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ANALYSIS OF MELIACEAE DERIVED PHYTOCHEMICALS TARGETING RNA-DEPENDENT RNA-POLYMERASE OF FLAVIVIRIDS THROUGH COMPUTER AIDED PROTOCOLS

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Abstract

Flavivirids are single stranded, positive-sense RNA viruses that belong to family *Flaviviridae*. Being responsible for ever evolving diseases and various health problems, flavivirids are big threat to world's health. Plant secondary metabolites as drug candidates may interfere flavivirids' replication. This study is an effort to computationally analyze the phytochemicals from the family Meliaceae targeting RNA-dependent RNA-polymerase protein of the type species from each of the four genus of the family *Flaviviridae.* Selection and screening through ADMET analysis, modeling and validation of targeted proteins, molecular docking, and the DFT analysis were carried out. Significant number of phytochemicals were determined as potential inhibitors of the targeted proteins. However, only four phytochemicals – *3-beta-hydroxystigmast-5-en-7-one*, *Kulinone*, *Odoratone*, and *Quercetin-3-O-L-Rhamnoside* – were selected for further analysis being promising candidate drugs. This study not only infer the phytochemicals from family Meliaceae as potential drug inhibitors, it may also expedite a way to further validation.

Keywords: CADD, Dengue virus, Hepacivirus, *In silico* analysis, NS5B

Introduction

Three recurring elements such as environmental changes, human conflicts and infectious diseases have contributed to shape the human development. In this regard, viruses are the most important agents, although they require host cell for survival and multiplication yet they recognize and attach to the suitable host through genetic material and proteins present in their outer surface. After which they multiply by deceiving the healthy cell to divide the viral nucleic acid and in turn kill or alter the function of host cells, which affects the host health through infectious diseases. (Coffey, 2017; Smith, 2007; Villarreal, 2005). Till date, various viruses has been discovered and classified into different families. The viruses with icosahedral, monopartite and enveloped virions encapsulating single-stranded (ss) positive-sense (+) RNA genome are classified within four genera of family *Flaviviridae*. The classification of the family into four genera is based upon differences ranging from host to the mode of transmission (Monath, 1987).

Flaviviridae is consisted of large number of viruses, *i.e.*, the genus flavivirus is consisted of more than 90 viruses. Most viruses belonging to this genus are arthropod-borne human pathogens that cause diseases ranging from mild fevers to life threatening hemorrhagic fevers and hepatic necrosis. *Yellow fever virus* is the worldwide and most leading cause of hemorrhagic fever and related mortalities (Monath, 1987). The flavivirus is known to spread through *Aedes* and *Haemogogus* insect species that acquire it from monkeys and after getting infected, transmit it to humans. (Payne, 2017). *Pestivirus* is another genus containing veterinary important pathogens responsible infecting major economically important life stock and large range of conditions such as acute hemorrhagic syndrome, acute diarrhea and wasting syndrome (Smith *et al*, 2017; Tautz *et al*, 2015). Moreover, *Pestivirus* A (designated as *Bovine* viral diarrhea virus 1) is one of the most important causes of mortality and morbidity in dairy and beef cattle. This acute and epizootic infection is highly abortifacient in goats defense (Smith *et al*, 2017; Tautz *et al*, 2015). On the other hand, genus *Hepacivirus* contains multiple species including *Hepacivirus hominis* of which hepatitis C virus (HCV) is the most widespread virus responsible for acute and cirrhotic liver diseases. (Drexler *et al.*, 2013; Firth *et al*., 2016; Hartlage, 2016; Scheel *et al*., 2015). Genus *Pegivirus* is the recently established genus which contains number of viruses associated with the human and mammalian diseases. It's been said that the members of this genus results in verimia but still there is no evidence that can describe their involvement in such diseases. The genus *Pegivirus* has species including *Pegivirus hominis* of which hepatitis G virus/GB virus type C are noted isolates first discovered in GB agent-infected tamarin and human (Neyts, 1999; Reisen, 2017; Simmonds *et al*, 2017).

