COMPUTER AIDED PERSPECTIVE OF SELECTION OF PLANTS AGAINST VIRUSES

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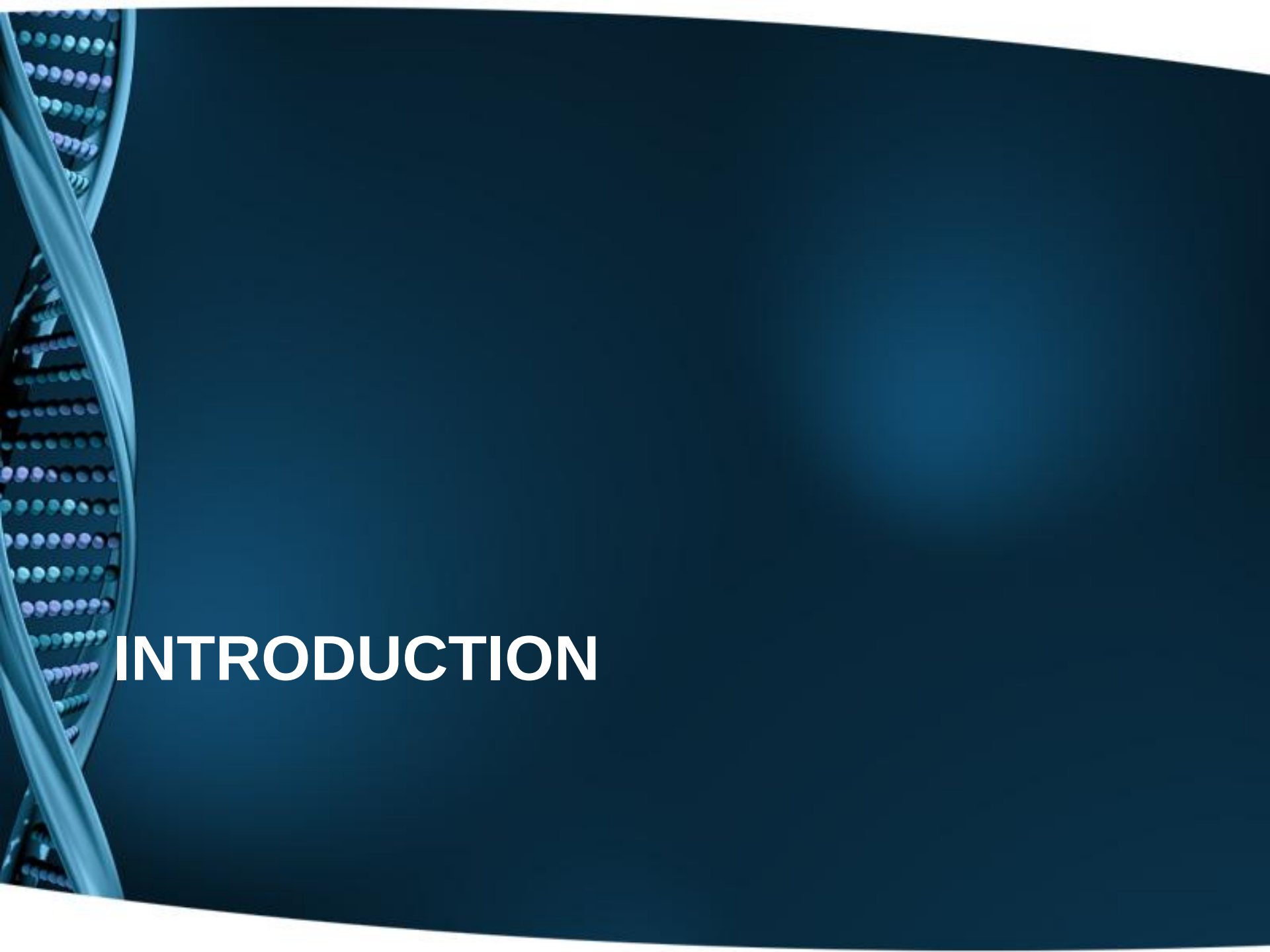
INTRODUCTION

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- Plants having different biosynthetic pathways are great sources of natural compounds, which can be used for various therapeutic purposes.
- More than 100 compounds acting as anticancer and anti-infective agents, at different stages of clinical development are derived from natural sources.
- The main aim of a medicinal chemist is to get active extracts, fractions or compounds against a particular target.

- In the recent times, computational chemistry has become an economic solution for drug discovery and identify of lead molecules.
- In order to estimate the biological activities of various chemical constituents of twenty different plants, docking was done on Maestro (Glide) and Lead IT (FlexX).
- Chemical moieties that got good docking scores were further docked in Autodock in order to estimate the inhibition constant.

