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Investigating the Effects of Columbin on Yellow Fever: A Computational Approach

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ABSTRACT: Infectious diseases are always a burden to the Global Health. The disease called Yellow Fever (YF) has re-emerged and caused a major loss to human and economy in many countries like Brazil and Africa. Many countries are also on the verge of having this disease in their countries. Due to scarcity and less accessibility of vaccine against this disease it is becoming hard to stop this spread. Naturally occurring Columbin in Tinospora Cordifolia is suggested to be the potent inhibitor of many viral disease. Current research discusses the in-silico studies done with Columbin on Envelope protein of Yellow Fever Virus (YFV). It has been reported that Columbin has the potential of inhibition of YFV. Further research will aid the in-vitro studies and will save time in discovering a drug against Yellow fever.

KEYWORDS: AutoDock, Binding energy, Columbin, Tinospora Cordifolia, Yellow fever Virus (YFV), Ribonucleic Acid (RNA).

INTRODUCTION

Yellow fever (YF) is triggered by positive-strand RNA viruses named as Yellow Fever Virus (YFV). This virus is a member of *Flaviviridae* family of viruses, transmitted to a healthy person from an infected person by the help of a mosquito breed named as *Aedes aegypti* Yellow fever is responsible for a major loss to human life in 18th century and start of 20th century. This disease is a global health burden and has re-emerged again with significant morbidity and mortality rate. Although an effective vaccine is available but still an estimate of 30,000 death and 200,000 cases are noted every year. This failure is primarily due to the poverty, inaccessibility of vaccine to rural areas, war between countries. And this has led to a situation of emergency in many countries [1].

Elements required in an area for YF

There are some elements that are necessary for the transmission of this disease from one place to another. Firstly and mainly a source of virus which can be an infected person or infected non-human primates. A competent transmitting agent is a competent mosquito in an area. The transmitting agent should be accessible to the source of virus. Other environment factors like rainfall, temperature, humidity should favour the mosquito to allow the transmission and lastly a non-immune normal human that will be infected by the bite of the transmitting agent [1][2].

Transmission of Virus

Virus can be transferred to a normal non-immune person when a mosquito takes a blood meal from an infected human and transmits the virus via saliva during feeding on another normal human. Virus usually is transmitted from the viremic human or host to the transmitting agent that is mosquito and then this mosquito infects the animal or human as shown in figure 1. There is one more mechanism is that when a female *Aedes aegypti* lays infected eggs.

The mosquitoes that are developed from these eggs may carry the YF virus. The eggs of these mosquitoes are desiccation resistant, they are not degraded in dry conditions and when they receive rain they can yield a viable mosquito with inherited virus [3].

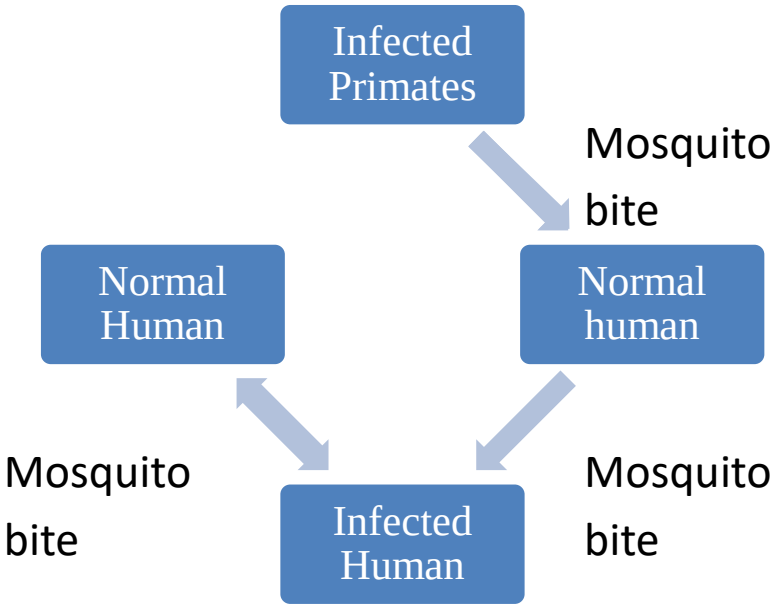

Figure 1: Transmission of Yellow fever

Structure of Yellow fever Virus

YFV is a single stranded Ribonucleic acid (ssRNA) genome with an open reading frame (ORF) of 10233 base pairs. This ORF codes for 3 structural proteins Envelope (E), pre Membrane (pM), and Capsid (C)) along with seven non-structural proteins (NS5, NS4b, NS4a, NS3, NS2b, NS2a, NS1) are shown in Fig. 2. Protein E, because of its high potential for anti-viral, is the most studied one. It has been reported that E protein in some major events like fusion, penetration, binding site for viral attachment, cell tropism and hemagglutination [4].

