



COMPUTER AIDED PERSPECTIVE OF SELECTION OF PLANTS AGAINST VIRUSES

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ABSTRACT

Discovery of drugs against viruses is always an attractive area of research. Plants are important natural source for discovery of drugs against viruses. Here in this article we targeted at two points. First the selection of a particular plant against viruses can be done more rationally by using the latest computer techniques i.e. docking studies of the known phytochemicals on various viral protein targets. Second with help of literature search and docking studies we identified some important natural compounds that can be used as natural leads. For this, we selected important phytoconstituents from 20 plants for docking studies using Maestro (Glide) and Lead IT (FlexX). Later those phytochemicals which got good docking scores were further docked in Autodock 4.2 in order to find out the estimated inhibition constant. Flavonoids, curcumin and other compounds gave good docking scores and better inhibition constant. These compounds can be used as natural leads and analogues and derivatives can be synthesized to get effective antiviral agents. In addition this approach gives a way to avoid random selection of plant for a particular activity, in order to save time, chemicals and effort of the medicinal chemist.

Keywords: Viruses, Docking, Phytochemicals, Maestro, Lead IT, Autodock.



INTRODUCTION

Plants having different biosynthetic pathways are great sources of natural compounds, which can be used for various therapeutic purposes.¹ More than 100 compounds acting as anticancer and anti-infective agents, at different stages of clinical development are derived from natural sources.²

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. This when coupled with natural products makes a medicinal chemist to explore more efficiently with less work.³ In this attempt we initially docked various phytochemicals present in different plants selected from ayurvedic literature. To our expectation, some natural compounds like curcumin, quercetin etc. which gave good docking scores was already reported as potential antiviral agents. In other case, the plants containing these HITs were found to have reported antiviral activities. This supports our method of using computational chemistry for better selection of plants against viruses. Further, we were successful in bringing out some of the major natural leads that can be used as antiviral agents.

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. 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. Analogues and derivatives can be synthesized to get effective antiviral agents. An attempt has also been made to compare docking studies with the co-crystallized molecules. A brief introduction on the life cycle of viruses selected is given in order to have a better understanding of various drug targets for the reader.

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.⁴

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 structural proteins include capsid protein, premembrane protein and envelope protein. 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.⁵

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 synthesizes two strands of RNA, one strand synthesizes the requirements of virus like reverse transcriptase, integrase and structural proteins etc. Other strand synthesizes genetic material of virus. Once the proteins are synthesized, they must be broken down into individual proteins and this can be achieved by protease enzyme. 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.⁶

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 proteinase activity, NSP3 has replication property and NSP4 has polymerase activity. These proteins together form a viral replication complex which synthesizes a negative RNA strand intermediate and this acts as a template for synthesis of genomic (49S) and subgenomic (26S) RNA. Subgenomic RNA carries out translation and drives expression of C-pE2-6K-E1 which on processing by autoprotease and signalase release Capsid (C), pE2 and E1 glycoprotein. Binding of viral nucleocapsid and viral RNA promotes viral assembly which leads to budding at cell membrane, which transforms into mature virions.⁷

MATERIALS AND METHODS OF VIRTUAL SCREENING

The following proteins (Table 1) used as drug targets were downloaded from Protein Data Bank.

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)

The chemical constituents from the following plants (Table 2) were used for docking studies.