OBJECTIVE

- The main objective behind these docking studies is to suggest the use of docking studies in selection of plants against viruses.
- Secondly, here we identified some natural leads that were proved to be potential antiviral agents, based on docking studies and literature search.
- An attempt has also been made to compare docking studies with the co-crystallized molecules.


VIRAL LIFE CYCLES AND
IMPORTANT DRUG TARGETS

Influenza

- Influenza viruses are a group of RNA viruses that causes common flu. There are mainly two types of Influenza virus, A and B. Virus is mostly spherical having lipid bi-layer. Influenza virus has negative stranded RNA (having 8 segments code for 11 proteins). Influenza uses the plasma membrane of the host cell for formation of viral particles and migrate to the neighboring host cells and these viral particles had double lipid layer. Viruses protrude out from apical side and so HA, NA and M2 move towards the apical side. M2 tail is important for viral formation. M1 is present under lipid bilayer and is important for budding of new viruses. Before leaving the virus has to cleave from sialic acid residues from glycoproteins and this can be done with the NA.

Dengue

- Dengue fever caused by Dengue virus (DENV 1-4) is a mosquito borne disease. Dengue virus belongs to the family *Flaviviridae* with four different serotypes (DENV 1-4) causes dengue fever and dengue hemorrhagic fever. DENV is positive stranded RNA virus. The non-structural proteins include NS1, NS2a, NS2b, NS3, NS4a, NS4b, NS5. The capsid protein orients towards the cytoplasmic side of RER envelope protein and premembrane protein towards the lumen side of RER. Once the translation is complete and folding of proteins occurs, the NS proteins stimulate the synthesis of new RNA. This RNA is then capped by capsid protein and will get formed into nucleocapsid. Then it enters the lumen side where it is further enveloped with premembrane protein and envelope protein. This immature virions then pass through the golgi apparatus where they mature and can cause infection, are released from the cell.

Human Immunodeficiency Virus (HIV)

- Human Immunodeficiency virus simply called HIV causes Acquired Immunodeficiency Syndrome (AIDS). HIV is a retrovirus, viruses having the RNA but get converted to DNA in the host cell. The first step is the attachment of the virus to the T-cell surface. This can be achieved by two proteins namely gp120 and gp41 which attach to the CD4 and CCR5/CXCR4 receptors. Then viral RNA is converted into double stranded DNA by a process referred to as reverse transcription assisted by enzyme reverse transcriptase. The viral DNA synthesize two stands of RNA, one strand synthesize the requirements of virus like reverse transcriptase, integrase and structural proteins etc. Other strand synthesizes genetic material of virus. This is followed by aggregation of various HIV components to form new virus. The newly formed virions move themselves outside the host cell called as budding.

Chikungunya

- Chikungunya is a class of Arbovirus. It enters the host cell by endocytosis. The decrease in pH causes conformation changes in envelope protein, exposing E1 peptide. This peptide helps in fusing viral membrane with host membrane. This releases viral genome into the cytoplasm. Translation of viral mRNA leads to formation of two precursors of non structural proteins and cleavage of these proteins leads to formation of NSP1-NSP4. NSP1 along with NSP2 is involved in catalyzing the synthesis of negative strand of RNA and have RNA capping properties, NSP2 shows RNA helicase, RNA phosphatase and proteinease activity, NSP3 has replication property and NSP4 has polymerase activity. These proteins together forms a viral replication complex which synthesizes a negative RNA strand intermediate and this acts as an template for synthesis of genomic (49S) and subgenomic (26S) RNA.

MATERIALS AND METHODS OF VIRTUAL SCREENING

Table 1: Different Viral protein targets with their respective PDB codes

Viral Protein	Influenza	Dengue	HIV	Chikungunya
Protease		2FOM	1ODY	3TRK
Methyl transferase		2P40		
Reverse transcriptase			2RF2	
Neuramidase	1L7F			

All the proteins were downloaded from Protein Data Bank (www.rcsb.org)

