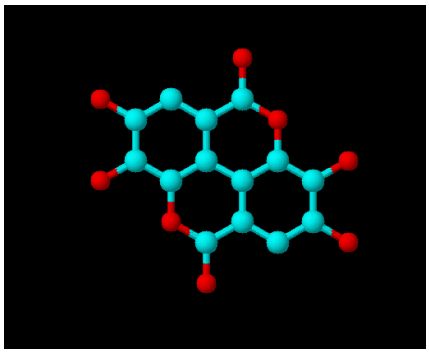
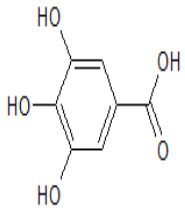
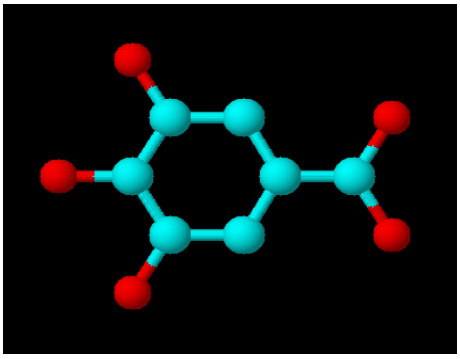
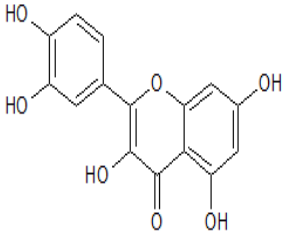
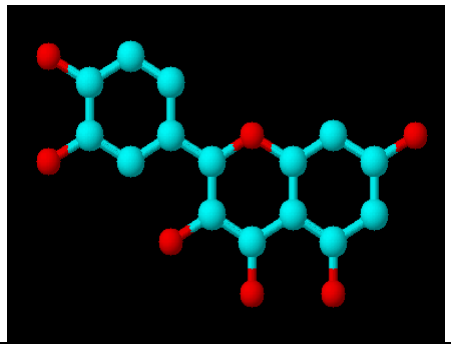
Mutant H-RAS and Methylgallate