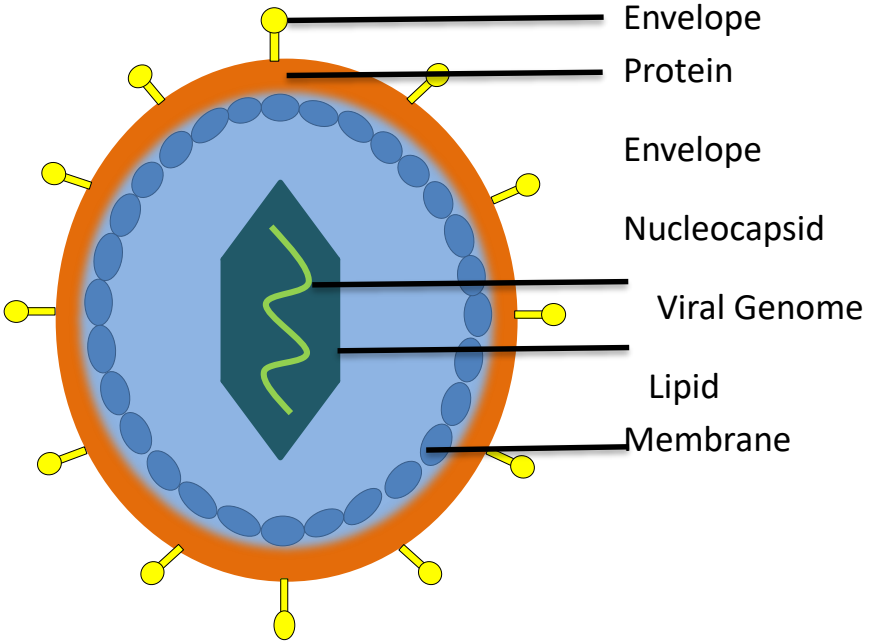

Figure 2: Structure of yellow fever virus

Several researches have been carried out to explore the approaches that have the potential and can be the promising anti-viral against yellow fever virus. Chemical compounds that the potential to interfere at virus replication or inhibiting the host pathway of viral entry or specific pathway that are necessary for virus replication, compounds that show less toxicity to host and have the potential to inhibit the virus. Some researches discusses the strategies that aims at modulating the host immune response against a specific disease by forming antibodies, or using immuno modulators and corticosteroids. Some of these researches have shown promising results but none of them have been approved for the use. Some studies require more research or they are still under trial.

Natural phyto-compounds are a major source to be searched as it consists of many potent phyto-compounds that can develop anti-viral drugs. Many plant products are used traditionally to treat many disease, these plants can be selected on their ethno-medicinal use. Several studies have shown that *Tinospora Cordifolia* or Giloy has been used to treat many viral diseases like malaria. It has been reported that it acts as an immuno booster and have a major amount of medicinal properties. Nonetheless, a very little knowledge is reported about the Columbin, major component of *Tinospora Cordifolia*, against viral disease. To our knowledge Columbin can be a potent phyto-compound to overcome this disease [5].

LITERATURE REVIEW

Meneses, Rocío, *et al*. conducted in-vitro study to report the effects of essential oil of *Lippia alba, Lippiaoriganoides, Artemisia vulgaris* and *Oreganum vulgare* on the viral replication mechanism of yellow fever virus. They performed CC_{50} (Cytotoxicity) analysis using 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) reduction method. In this research they found out that selected essential oil were having significant effects on the virus directly. They stated that virus inactivation can be due to disturbance in the viral membrane. These disturbances can be due to liphophilic compounds, but still the proper and perfect mechanism was not discussed [6]. Another study by Cheng, Sen-Sung, *et al*. described the effects of 14 essential oils from 5 different plants. They conducted Brine shrimp lethality test on Shrimp's larvae and Mosquito Larvicidal test on larvae of *A. Aegypti*. They used various plant essential oils to control the growth and minimize the growth of larvae of *A. Aegypti*. They concluded in their research that essential oils of *C. Japonica* is the potential essential oil that should be used to minimize the growth of *A. Aegypti* larvae [7]. There is little knowledge in the literature about the use of phyto-chemicals against the yellow fever. Researches are performed to stop the growth of their larvae of transmitting agent or producing a vaccine against it.Yet no drug is available to treat this disease in those people who didn't had vaccine against YFV or are non-immune. Current research will be discussing the in-silico studies performed with Columbin on the Yellow fever.