Flavivirids encode three structural proteins: capsid protein (C), pre membrane or membrane (prM and M), envelop protein (E) and at least 7 non-structural (NS) proteins. The non-structural proteins such as RNA-dependent RNA-polymerase (RdRp), helicase and protease are generally involved in genome replication as well as immune responses (Blitvich and Firth, 2017; Payne, 2017). Potential strategies for antiviral therapy can be undertaken by interference in the steps of the replication cycle and activities of various enzymes involved in the proliferation of viruses. However, this depends upon the interaction of viral molecules which bind to receptor or cognate receptors (Gupta *et al.*, 1991). The proteins encoded by Flavivirids have distinct characteristics and each protein can serve as a potential target. For example, the non-structural proteins essential for replication are serine protease, RNA helicase and RdRp (Simmonds *et al*, 2017).

NS1 protein plays essential role during the replication of flavivirids, this protein take part in the formation of replication complex, morphogenesis and immune system evasion. Moreover, it is unique to flaviviruses and plays early role in replication while NS2B is required for proteolytic activity. (Sironi *et al.*, 2016). NS3, and its cofactor NS4A or NS2B, in flaviviruses are the main viral protease and also play helicase and nucleoside triphosphatase (NTPase) activities in order to bring about successful replication. On the other hand, NS5 is found to have two activities based on in vitro experiments *i.e.*, primer dependent RdRp and a terminaltransferase (TNTase) activity. The cleavage of NS5 protein is done through the

NS3/NS4A protease complex in order to produce two viral polypeptides, namely NS5A and NS5B in hepaciviruses. NS5B protein is the membrane-associated phosphoprotein with an RdRp activity and plays a crucial role in replication and poly protein progression. That is why it is the key enzyme for the synthesis of RNA strands progeny. (Argos, 1988; Miller and Purcell, 1990).

Though synthetic chemistry is the dominated when one talks about the discovery and production of drugs but still the potential of therapeutic plants and their bioactive compounds are important, as they provide new and novel ways and products for disease treatment (Raskin *et al.*, 2003). Moreover in comparison with the synthetic chemistry plant derived natural products provide a fascinating source of biologically active compounds as they are natural and have affordable prices as well (Ghosh *et al.,* 2008). Family Meliaceae, also known as mahogany family, is native to tropical and sub-tropical region. More than twenty two genera of family Meliaceae have been investigated by scientists to determine the bioactive substances and compounds that are present in their species. Variety of compounds such as limonoids, terpenoids (mono, di, sesqui and triterpenoids) lignans, flavonoids, phenolics, coumarins and various chromones has been isolated from these species. These vast range of active compounds are reported to have anti-inflammatory, anti-bacterial, anti-diabetic and most importantly anti-viral activities (Koriem, 2013).

Researches have been executed previously to find out effective cure against flavivirids but due to less effective results, the need of urgent researches has been increased even more. However, previous researches has pointed out toward the role and significance of non-structural proteins in the RNA replication and processing of flavivirids. In this regard, NS5 region was highlighted as more significant region as it encodes RdRp. This region can be targeted through structural based drug designing which in turn can serve to suppress and inhibit the viral proliferation and replication(Papageorgiou *et al.,* 2016).

The present study is therefore designed to screen potential phytochemicals from the plant's family Meliaceae against viruses belonging to family *Flaviviridae* using computer aided analysis. The research methodology was elucidated to select potential phytochemicals with high binding and inhibitory affinity specifically against the RdRp of flavivirids.

Materials and Methods

The implementation of this research was aimed at the identification of potential and possible inhibitors of the targeted RdRp of the flavivirids. Sequence of computation based steps for the aimed analysis and investigations were computed as shown in Figure 1.

Figure 1: Flowchart showcasing many steps of methodology.

Selection of phytochemicals

Plant species within the family Meliaceae were selected for the study. The information about the compounds extracted from the selected plant species was gathered through various literature sites and Dr. Duke's Phytochemical and Ethnobotanical Databases. Based upon the beneficial effects and activities, several phytochemicals were selected. The 3D and 2D structures of these selected phytochemicals were searched through PubChem database. For the visualization of these structures, UCSF Chimera 1.14 was used, which is free software. Moreover, these structures were converted to PDB files, for further use.