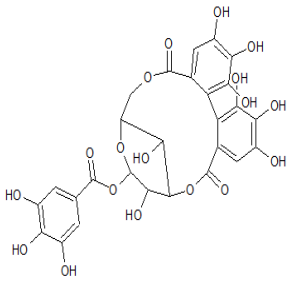
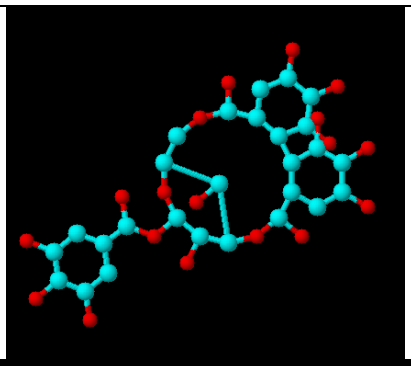
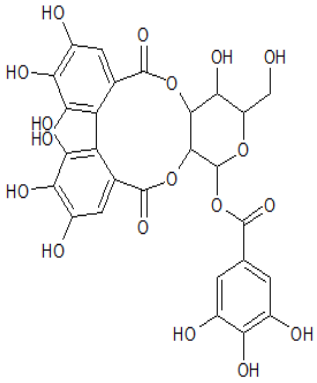
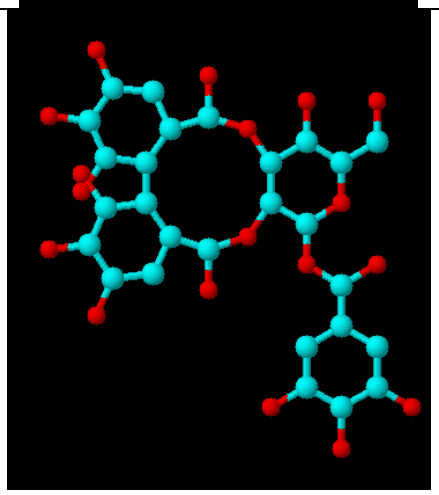
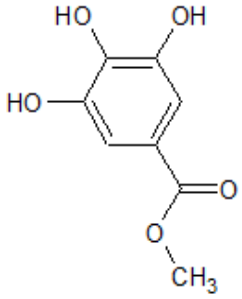
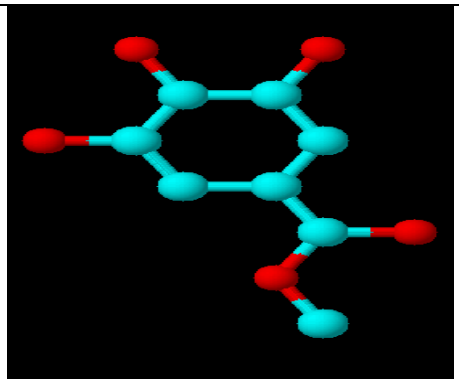
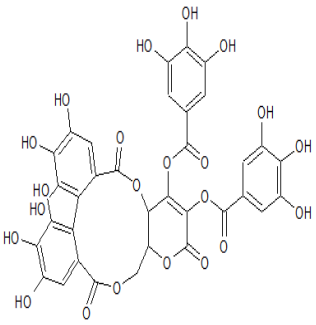
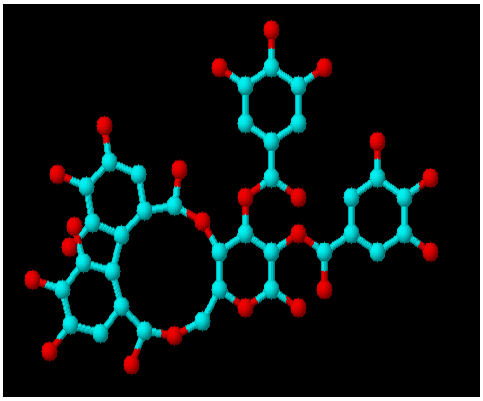
Docking of mutant H-ras against methylgallate produced three clusters of conformers using RMSD tolerance of 2.0 Å out of 10 docking runs. Cluster Rank 1 with binding energy -8.76 kcal/mol at tenth run has formed six hydrogen bond with active binding sites of mutant H-ras shown in the figure4. Docking conformation between the methylgallate and mutant H-ras the is shown in Figure 4(a), docking score is shown in Figure 4(b). The interactions between atoms of methylgallate and atoms of aminoacids of mutant H-ras is shown in Figure 4(c). Hydrogen bond distance between the donor and acceptor atoms was shown in table-5.

Mutant H-RAS and Emblicanin A

Docking of mutant H-ras against emblicanin Aproduced four clusters of conformers using RMSD tolerance of 2.0 Å out of 10 docking runs. Cluster Rank 1 with binding energy -11.68 kcal/mol at sixth run has formed three hydrogen bond with active binding sites of mutant H-ras shown in the figure5.

Table.1 Showing the compounds extracted from *Phyllanthus emblica*

S.No	Compounds	Molecular formula	2D structure	3D structure
1.	Apigenin	$C_{15}H_{10}O_5$		
2.	Ellagic acid	$C_{14}H_6O_8$		
3.	Gallic acid	$C_7H_6O_5$		
4.	quercetin	$C_{15}H_{10}O_7$		

5.	Corilagin	$C_{27}H_{22}O_{18}$		
6.	Isostrictiniin	$C_{27}H_{22}O_{18}$		
7.	Methylgallate	$C_8H_8O_5$		
8.	Emblicanin A	$C_{34}H_{22}O_{22}$		