Table 2: Different chemical constituents from plants docked

Sl. No.	Plant	Chemical constituents docked
1	<i>Curcuma longa</i> ⁸	Curcumin (1), demethoxycurcumin (2), β -phellandrene (3), p-cymene (4), α -turmerone (5)
2	<i>Ficus religiosa</i> ⁹	Kaempferol (6), myricetin (7), quercetin (8), methyl oleanolate (9), β -sitosterol (10), stigmasterol (11), lanosterol (12)
3	<i>Cuminum cyminum</i> 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	<i>Punica granatum</i> ¹¹	Citric acid (22), mallic acid (23), gallic acid (24), catechin (25), quercetin (8), α -tocopherol (26), linoleic acid (27), oleic acid (28), β -sitosterol (10)
5	<i>Oroxylum indicum</i> ¹²	Baicalein (29), oroxyline (30), pinostrobin (31), stigmast-7-en-3-ol (32)
6	<i>Mangifera indica</i> ¹³	Mangiferin (33), protocatechuic acid (34), catechin (25), shikimic acid (35), mangostin (36), gallic acid (24), ethyl gallate (37)
7	<i>Achyranthes aspera</i> ¹⁴	D-Glucuronic acid (38), oleanolic acid (39), achyranthine (40), ecdysterone (41)
8	<i>Barleria prionitis</i> ¹⁵	Barlerinoside (42), barlerin (43)
9	<i>Terminalia chebula</i> ¹⁶	gallic acid (24), chebulanin (44), chebulinic acid (45)
10	<i>Pterocarpus marsupium</i> ¹⁷	kinotannic acid (46), pterocarpol (47), liquiritigenin (48), gallic acid (24)
11	<i>Cajanus cajan</i> ¹⁸	Cajanin (49), pinostrobin (50), longistylin A (51), cajanuslactone (52), vitexin (53)
12	<i>Acacia nilotica</i> ¹⁹	Gallic acid (24), Apigenin (54), protocatechuic acid (34), rutin (55)
13	<i>Zingiber officinale</i> ²⁰	Curcumene (56), fernesene (57), gingerene (58)
14	<i>Piper longum</i> ²¹	Piperine (59), asarinine (60), sesamin (61), caryophyllene (62), gingerene (63), p-cymene (4)
15	<i>Euphorbia hirta</i> ²²	Quercetin (8), myricetin (7), rutin (55), kaempferol (6), gallic acid (24), protocatechuic acid (34)
16	<i>Cissus quadrangularis</i> ²³	Ascorbic acid (64), β -sitosterol (10), quercetin (8), amyirin (65)
17	<i>Ocimum sanctum</i> ²⁴	Eugenol (66), ursolic acid (67), carvacrol (68), linalool (21), caryophyllene (62), estragole (69)
18	<i>Tabernaemontana divaricata</i> ²⁵	Conophylline (70), Dregarnine (71), tabermontanine (72)
19	<i>Hibiscus sabdariffa</i> ²⁶	Hibiscitrin (73), β -sitosterol (10), citric acid (22), delphinidin-3-glucoside (74), protocatechuic acid (34), quercetin (8)
20	<i>Allium sativum</i> 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 with the following parameters. Runs: 50, population size: 150, number of evaluations: 2500000 and generations: 27000.

RESULTS AND DISCUSSION

Flavonoids gave good docking scores and inhibition constant values. Apart from flavonoids, gallic acid gave good docking score against Dengue, Chikungunya and HIV proteins. The docking scores of HITs were tabulated (Table 3 & 4). The inhibition constants of HITs were estimated using Autodock 4.2 and was tabulated (Table 5), following Maestro and FlexX docking scores.

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	37.83 μ M
Reverse transcriptase of HIV (2RF2)	-8.206	ND	ND
Neuramidase of influenza (1L7F)	-8.288	-28.635	149.05 μ M

*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-Methyl transferase of Dengue, 3TRK-Chikungunya protease, 1ODY-HIV protease, 2RF2-HIV Reverse transcriptase, 1L7F-Neuramidase of Influenza

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.	Cajaniin	-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-Methyl transferase of Dengue, 3TRK-Chikungunya protease, 1ODY-HIV protease, 2RF2-HIV Reverse transcriptase, 1L7F-Neuramidase of Influenza

Table 6: Autodock scores (Inhibition constant)