Table 2: Different chemical constituents from plants docked

Sl. No.	Plant	Chemical constituents docked
1	*Curcuma longa*[8]	Curcumin (1), demethoxycurcumin (2), β-phellandrene (3), p-cymene (4), α-turmerone (5)
2	*Ficus religiosa*[9]	Kaempferol (6), myricetin (7), quercetin (8) , methyl oleanolate (9), β-sitosterol (10), stigmasterol (11) ,lanosterol (12)
3	*Cuminum cyminum*[10]	Cuminaldehyde (13), limonene (14), α-pinene (15), β-pinene (16), o-cymene (17), α-terpinene (18), γ-terpinene (19), safranal (20), linalool (21)
4	*Punica granatum*[11]	Citric acid (22), mallic acid (23), gallic acid (24), catechin (25), quercetin (8), α-tocopherol (26), linoleic acid (27), oleic acid (28), β-stitosterol (10)
5	*Oroxylum indicum*[12]	Baicalein (29), oroxyline (30), pinostrobin (31), stigmast-7-en-3-ol (32)
6	*Mangifera indica*[13]	Mangiferin (33), protocatechuic acid (34), catechin (25), shikimic acid (35), mangostin (36), gallic acid (24), ethyl gallate (37)
7	*Achyranthes aspera*[14]	D-Glucuronic acid (38), oleanolic acid (39), achyranthine (40), ecdsysterone (41)
8	*Barleria prionitis*[15]	Barlerinoside (42), barlerin (43)
9	*Terminalia chebula*[16]	gallic acid (24), chebulanin (44), chebulinic acid (45)
	Pterocarpus marsupium[17]	kinotannic acid (46), pterocarpol (47), liquiritigenin (48), gallic acid (24)

11	***Cajanus cajan*[18]**	Cajanin (49), pinostrobin (50), longistylin A (51), cajanuslactone (52), vitexin (53)
12	***Acacia nilotica*[19]**	Gallic acid (24), Apigenin (54), protocatechuic acid (34), rutin (55)
13	***Zingiber officinale*[20]**	Curcumene (56), fernesene (57), gingiberene (58)
14	***Piper longum* [21]**	Piperine (59), asarinine (60), sesamin (61), caryophyllene (62), gingiberene (63), p-cymene (4)
15	***Euphorbia hirita* [22]**	Quercetin (8), myricetin (7), rutin (55), kaempferol (6), gallic acid (24), protocatechuic acid (34)
16	***Cissus quadrangularis* [23]**	Ascorbic acid (64), β-sitosterol (10), quercetin (8), amyrin (65)
17	***Ocimum sanctum* [24]**	Eugenol (66), ursolic acid (67), carvacrol (68), linalool (21), caryophyllene (62), estragole (69)
18	***Tabernaemontana divaricata* [25]**	Conophylline (70), Dregarnine (71), tabermontanine (72)
19	***Hibiscus sabdariffa* [26]**	Hibiscitrin (73), β-sitosterol (10), citric acid (22), delphinidin-3-glucoside (74), protocatechuic acid (34), quercetin (8)
20	***Allium sativum* [27]**	Allixin (75), propiin (76)

Docking Software

- **Maestro:** The computation studies were carried using Maestro 8.5. The chemical constituents were obtained from literature search. Glide is used for docking natural compounds into the protein molecules. The molecules were docked using standard precision. Residues interaction scores were taken within 12 Å range.

- **Lead IT:** The ligands prepared in Maestro 8.5 were used for docking. They were saved in .sdf format and used for docking studies. The docking is done using default parameters using hybrid approach, followed by visualization using Pose View
- .
- **Autodock:** Proteins prepared in Maestro saved in .pdb format were converted to Autodock compatible atom type using OpenBabel. Ligands were prepared in Maestro and saved in .pdb format. Docking was done using Autodock 4.2

RESULTS

Table 3: Docking scores of co-crystallized molecules with their respective proteins

Viral Protein	Maestro	Lead IT	Autodock
Methyl transferase of dengue (2P40)	-7.812	-23.436	17.02 mM
Protease of HIV (1ODY)	-8.319	ND	37.83µM
Reverse transcriptase of HIV (2RF2)	-8.206	ND	ND
Neuramidase of influenza (1L7F)	-8.288	-28.635	149.05µM

**ND- Not docked*

Table 4: Maestro scores of HITs

Sl. No.	Chemical constituent	1L7F	2FOM	2P40	3TRK	1ODY	2RF2
1.	**Kaemferol**	-4.893	-5.240	-6.299	-6.819	-5.920	-8.117
2.	**Myricetin**	-5.340	-5.574	-6.337	-5.564	-6.210	-8.107
3.	**Quercetin**	-5.270	-4.551	-6.357	-5.992	-7.050	-7.742
4.	**Gallic acid**	-4.611	-6.084	-6.408	-6.153	-6.243	-7.116
5.	**β-sitosterol**	-4.771	-4.499	-3.893	-3.781	-4.130	-4.381
6.	**Achyranthine**	-5.777	-5.421	-5.021	-5.755	-6.091	-8.360
7.	**Linalool**	-9.430	-0.622	-5.527	-2.730	-6.905	-6.880
8.	**Delphinidin**	-6.172	-5.886	-5.991	-6.364	-7.130	-7.558
9.	**Piperine**	-4.264	-4.138	-4.655	-6.451	-6.261	-3.821
10.	**Mangiferin**	-6.210	-5.360	-5.720	-5.210	-7.740	-3.520