RESEARCH QUESTION

What is the effect of naturally occurring Columbin on Yellow fever Virus?

METHODOLOGY

Design

As shown in figure 3, files with the three dimensional conformations of protein and ligand were downloaded from the online web servers. AutoDock is a freely accessible software that is used to perform the docking of Columbin (Ligand) on the E protein of YFV (Receptor). During the docking various types of files are formed like .pdbqt, .glg and .dlg file. DLG file type is the docked

models files which is further visualized with the help of BOVIA drug discovery studio and aster visualisation distance between bonds and binding energy is calculated.

Binding energy is the energy that is required to remove a particle from a system or a system from a particle. The negative value of binding energy is the indicator of the stability of a complex. The more the negative value depicts that the complex formed between ligand and receptor is more stable. Binding energy is the sum of the inter-molecular interactions amongst protein and ligand and internal steric energy of ligand.

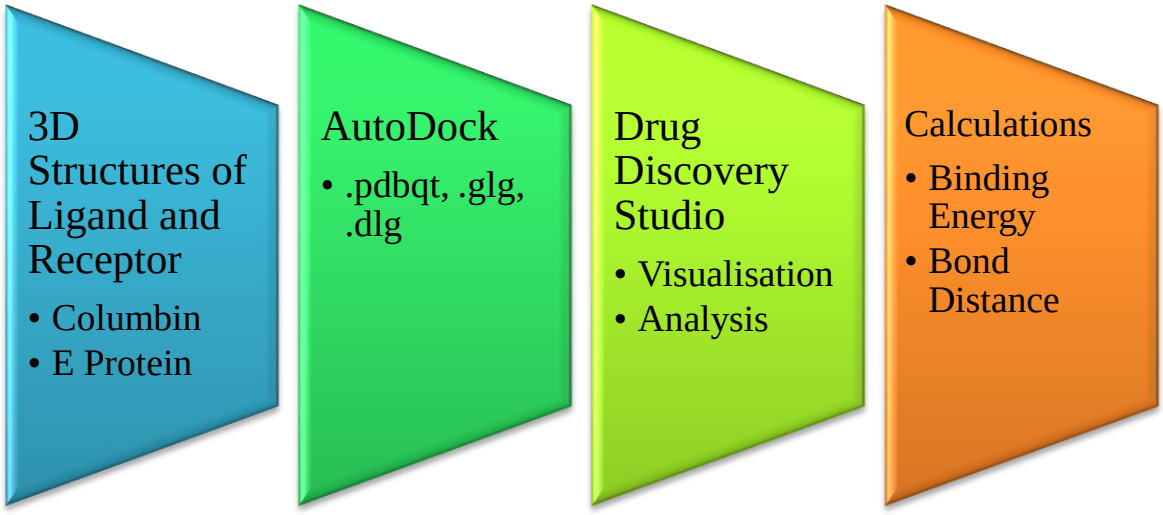

Figure 3: Flow Chart representing the steps that were followed

Sample

Files containing the 3D structure of ligand and receptor were downloaded from PubChem and protein data bank respectively. Fig. 4 is representing the ball and stick model of Columbin, Green balls represents the carbon, red represents the oxygen and white ball represents the hydrogen molecule. Single stick between the balls is for single bond while the double stick represents the double bond. In figure 5, Quaternary structure of protein is shown where red spirals are representing the α-helices and the cyan coloured ribbons are the β-plated sheets of the Envelope protein YFV.

Instrument

Public Chemistry (PubChem) is accessed to download the 3D structure of Columbin in SDF format. PubChem is a freely accessible database of chemical information server which is launched by National Institute of Health (NIH) in 2004. Anyone can access this information by the means of a computer and an internet, you can upload the chemical information and it can be accessed by anyone worldwide. It consists of chemical information like Molecular structure, their patents and toxicity information and chemical properties. This chemical information is very useful to many researches in finding out many important drugs.