Pharmacokinetic screening of phytochemicals

In order to estimate the drug likeliness of selected phytochemicals, the evaluation of ADMET (absorption, distribution, metabolism, secretion and toxicity) properties was carried out through the help of three computer based approaches; Swiss ADME, PreADMET online server and Way2drug. Lipinski's rule of five was used to set the following criteria for further screening.

Protein structure retrieval and Homology Modeling

The focus of this study was NS5B (RdRp) of the type species from each genus of family *Flaviviridae*. For this purpose the estimation of the type specie was done through the ICTV Taxonomy. RCSB PDB was used to search the target proteins however, due to the unavailability of the target proteins homology modeling was carried out. For this purpose the accession number for each type species was noted and further searched through the NCBI to download the coding genome sequence and poly protein sequence in FASTA format. Before going further, these structures were compared, aligned and evaluated through CLUSTW to check the similarity between sequences of each RdRp protein. These polyprotein precursor sequences were ran through BLASTp and four sequences having the maximum query cover were selected as templates for the modeling purpose. The protein modeling was done through Modeller 9.22, where five script files, one template sequence files and the four query sequence files were ran through it. The resultant models were evaluated through ModEval. The structures were visualized through Raswin and Pymol. For the validation, Ramachandran plots were drawn through MolProbity and SAVES v6.0. Minimization of the modeled structures was done through UCSF Chimera 1.14. Estimation of the homologues proteins was done through BLAST psi from where the proteins having maximum sequence similarity were selected to analyze the conserved regions. The superimposition of the selected homologous proteins with the target proteins were done through the match maker command of UCSF Chimera 1.14. PockDrug was used to estimate the validity of the predicted protein pockets and their affinity to bind drug like molecules.

Molecular Docking

Molecular docking is an essential step to estimate the binding modes, affinity and inhibitory constant of the screened phytochemicals against the target proteins. For this purpose, molecular docking analysis was carried out through; Autodock Vina and Autodock tool. Screened phytochemicals were used as ligands to perform docking against target proteins. PDBQT formats were obtained by receptor preparation done through the addition of hydrogen bonds and the modification along with torsion

adjustment of ligands. For the specified ligand- protein interactions; x, y, z dimensions were specified and grid box was generated. The visualization of resulting complexes was done through Pymol, 2D and 3D structural images of resulting complexes and binding interactions were generated through Discovery Studio Visualizer. In order to obtain the complexes with highest binding and inhibitory affinity, the threshold values were set. The binding energies were noted while the inhibitory constant Ki of the resulting complexes were calculated by Eq. (1).

$$Ki = \frac{\Delta G}{e^{R_{XT}}} \quad \text{…….. (1)}$$

Where Ki is the inhibitory constant, temperature is denoted by T, which is 298.15 K. Gas constant is denoted by R while ΔG is the docking energy. The value of R is 1.9872036 cal/mol.

DFT Analysis

Density functional theory analysis was executed in order to predict the transition energies of the selected phytochemicals. DFT methods, chiefly use Becke's three parameter hybrid function in combination with the Lee-Yang-Parr correlation functional (B3LYP) to evaluate the reactivity and proficiency of phytochemicals (Rasool *et al*, 2021). For the estimation of quantum computational properties, DFT analysis was carried out through the use of GaussView 6.0 and Gaussian 09W. The optimization of the lead compounds was carried out at the ground state. The highest occupied molecular orbital (HOMO) and lowest unoccupied molecular orbital (LUMO) energies were used in this study. The band energy gap (ΔE) was calculated using the expression. The basis set that was selected is 6-31G (d, p, ++).

Results

Selection and pharmacokinetic screening of phytochemicals

Although family Meliaceae is consisted of large number of plant species, but during this study, three species *Melia azedarach*, *Azadirachta indica* and *Cederala ordata* were chosen for active compound analysis. Based upon the activities reported in previous literature 75 phytochemicals were selected and analyzed for their ADMET properties. However, 45 phytochemicals didn't follow the criteria set previously for Lipinski's rule, *i.e.,* Lipinski's violation = 0, 2 solubility = high to low, GI absorption = high or moderate, blood-brain barrier permeability = Nil, and toxicity = medium/low. The remaining phytochemicals were further analyzed for approach BBB (blood-brain barrier) permeability, mutagenicity and carcinogenicity effects and finally 30 phytochemicals (18 from *Melia azedarach*, 09 from *Azadirachta indica* and 03 from *Cederela ordata* were screened for further analysis (Table 1).