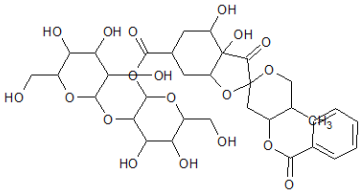
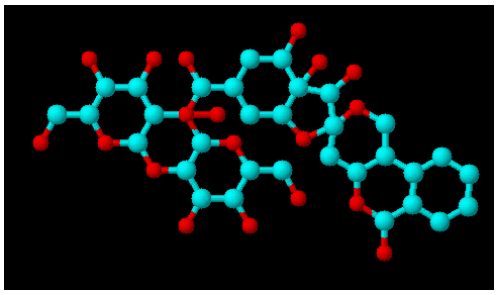
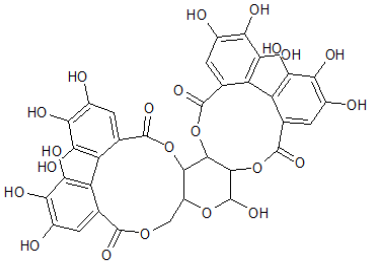
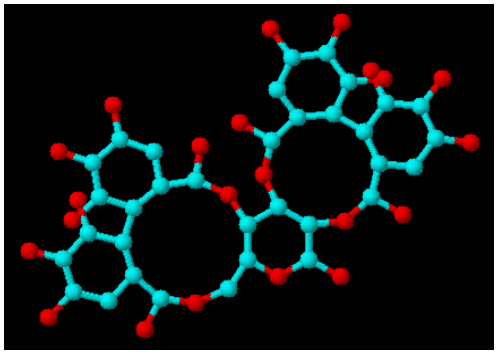
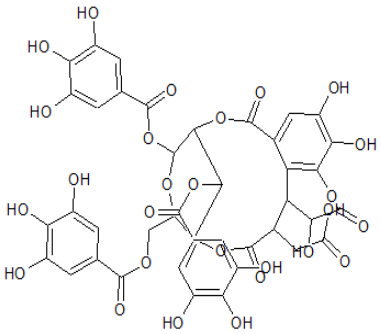
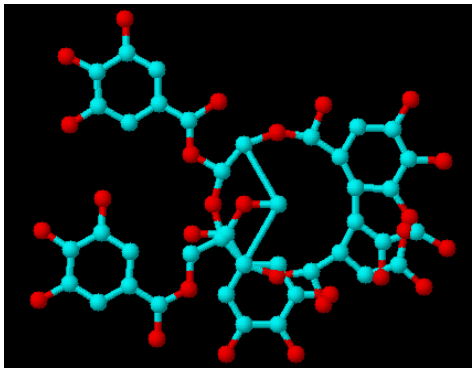
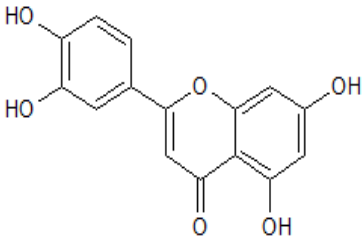
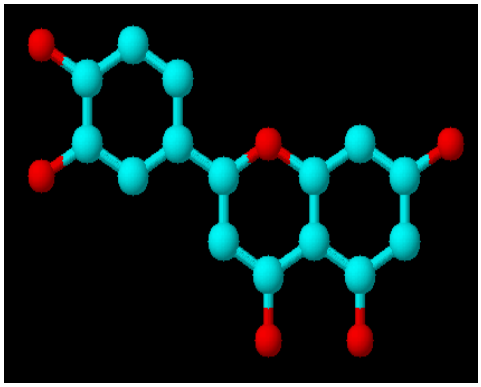
9.	Phyllaemblicin B	$C_{33}H_{44}O_{19}$		
10.	Pedunculagin	$C_{34}H_{24}O_{22}$		
11.	chebulinic acid	$C_{41}H_{32}O_{27}$		
12.	Luteolin	$C_{15}H_{10}O_6$		

Table.2 Shows the docking interaction between MUTATE H-RAS and ISOSTRINIIN

MUTATE H-RAS		ISOSTRICTINIIN	Distance (A)	Docking energy (Kcal / mol)
RESIDUES	ATOM			
SER-17	N	O	3.2	-11.64
LYS-16	N	O	3.0	
THR-35	OG1	O	3.2	
ASP-33	N	O	2.8	
TYR-32	N	O	3.2	
VAL-29	O	O	2.6	
ASN-116	ND2	O	3.2	
ASP-119	OD1	O	2.9	
ASP-119	N	O	2.7	
ALA-146	OG	O	2.7	
SER-145	N	O	3.1	
LYS-147	O	O	3.0	
ASP-33	OD2	O	3.1	
ASP-119	OD1	O	2.7	
LYS-147	O	O	3.3	
SER-17	N	O	3.0	