3D structure of Envelope protein YFV was downloaded from Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (PDB) in PDB format. It is an open database led by Helen M. Berman. RCSBPDB, here all the information about the different viral, bacterial and any other protein information can be found and can be downloaded. This information is used by many researchers to complete their research and further help the world. Open Babel is an easily

accessible tool that coverts the different chemical molecules file formats to another format. It is used to convert the SDF format of Columbin 3D structure file to PDB format.

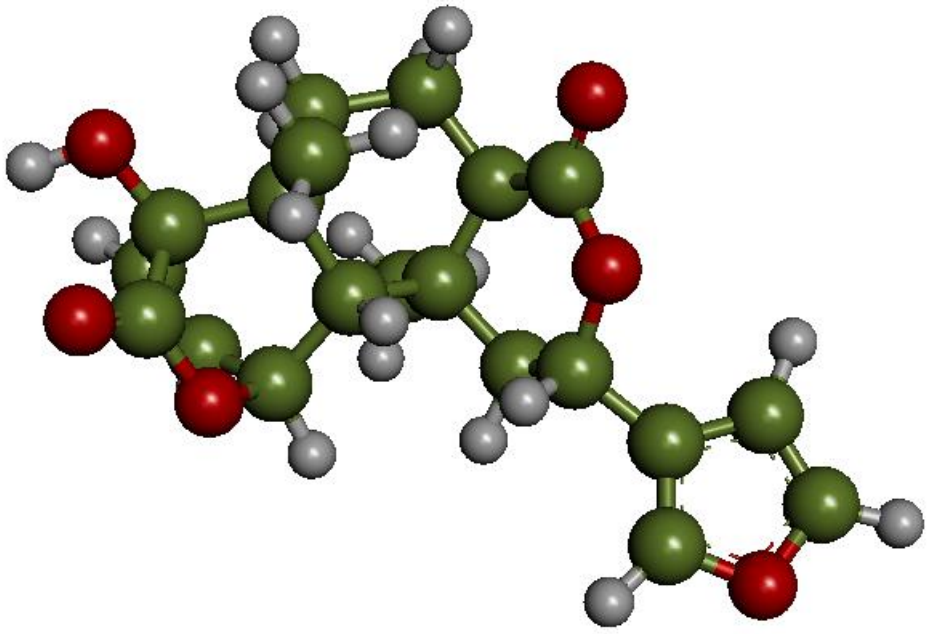

Figure 4: Ball and Stick model of Columbin

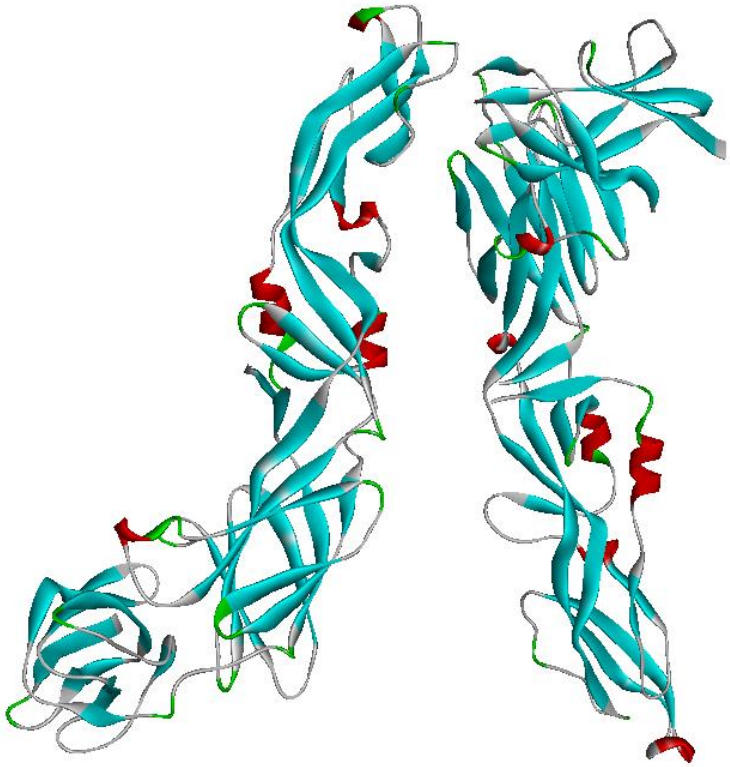

Figure 5: Quaternary structure of E protein YFV (6IW4)