Table 1: ADMET Properties of the screened compounds.

No	Species	Compound	ESOL Class	GI Absorption	BBB Penetration	BBB Permeability	Lipinski Violation	Carcinogenicity (Positive)	Toxicity (Herg)	Drug Likeness	Mutagenicity
1	***Melia .azedarach***	*17-Epiazadiradione*	Moderately Soluble	High	No	Yes	0	Mice/Rat	Medium	Yes	No
2		*3-Beta-Hydroxystigmast-5-En-7-One*	Poorly Soluble	Low	No	Yes	2	Mice/Rat	Medium	Yes	No
3		*Geranyl Acetate*	Soluble	High	Yes	Yes	0	Mice/Rat	Low	Yes	No
4		*Kulactone*	Poorly Soluble	Low	No	Yes	1	Mice/Rat	Low	Yes	No
5		*Kulinone*	Poorly Soluble	Low	No	Yes	1	Mice/Rat	Low	Yes	No
6		*Linalyl-Acetate*	Soluble	High	Yes	Yes	0	Mice	Low	Yes	No
7		*Meldenin*	Moderately Soluble	High	No	Yes	0	Rat	Medium	Yes	No
8		*Melianone*	Poorly Soluble	High	No	Yes	1	Mice/Rat	Low	Yes	No
9		*N-Heptanol*	Very Soluble	High	Yes	Yes	0	No	Low	Yes	No
10		*N-Hexanol*	Very Soluble	High	Yes	Yes	0	No	Low	Yes	No
11		*Nimbinin*	Moderately Soluble	High	No	Yes	0	Rat	Medium	Yes	No
12		*Nimbiol*	Moderately soluble	High	Yes	No	0	No	Low	Yes	No
13		*Nimbolidin-A*	Poorly soluble	Low	No	Yes	2	Rat	Low	Yes	No
14		*Ohchinin*	Moderately soluble	High	No	Yes	1	Mice/Rat	Medium	Yes	No
15		*Ohchinolide-B*	Moderately Soluble	Low	No	Yes	1	Rat	Medium	Yes	No
16		*Quercetin-3-O-L-Rhamnoside*	Soluble	Low	No	No	2	No	High	Yes	No
17		*Stigmast-4-En-3-One*	Poorly Soluble	Low	No	Yes	1	Mice	Low	Yes	No
18		*Triacontanol*	Poorly Soluble	Low	No	Yes	1	Mice	Low	Yes	No
19	***Azadirachta indica***	*Azadiradione*	Moderately Soluble	High	No	Yes	0	Rat	Medium	Yes	No
20		*Beta-Sitosterol*	Poorly Soluble	Low	No	Yes	1	Mice	Low	Yes	No
21		*Epoxyazadiradione*	Moderately Soluble	High	No	Yes	0	Mice/Rat	Medium	Yes	No
22		*Gedunin*	Moderately Soluble	High	No	No	0	Mice/Rat	Low	Yes	No
23		*Nimbaflavone*	Poorly Soluble	High	No	No	0	Rat	Medium	Yes	No
24		*Oleic Acid*	Moderately Soluble	High	No	Yes	1	Mice/Rat	Medium	Yes	No
25		*Scopoletin*	Soluble	High	Yes	Yes	0	Rat	Low	Yes	Yes
26		*Stearic-Acid*	Moderately Soluble	High	No	Yes	1	Rat	Medium	Yes	No
27		*Sugiol*	Moderately Soluble	High	Yes	No	0	No	Low	Yes	No
28	***Cederala. Ordata***	*Angolensic-Acid-Methyl-Ester*	Moderately Soluble	High	No	No	0	Rat	Medium	Yes	No
29		*Mexicanolide*	Moderately Soluble	High	No	Yes	0	Rat	Low	Yes	No
30		*Odoratone*	Poorly Soluble	High	No	Yes	1	Mice	Low	Yes	No