2FOM-Dengue protease, 2P40-Methy transferase of Dengue, 3TRK-Chikungunya protease, 1ODY-HIV protease, 2RF2-HIV Reverse transcriptase, 1L7F-Neuramidase of Influenza

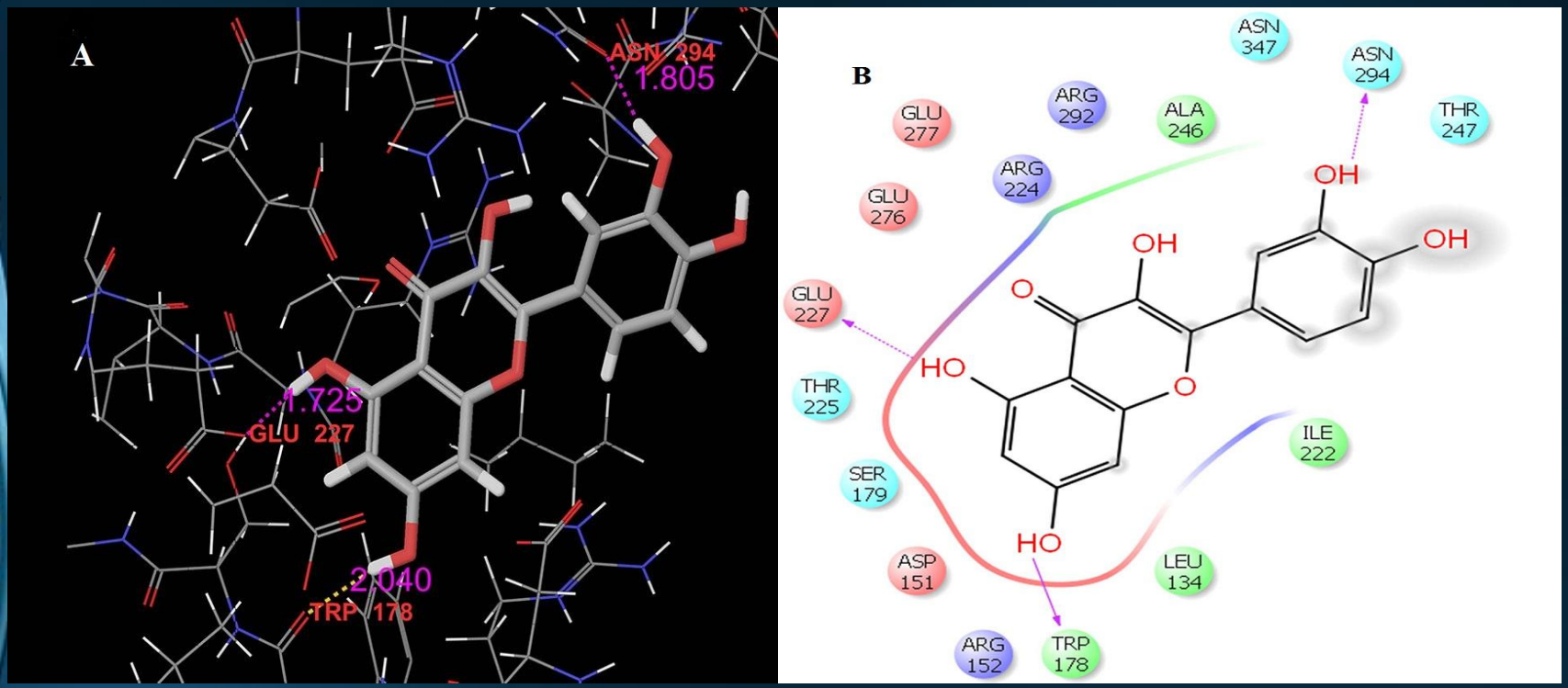
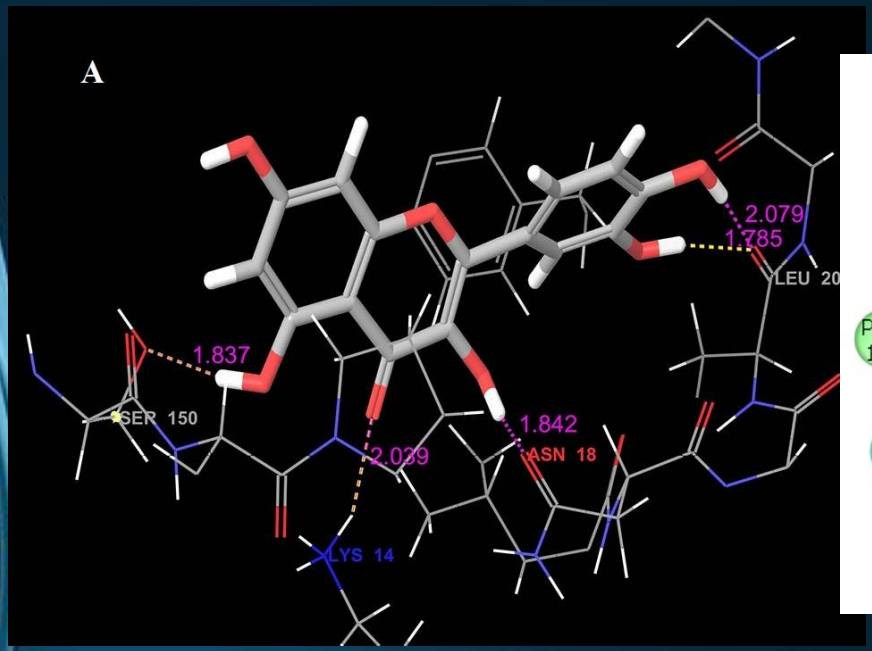