These structure are further used for the molecular docking experiment using AutoDock4. It is an open and easily accessible tool that is used to calculate how one small molecule will bind or how

will it interact with the receptor of a known structure in 3D. Apart from docking it also calculates the binding affinity that helps in finding a better binding molecule to a receptor. After retrieving the docked complex, it was visualised in Drug Discovery Studio. Distance between bonds, type of bonds formed were calculated and binding energy were calculated. BOVIA Drug Discovery Studio is used to visualise the bonds and distance between the formed bonds. It is a molecular modelling suite that has various features that helps in molecular modelling and simulation.

Data Collection

After forming the DLG file type with the help of AutoDock, different conformations were analysed and based in the different binding energies model with the least binding energy was selected and it was visualised using AutoDock and BOVIA Drug Discovery Studio as shown in Fig. 6. For better understanding 3D structure of docked complex was visualised and distance between bonds, type of bonds formed as shown in figure 7 was recorded.

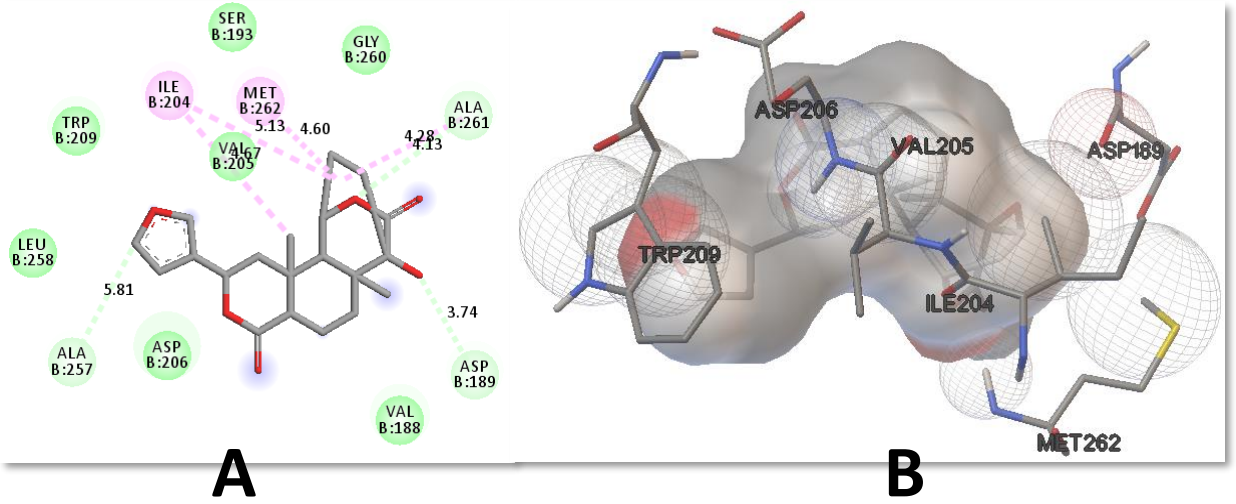

Fig. 6 A: 2D Representation of Docked complex showing bonds formed and distance between bonds in BOVIA Drug Discovery Studio; B: 3D Representation of Docked complex showing bonds formed in AutoDock.

Data Analysis

Collected data was analysed using BOVIA Drug Discovery Studio and it was recorded that Columbin or ligand (UNL1) was forming seven bonds with the Envelope protein. ILE204, MET262, ILE204 and ALA261 formed hydrophobic bonds with the ligand by distance between these bonds was 4.9, 4.4, 4.8 and 5.2 respectively whereas three hydrogen bonds with ALA257, ALA261 and ASP189 were formed with the distance of 3.6, 3.5 and 3.5 respectively as mentioned in Table 1.