Table.3 Shows the docking interaction between MUTATE H-RAS and QUERCETIN

MUTATE H-RAS		QUERCETIN	Distance (A)	Docking energy (Kcal / mol)
RESIDUES	ATOM			
ASP-33	OD1	O	2.8	-9.67
ASP-38	OD2	O	3.2	
THR-35	OG1	O	2.6	
SER-17	OG	O	2.6	
GLU-31	O	O	2.9	
GLU-31	O	O	2.6	
GLY-15	N	O	3.1	
VAL-14	N	O	2.7	
LYS-16	NZ	O	3.6	
LY-S-16	NZ	O	2.9	
ASP-33	N	O	3.1	
THR-32	OH	O	3.4	
GLY-13	N	O	3.1	
ALA-11	O	O	3.4	
GLY-13	N	O	2.7	

Table.4 Shows the docking interaction between MUTATE H-RAS and GALLIC ACID

MUTATE H-RAS		GALLIC ACID	Distance (A)	Docking energy (Kcal / mol)
RESIDUES	ATOM			
GLU-31	O	O	3.4	-8.48
ASP-33	O	O	3.5	
THR-35	OG1	O	2.5	
SER-17	OG	O	2.7	
LYS-16	N	O	2.6	
GLY-15	N	O	3.0	
VAL-14	N	O	3.3	
ASP-33	N	O	3.2	
LYS-16	N	O	2.7	
LYS-16	N	O	2.9	
ASP-33	O	O	2.5	
ASP-33	O	O	3.2	

Table.5 Shows the docking interaction between MUTATE H-RAS and METHYLGALLTE

MUTATE H-RAS		METHYLGALLATE	Distance (A)	Docking energy (Kcal / mol)
RESIDUES	ATOM			
ALA-11	O	O	3.4	-8.76
GLY-13	N	O	3.1	
VAL-14	N	O	2.8	
GLY-15	N	O	2.9	
LYS-16	N	O	3.3	
LYS-16	NZ	O	2.6	
LYS-16	NZ	O	2.7	
ALA-18	N	O	2.8	
THR-35	N	O	2.7	

Table.6 Shows the docking interaction between MUTATE H-RAS and EMBLICANIN A

MUTATE H-RAS		EMBLICANIN A	Distance (A)	Docking energy (Kcal / mol)
RESIDUES	ATOM			
ASN-26	ND2	O	3.3	-11.68
GLN-25	NE2	O	2.7	
LYS-42	NZ	O	3.3	
ARG-41	NE	O	2.9	
GLN-25	O	O	3.3	
GLN-43	N	O	3.0	
GLN-25	NE2	O	3.2	
GLN-25	NE2	O	2.9	
GLN-25	NE2	O	3.2	
LYS-42	NZ	O	3.3	
GLN-25	O	O	3.0	
GLN-43	N	O	3.2	

Table.7 Shows the docking interaction between MUTATE H-RAS and ELLAGIC ACID

MUTATE H-RAS		ELLAGIC ACID	Distance (A)	Docking energy (Kcal / mol)
RESIDUES	ATOM			
ASP-30	O	O	3.0	-7.71
ALA-146	N	O	2.8	
ASP-119	OD1	O	2.3	
ASP-30	O	O	3.1	
ASN-116	ND2	O	2.5	
SER-145	OG	O	2.5	
LYS-147	N	O	3.2	
ALA-146	N	O	3.0	
ASP-119	OD1	O	3.6	