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

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

Quercetin and Kaemferol, which are flavonoids, gave good docking scores against most of the viral proteins. In Maestro, quercetin interacted well with Neuramidase of Influenza (1L7F) and it gave a docking score of -7.742. It made interactions with three residues. With Glu 227, the hydrogen bond length was 1.72 Å, with Asn 294, it is 1.81 Å and with Trp 178, it is 2.04 Å (Fig. 1). The same compound was docked with methyl transferase of dengue (2P40) in Maestro gave docking score of 6.36. It made four interactions, Ser150 having H-bond length 1.84 Å, Lys14 having H-bond length 2.04 Å, Leu20 having H-bond lengths 2.08 Å, 1.79 Å, and Asn18 having H-bond length 1.84 Å (Fig. 2). In Lead IT, kaemferol made five interactions with 1L7F giving docking score of -20.468. The hydrogen bonding lengths were 2.34 Å with Arg 371, 1.52 Å with Arg 118, 2.02 Å with Asp 151, 1.86 Å with Trp 178 and 2.13 Å with Asn 347 (Fig. 3). In Autodock, Kaemferol made two interactions with 1L7F, one with Ile 427 having H-bond length 1.88 Å and other with Ile 149 having H-bond length 1.99 Å, The estimated inhibition constant value was found to be 4.35 μ M (Fig. 4). Quercetin made three interactions with 1L7F in Autodock, with Arg 371 having H-bond length 1.99 Å, with Ser 404 having H-bond length 2.16Å and with Asp 151 having H-bond length 1.99 Å. The estimated inhibition constant was found to be 2.83 μ M.

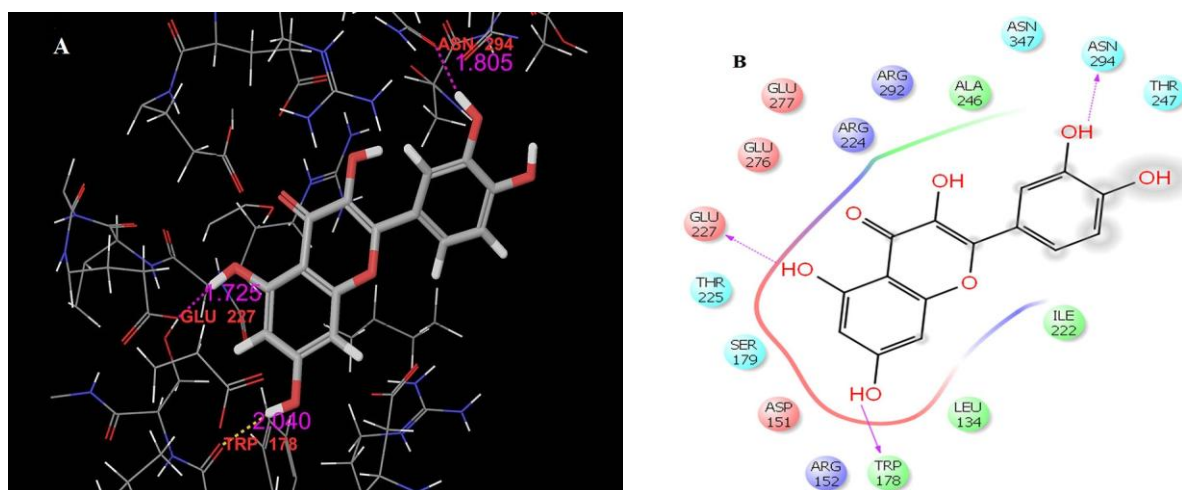


Fig. (1): Maestro: (A) Interaction of Quercetin with 1L7F with a docking score -7.742 interacting with Glu 227 (H-bond length 1.72 Å) Asn 294 (H-bond length 1.81 Å), Trp178 (H-bond length: 2.04 Å) (B) Interactions in ligand interaction viewer