Fig. (1): Maestro: (A) Interaction of Quercetin with 1L7F with a docking score -7.742 interacting with Glu 227 (H-bond length 1.72 Å) Asn 294 (H-bond length 1.81 Å), Trp178 (H-bond length: 2.04 Å) (B) Interactions in ligand interaction viewer



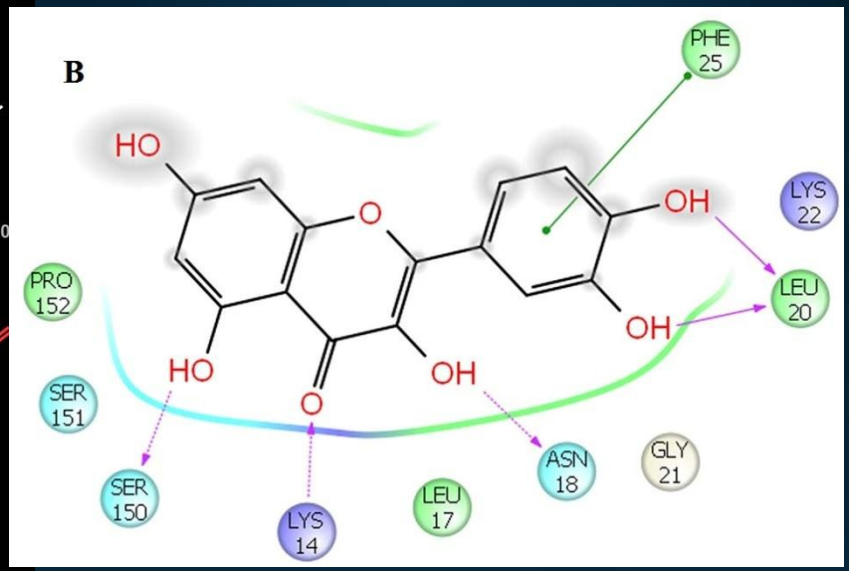


Fig. (2): Maestro: (A) Interaction of Quercetin with 2P40 interacting with docking score -6.36 interacting with Ser150 (H-bond length 1.84 Å), Lys14 (H-bond length 2.04 Å), Leu20 (H-bond lengths 2.08 Å, 1.79 Å), and Asn18 (H-bond length: 1.84 Å) (B) Interactions in ligand interaction viewer

Table 5: FlexX scores of HITs

Sl. No.	Chemical constituent	1L7F	2FOM	2P40	3TRK	1ODY	2RF2
1.	**Curcumin**	-22.870	-9.567	-19.068	ND	-33.973	ND
2.	**Gallic acid**	-39.539	-7.961	-25.987	ND	19.090	-14.954
3.	**Achyranthine**	-43.696	-6.543	-17.903	-6.312	-19.764	-10.988
4.	**Mallic acid**	-43.417	-4.598	-16.308	-0.511	-22.396	-12.338
5.	**Citric acid**	-46.410	-2.359	-17.156	2.398	-25.051	-10.473
6.	**Quercetin**	-22.455	-10.339	-19.899	ND	-24.165	-21.230
7.	**Cajanin**	-20.545	-12.245	-14.063	4.863	-23.821	-14.063
8.	**Kaemferol**	-20.468	-10.282	-17.644	ND	-25.031	-19.561
9.	**Myricetin**	-19.520	-9.556	-18.992	ND	-25.756	-19.052
10.	**β-sitosterol**	ND	-6.0841	-3.040	ND	ND	-21.130