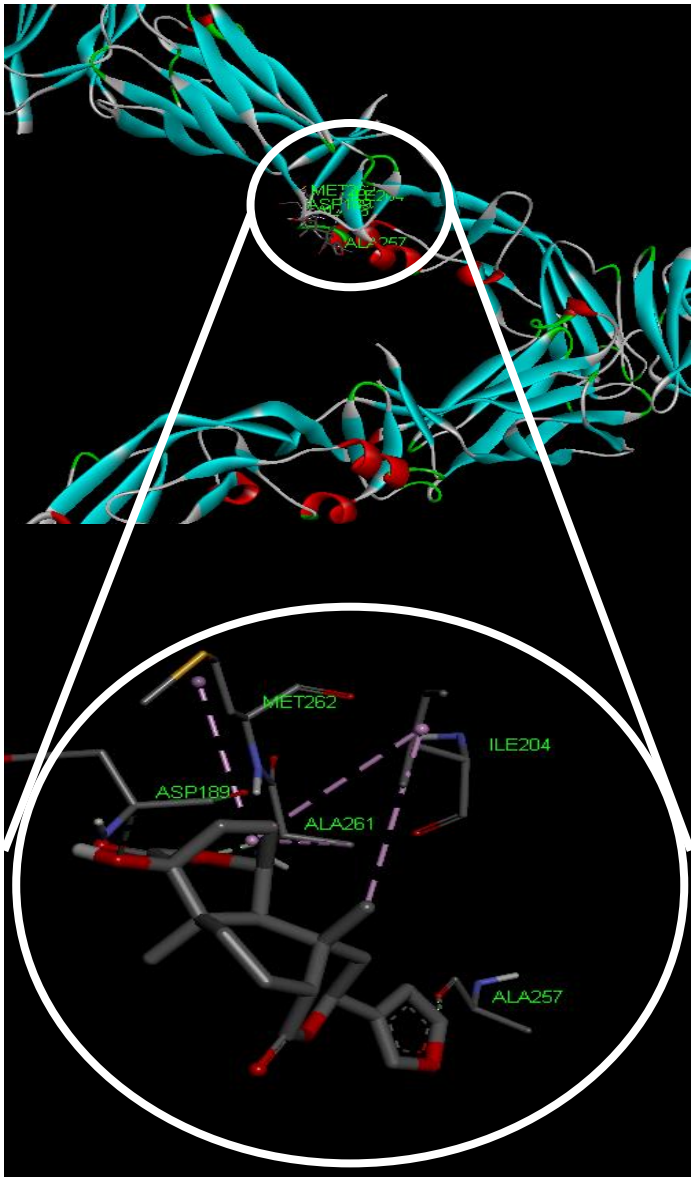

Figure 7: Docked complex of Columbin and E-Protein

Table 1: Table showing the distance and type of bonds between the Ligand:Receptor

Sl. No.	Ligand:Receptor	Distance	Type of Bond
1.	UNL1 : ILE204	4.93564	Hydrophobic
2.	UNL1 : MET262	4.47074	Hydrophobic
3.	UNL1 : ILE204	4.80609	Hydrophobic
4.	UNL1 : ALA261	5.2001	Hydrophobic
5.	UNL1 : ALA257	3.69476	Hydrogen Bond
6.	UNL1 : ALA261	3.59803	Hydrogen Bond
7.	UNL1 : ASP189	3.54018	Hydrogen Bond

RESULT AND DISCUSSION

Binding energy of a compound toward its target is used as the measurable quantity in designing a drug, the negativity of binding energy determines the stability of Ligand-receptor complex. More negative the binding energy means the more stability of the complex. Binding energy is the total of Inter-molecular Energy, Torsional Free Energy and Total Internal Energy while Unbound System's Energy is being subtracted from the total. Unbound Systems' Energy was-0.90kcal/mol, Torsional Free Energy was +0.60 kcal per mol, Total Internal Energy was -0.90 kcal per mol and Intermolecular Energy was -7.02 kcal per mol,. After performing the calculations it was noted that the binding energy was -6.43 kcal/mol.

CONCLUSION

Yellow fever virus has re-emerged as a deadly disease and due to less accessibility and availability of vaccine people are dying at a rapid rate. People who are non-immune to this disease die within 7-10 days of infection. Its deadliness has increased an urgent demand of drug for non-immune people. Current research was performed to find a potent phyto-compound that can be used to target the specific protein to stop the viral entry in the host cell. Columbin is used to target the Envelope protein of Yellow fever virus and it was found out that they were binding with a binding energy of -6.43kcal/mol. This suggests that Columbin have the potential to inhibit the viral entry into the host cell. Further in-vitro studies are required to gain knowledge about the toxicity and other parameters before the use of Columbin formed drug in clinical trials.

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