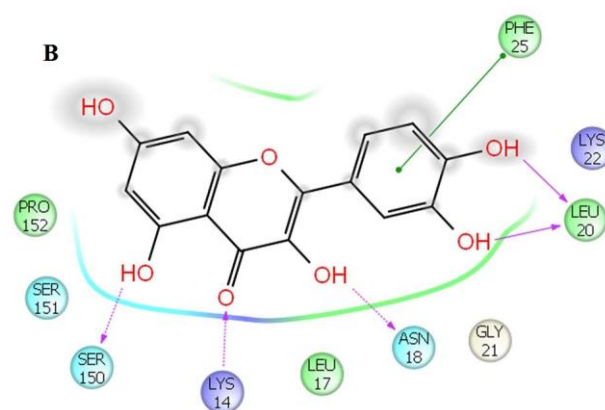
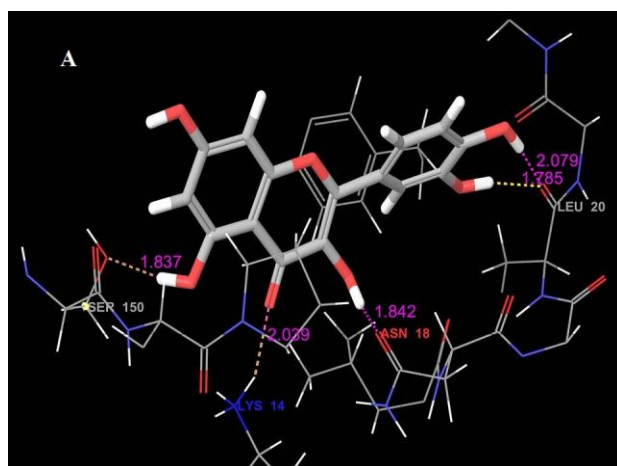


Fig. (2): Maestro: (A) Interaction of Quercetin with 2P40 interacting with docking score -6.36 interacting with Ser150 (H-bond length 1.84 Å), Lys14 (H-bond length 2.04 Å), Leu20 (H-bond lengths 2.08 Å, 1.79 Å), and Asn18 (H-bond length: 1.84 Å) (B) Interactions in ligand interaction viewer

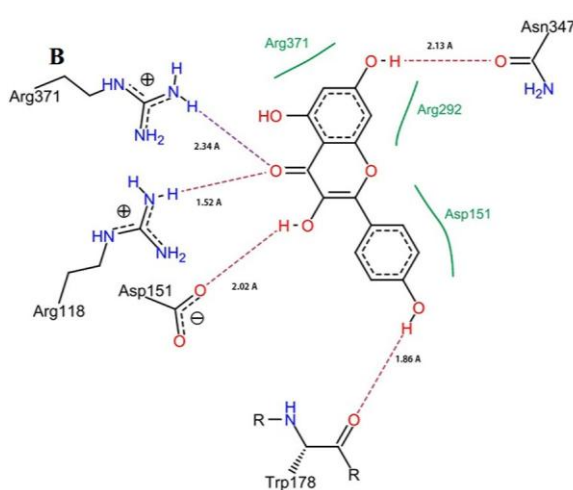
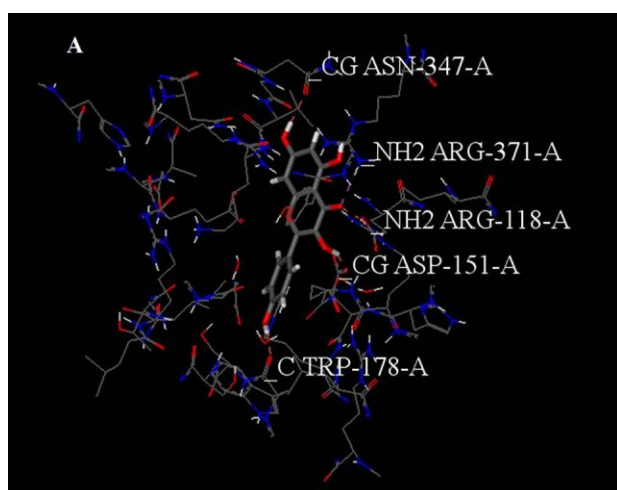


Fig. (3): Lead IT: (A) Interaction of Kaemferol with 1L7F with a docking score of -20.468 interacting with Arg 371 (H-bond length 2.34 Å), Arg 118 (H-bond length 1.52 Å), Asp 151 (H-bond length 2.02 Å), Trp 178 (H-bond length 1.86 Å) and Asn 347 (H-bond length: 2.13 Å) (B) Display of interactions in pose viewer