Table.8 Shows the docking interaction between MUTATE H-RAS and CORILAGIN

MUTATE H-RAS		CORILAGIN	Distance (A)	Docking energy (Kcal / mol)
RESIDUES	ATOM			
THR35	OG1	O	3.5	-12.32
THR35	OG1	O	2.9	
GLU31	O	O	2.6	
VAL29	O	O	3.0	
ALA11	O	O	2.7	
LYS16	NZ	O	2.7	
GLY15	N	O	2.9	
VAL14	N	O	2.8	
ALA18	N	O	2.7	
LYS16	NZ	O	3.3	
LYS16	NZ	O	2.6	
ALA11	O	O	3.3	
GLY15	N	O	3.3	

Table.9 Shows the docking interaction between MUTATE H-RAS and APIGENIN

MUTATE H-RAS		APIGENIN	Distance (A)	Docking energy (Kcal / mol)
RESIDUES	ATOM			
ASP33	O	O	3.6	-8.17
THR35	OG1	O	2.7	
LYS16	NZ	O	2.7	
SER17	OG	O	2.9	
GLY13	N	O	3.1	
ASP33	O	O	2.4	

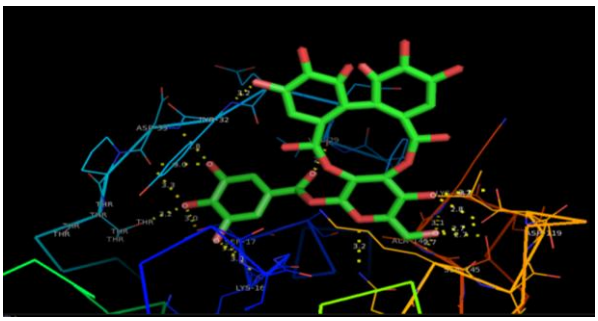
Table.10 Shows the docking interaction between MUTATE H-RAS and APIGENIN

MUTATE H-RAS		PEDUNCULAGIN	DISTANCE (A)	DOCKING ENERGY(Kcal / mol)
RESIDUE	ATOM			
ASN-26	ND2	O	3.3	-11.68
LYS-42	NZ	O	3.4	
LYS-42	NZ	O	3.3	
ARG-41	NH2	O	3.5	
ARG-41	NE	O	2.8	
GLN-43	NE2	O	2.9	
GLN-43	O	O	3.3	
GLN-43	N	O	3.1	
GLN-43	N	O	3.0	
GLN-25	O	O	3.2	
GLN-25	O	O	2.9	
GLN-25	O	O	3.2	

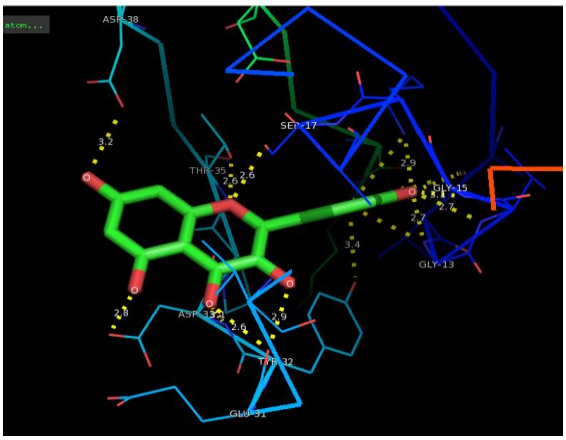
Key residues of mutant H-RAS, Hydrogen bond and Docking score

COMPOUNDS	KEY RESIDUES	Docking energy (Kcal / mol)	NO OF INTERACTION
Apigenin	ASP33, THR35, LYS16, SER17, GLY13.	-8.17	6
Ellagic acid	ASP-30, ASN-116, SER-145, LYS-147, ALA-146, ASP-119.	-7.71	9
Gallic acid	GLU-31, ASP-33, THR-35,SER-17, LYS-16, GLY-15, VAL-14.	-8.48	12
Corilagin	THR35, GLU31, VAL29, ALA11, LYS16, GLY15, VAL-14, ALA-18.	-12.32	13
Isostrictiniin	SER-17, LYS-16, THR, TYR-32, VAL-29, ASN-116, ASP-119 ,ALA-146, SER-145, LYS-147, ASP-33.	-11.64	16
Pedunculagin	ASN-26,LYS-42,ARG-41,GLN-43, LYS-42.	-11.68	12
Emblicanin A	ASN-26, GLN25, LYS-42, ARG-41, GLN-25, GLN-43.	-11.68	12
Quercetin	ASP-33, ASP-38, THR-35, SER-17, GLU-31, GLY-15, VAL-14, LYS-16, THR-32,GLY-13,ALA-11,GLY-13.	-9.67	15
Methylgallate	THR35, ALA18, LYS16, GLY15, VAL14, ALA11, LYS-16 GLY13.	-8.76	9