ND- Not docked; 2FOM-Dengue protease, 2P40-Methy transferase of Dengue, 3TRK-Chikungunya protease, 1ODY-HIV protease, 2RF2-HIV Reverse transcriptase, 1L7F-Neuramidase of Influenza

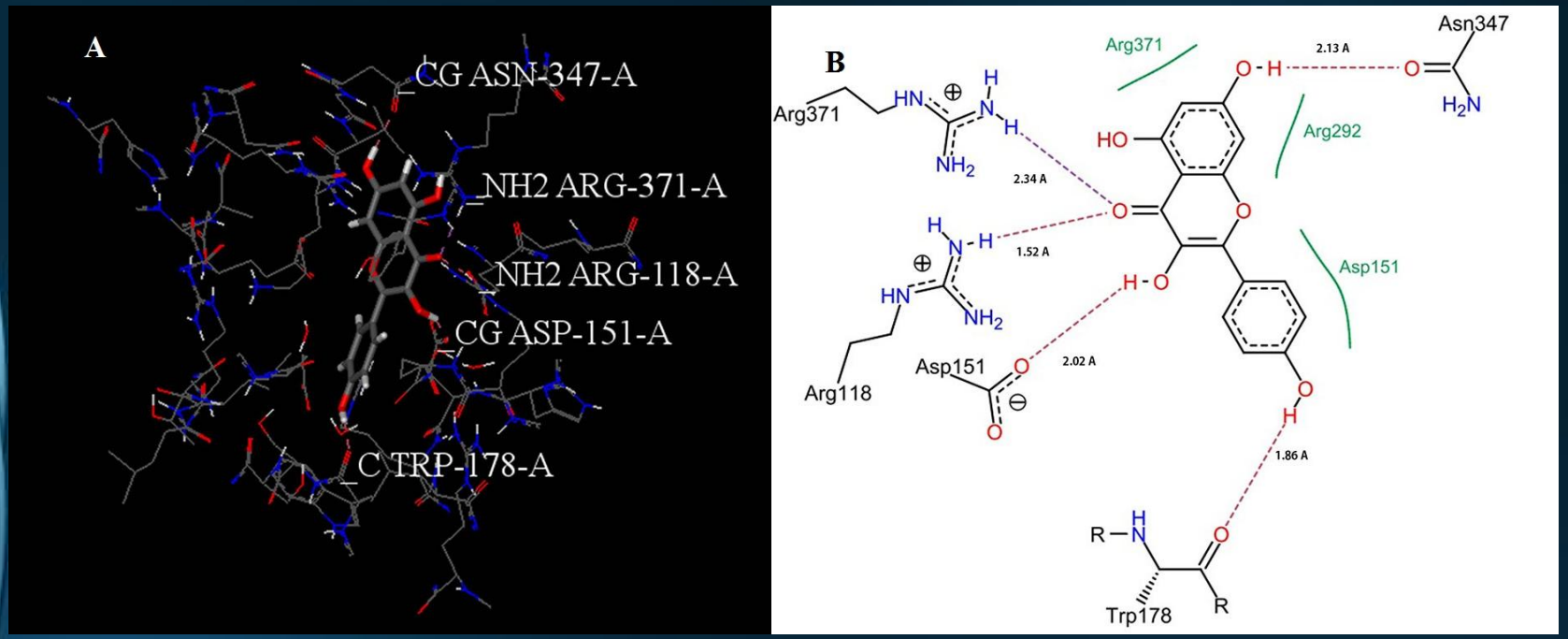


Fig. (3): Lead IT: (A)Interaction of Kaemferol with IL7F with a docking score of -20.468 interacting with Arg 371 (H-bond length 2.34 Å), Arg 118 (H-bond length 1.52 Å), Asp 151 (H-bond length 2.02 Å), Trp 178 (H-bond length 1.86 Å) and Asn 347 (H-bond length: 2.13 Å) (B) Display of interactions in pose viewer

Table 6: Autodock scores (Inhibition constant)