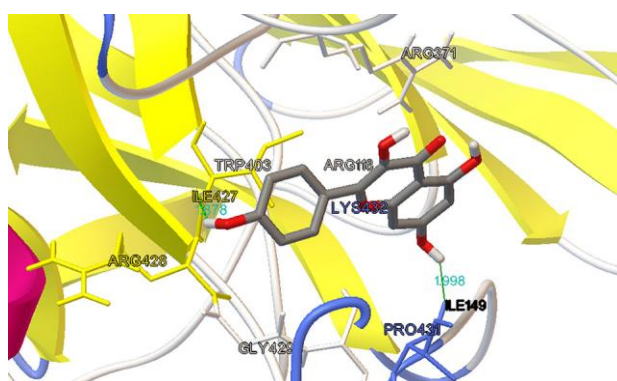


Fig. (4): Interaction of Kaemferol with 1L7F in Autodock with Ile 427 (H-bond length 1.88 Å) and Ile 149 (H-bond length 1.99 Å)

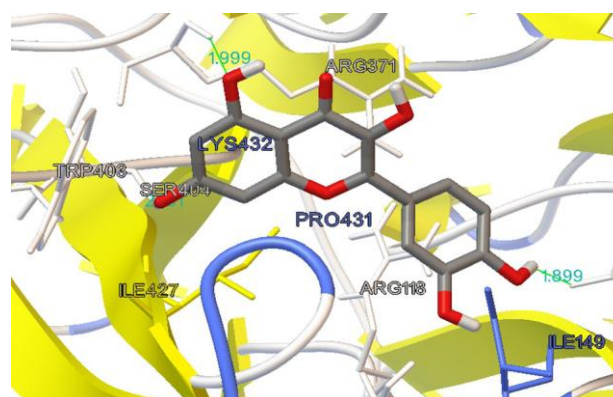


Fig. (5): Interaction of Quercetin with 1L7F in Autodock with Arg 371 (H-bond length 1.99 Å), Ser 404 (H-bond length 2.16 Å) and Asp 151 (H-bond length 1.89 Å)



Flavonoids gave excellent docking scores and inhibition constant values. Apart from flavonoids, gallic acid gave good docking score values against Dengue, Chikungunya and HIV proteins.

Chemical constituents of *Euphorbia hirta* gave good docking scores on dengue, HIV and influenza proteins. Agnes gyuris *et al.* (2009) has established anti-HIV properties of *Euphorbia hirta*. They found that the aqueous and methanol extracts containing the polyphenolic compounds were found to have anti-HIV properties and non-polar extracts were found to be inactive.²⁸ In another study it was found that flavonoids act against HIV by acting on reverse transcriptase and integrase enzymes.²⁹ Docking studies on these proteins found to be good. Another study indicated quercetin to be a potential agent against dengue virus and *Euphorbia hirta* was found to be effective against dengue virus.^{30, 31} *Ficus religiosa* fruits which are rich in flavonoids are believed to be good source of antiviral agents.

Curcumin, an active ingredient in *Curcuma longa* is proved to have potential antiviral properties against HIV.³² The same compound gave good docking scores against HIV proteins like reverse transcriptase and protease. Gingerols and shagoals are the active constituents of *Zingiber officinale* gave good docking scores on proteins selected. The same plant was proved to be potential agent against influenza.³³

Pundaleekappa (2012) has discovered anti-HIV properties of *Ocimum sanctum*. They found linalool as one of the component of extract having the anti-HIV properties.³⁴ Mangiferin and gallic acid are the main components of *Mangifera indica*, gave good docking studies on almost all the viruses. Mangiferin is reported to antagonize cytopathic effects of HIV gave good docking studies against HIV protease. The same compound was reported to have potential activity against HIV protease.³⁵ In another study gallic acid which gave excellent docking scores on HIV reverse transcriptase and found to have inhibitory activity on the same.³⁶

Dengue and chikungunya are viruses of contemporary time and based on the above studies, docking protocol can help for rationale selection of natural compounds and plants against these viruses.

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.

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