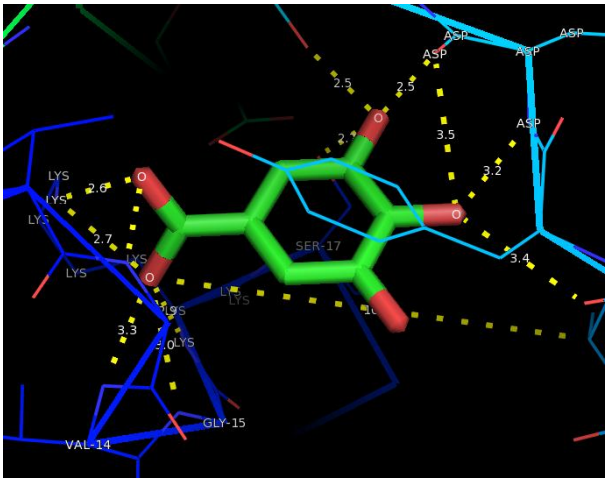
Mutant H-RAS and Isostrictiniin



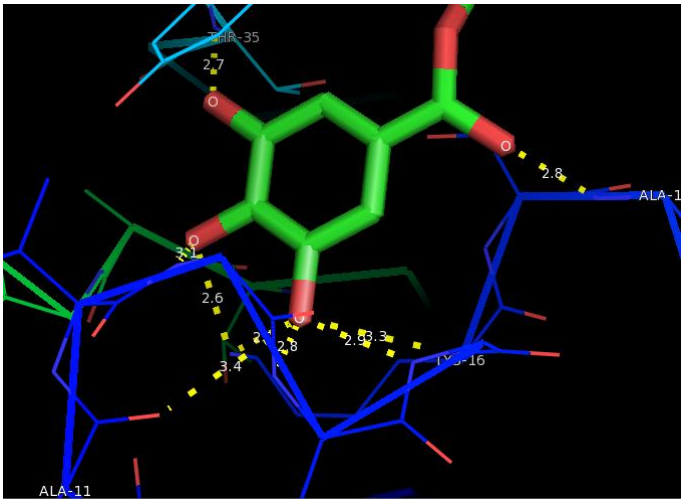
Mutant H-RAS and Quercetin



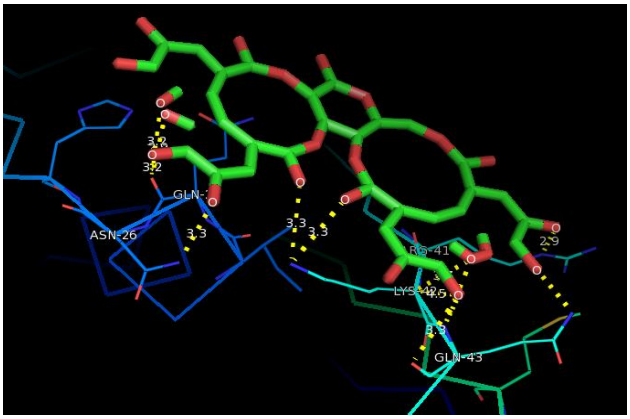
Mutant H-RAS and Gallic Acid



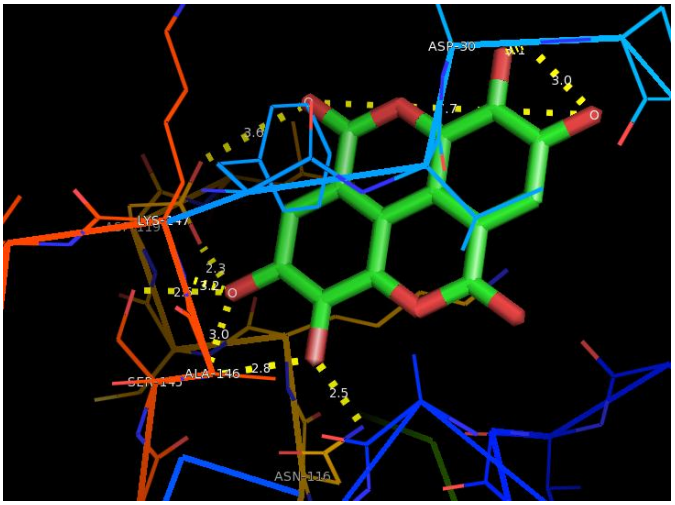
Mutant H-RAS and Methylgallate



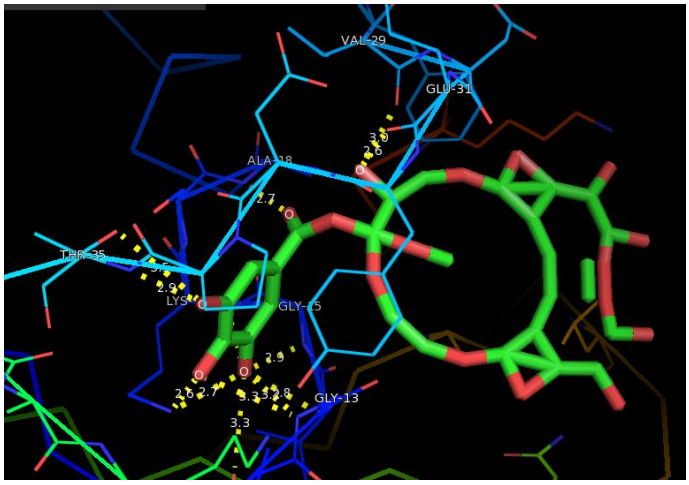
Mutant H-RAS and Emblicanin A



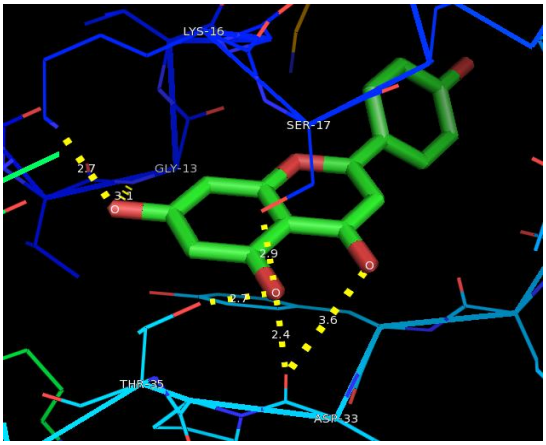
Mutant H-RAS and Ellagic Acid



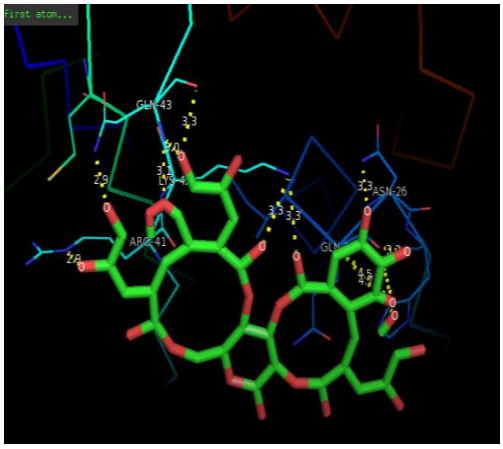
Mutant H-RAS and Corilagin



Mutant H-RAS and Apigenin



Mutant H-RAS and Pedunculagin



Docking conformation between the emblicanin A and mutant H-ras the is shown in Figure 5(a), docking score is shown in Figure 5(b). The interactions between atoms of emblicanin A and atoms of aminoacids of mutant H-ras is shown in Figure 5(c). Hydrogen bond distance between the donor and acceptor atoms was shown in table-6.