Sl. No.	Chemical constituent	1L7F	2FOM	2P40	3TRK	1ODY	2RF2
1.	**Kaemferol**	4.35µM	12.30µM	62.22µM	2.39µM	42.20µM	86.21µM
2.	**Myrecetin**	1.79µM	5.85µM	64.43µM	847.42nM	105.90µM	11.00µM
3.	**Quercetin**	2.83µM	6.64µM	124.80µM	2.68µM	47.67µM	74.45µM
4.	**Gallic acid**	100.10µM	176.80µM	23.86µM	377.00µM	4.10µM	180.50µM
5.	**Curcumin**	38.90µM	89.79µM	3.00mM	72.45µM	468.70µM	27.66µM
6.	**Achyranthine**	1.78mM	457.60µM	135.81µM	2.65mM	7.30mM	1.55mM
7.	**Mangiferin**	25.42µM	1.23µM	50.14µM	21.53µM	29.41µM	21.73µM
8.	**Shagoal**	125.95µM	87.30µM	311.45µM	40.51µM	1.50mM	52.84µM
9.	**Linalool**	333.43µM	779.80µM	1.79mM	413.90µM	4.67mM	577.75µM
10.	**Delphinidin**	9.78µM	31.49µM	27.19µM	26.33µM	210.74µM	220.74µM

2FOM-Dengue protease, 2P40-Methy transferase of Dengue, 3TRK-Chikungunya protease, 1ODY-HIV protease, 2RF2-HIV Reverse transcriptase, 1L7F-Neuramidase of Influenza

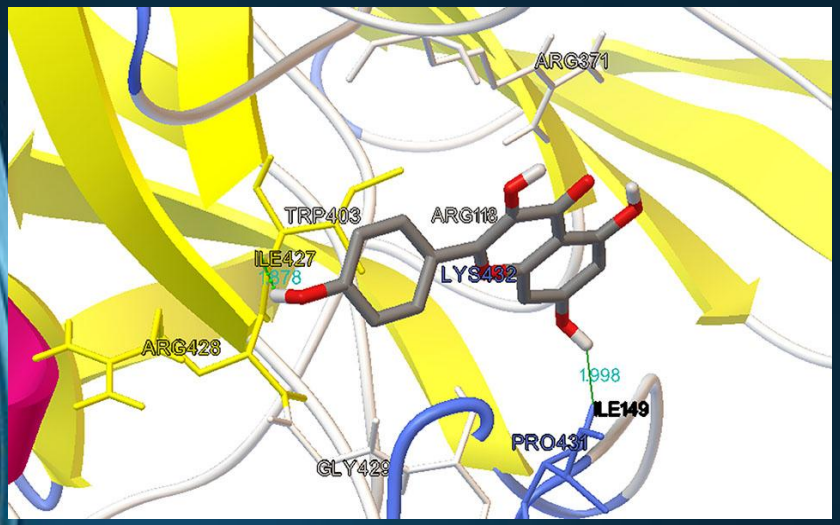


Fig. (4): Interaction of Kaemferol with 1L7F in Autodock with Ile 427 (H-bond length 1.88 Å) and Ile 149 (H-bond length 1.99 Å)

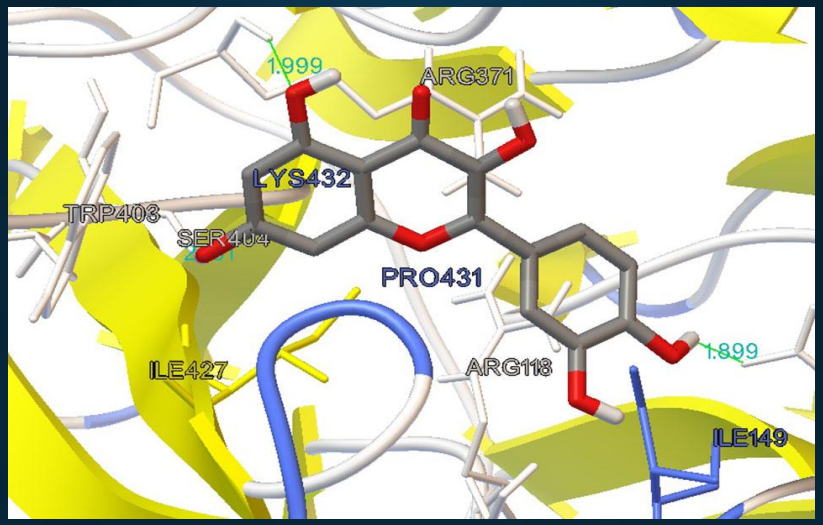
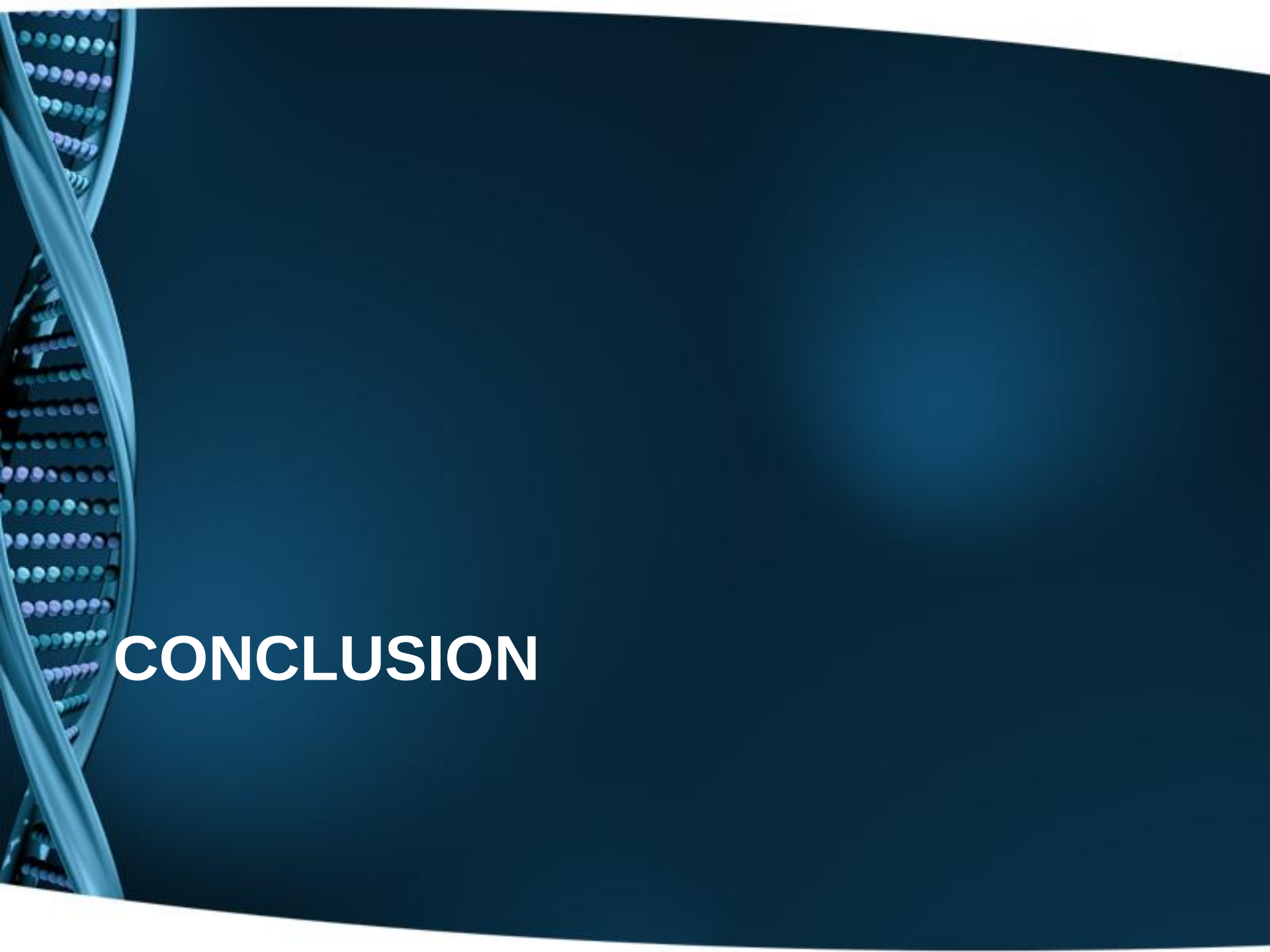


Fig. (5): Interaction of Quercetin with 1L7F in Autodock with Arg 371 (H-bond length 1.99 Å), Ser 404 (H-bond length 2.16 Å) and Asp 151 (H-bond length 1.89 Å)


CONCLUSION

Flavonoids are found to be good antiviral agents and *Euphorbia hirta*, which is rich in flavonoids is found to be a potential agent against HIV and dengue virus.

Other plants like *Cissus quadrangularis* and fruits of *Ficus religiosa* which are rich sources of flavonoids can also act as antiviral agents.

Curcumin, an important phytoconstituent of *Curcuma longa* has given good docking scores, also found to be a potential antiviral agent.

Gallic acid which is a main component of many plants have good docking interactions is also found to be potential antiviral agent.

Ocimum sanctum is another plant whose chemical constituents gave good docking scores, proved to have potential antiviral activity.

Chemical constituents from other plants like *Zingiber officinale* and *Achyranthes aspera* are also expected to have potential antiviral activity among the plants considered. By this attempt we can prove, use of computational chemistry is a reliable method for the better selection of plants against viruses.

In addition, flavonoids, curcumin, gallic acid, linalool and chemical constituents from *Zingiber officinale* and *Achyranthes aspera* can be used as natural leads based on docking studies and literature search. Their analogues and semi-synthetic derivatives can be synthesized to get effective antiviral agents in the future.


THANK YOU