Mutant H-RAS and Ellagic Acid

Docking of mutant H-ras against ellagic acid produced four clusters of conformers using RMSD tolerance of 2.0 Å out of 10 docking runs. Cluster Rank 1 with binding energy -7.71 kcal/mol at eigth run has formed four hydrogen bond with active binding sites of mutant H-ras shown in the figure6. Docking conformation between the ellagic acid and mutant H-ras the is shown in Figure 6(a), docking score is shown in Figure 6(b). The interactions between atoms of ellagic acid and atoms of aminoacids of mutant H-ras is shown in Figure 6(c). Hydrogen bond distance between the donor and acceptor atoms was shown in table-7.

Mutant H-RAS and Corilagin

Docking of mutant H-ras against corilagin produced eight clusters of conformers using RMSD tolerance of 2.0 Å out of 10 docking

runs. Cluster Rank 1 with binding energy -12.32 kcal/mol at second run has formed six hydrogen bond with active binding sites of mutant H-ras shown in the figure7. Docking conformation between the corilagin and mutant H-ras the is shown in Figure 7(a), docking score is shown in Figure 7(b). The interactions between atoms of corilagin and atoms of aminoacids of mutant H-ras is shown in Figure 7(c). Hydrogen bond distance between the donor and acceptor atoms was shown in table-8.

Mutant H-RAS and Apigenin

Docking of mutant H-ras against apigenin produced four clusters of conformers using RMSD tolerance of 2.0 Å out of 10 docking runs. Cluster Rank 1 with binding energy -8.17 kcal/mol at first run has formed five hydrogen bond with active binding sites of mutant H-ras shown in the figure8.

Docking conformation between the apigenin and mutant H-ras the is shown in Figure 8(a), docking score is shown in Figure 8(b). The interactions between atoms of apigenin and atoms of aminoacids of mutant H-ras is shown in Figure 8(c). Hydrogen bond distance between the donor and acceptor atoms was shown in table-9.

Mutant H-RAS and Pedunculagin

Docking of mutant H-ras against pedunculagin produced three clusters of conformers using RMSD tolerance of 2.0 Å out of 10 docking runs.

Cluster Rank 1 with binding energy -11.68 kcal/mol at ninth run has formed three hydrogen bond with active binding sites of mutant H-ras shown in the figure9. Docking conformation between the pedunculagin and mutant H-ras the is shown in Figure 9(a), docking score is shown in Figure 9(b). The interactions between atoms of pedunculagin and atoms of aminoacids of mutant H-ras is shown in Figure 9(c). Hydrogen bond distance between the donor and acceptor atoms was shown in table-10.

The bioactive compounds (apigenin, gallic acid, ellagic acid, quercetin, corilagin, isostrictiniin, methylgallate, emblicanin A, pedunculagin) from *Phyllanthus emblica* docked against mutant H-Ras resulted in protein and ligand complex. The docked structures were analyzed and the interaction were seen. Hydrogen bond interactions and the binding distance between the donors and acceptors were measure for the best conformers. The binding energy is correlated with the probability of affinity and stable bound between ligand and its receptor. Binding energy values may also predict the bioactivity value for ligands to the corresponding receptor.

From this *insilico* study and previously reported experimental data in literature, we conclude this compound are least binding energy and hydrogen bond are formed for this compounds (corilagin, isostrictiniin, emblicanin A, pedunculagin, quercetin) would be an effective lead to inhibits function of mutant H-Ras p21 protein, which will in turn arrest the process of cell growth and proliferation of the cancer cell. Two decades later, the RAS pathway still remains one of the most investigated pathways in human cancer, including melanoma, and our current understanding suggest that several possible mutation along this cascade lead to tumor-promoting physiology. Activation of point mutations in the Ras is one of the most frequent genetic alterations associated with human cancers, including melanoma cancer. Further, the four ligand molecule can be incorporated into the drug development phases and clinical trial.

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