Homology modeling and Retrieval of targeted proteins structures

For the Genus *Hepacivirus*, *Flavivirus*, *Pestivirus* and *Pegivirus*; isolates hepacivirus subtype 3a (HCV 3a), yellow fever virus (YFV), bovine viral diarrhea virus 1, strain NADL (BVDV) and hepatitis GB virus (GBV-A) having accession Numbers; AGQ17416, NC_002031, M31181 and U22303 respectively; were selected and accession numbers were saved through the information given in ICTV Taxonomy. Based upon the importance and function, NS5B (RdRp) was selected as the protein of interest (Target Protein). Through RCSB: PDB the RdRp for each species was searched to find the 3D structure but due to unavailability of these structures of interest were sent to BLASTp where four sequences with max scores were selected. Among the five generated models by Modeller 9.21 for each protein the lowest DOPE energy was selected for further analysis. The evaluation of Modeller scoring results was done through ModEval. While validations of the resulting structures was done through Ramachandran plots obtained by the use of MolProbity and SAVES v6.0 (Table 2)

Table 2: Reliability Assessment of Modeled Protein Structures Using Ramachandran Plot Analysis.

Protein	Most Favored Region (%)	Additionally Allowed Region (%)	Generously Allowed Region (%)	Disallowed Region (%)	reliability
BVDV-RdRp	89.6	10.6	04.0	0.0	Yes
GBV-A-RdRp	90.4	8.9	0.7	0.0	Yes
HCV-RdRp (subtype 3a)	93	7.0	0.0	0.0	Yes
YFV-RdRp	91.7	7.8	0.0	0.5	Yes

Molecular Docking Analysis

Prior to molecular docking the ligands and receptors were prepared through Autodock Vina and Autodock tools and grid size was estimated to cover the binding sites/active residues within the docking region. The application of the cut off values -8.0 kcal/mol was done in order to estimate the best complexes. Binding affinities and Ki values were reported (Table 3).

Kulinon displayed the highest binding affinity against RdRp of BVDV and formed Pi-Sigma bond with TYR_{186} along with Alkyl and Pi Alkyl bonds with MET_{185}, CYS_{189}, LEU_{182}, LEU_{122}, TYR_{196} (Figure 3). Apart from that 3 more compounds were found to have binding affinities ≥ -8.0kcal/mol (Table 4).

Hepatitis GB virus (PGV)

Odoratone made conventional hydrogen bond with ARG_{135} and Alkyl bond with ARG_{91}, ARG_{92}, and $ILE3_{81}$ with highest binding affinity against RdRp of PGV (Figure 3). However, 12 phytochemicals were also found to have binding affinity lesser than odoratone but ≥ -8.0kcal/mol (Table 4).

Table 3: Binding affinities and Ki values for docking of screened phytochemicals against RdRp of type species from each genus

No	Screened and Docked phytochemicals	Hepatitis C Virus Subtype3a (HCV)		Bovine viral Diarrhea Virus, 1 NADL (BVDV)		Hepatitis GB Virus (PGV)		Yellow Fever Virus (YFV)	
		Binding Energies (kcal/mole)	Ki (µM)	Binding Energies (kcal/mole)	Ki (µM)	Binding Energies (kcal/mole)	Ki (µM)	Binding Energies (kcal/mole)	Ki (µM)
1	***Azadiradione***	-9.6	0.090	-7.1	6.164	-8.1	1.138	-7.2	5.206
2	***Beta-sitosterol***	-7.3	4.397	-6.1	33.39	-6.8	10.23	-8.6	0.489
3	***Epoxyazadiradion***	-9.1	0.210	-7.5	3.136	-7.9	1.595	-9.2	0.177
4	***Gedunin***	-9.7	0.076	-7.7	2.237	-8.2	0.961	-8.2	0.961
5	***Nimbaflavone***	-8.9	0.294	-7.2	5.206	-7.4	3.713	-8.1	1.138
6	***Oleic acid***	-5.2	152.8	-3.4	3199	-5.1	180.9	-3.9	1374
7	***Scopoletin***	-6.3	23.82	-5.3	129.0	-6.4	20.11	-5.7	65.65
8	***Stearic-acid***	-6.1	33.39	-3.4	3199	-5.2	152.8	-4.0	1160
9	***Sugiol***	-8.1	1.138	-6.8	10.23	-7.6	2.468	-7.7	2.237
10	***Angolensic-acid-methyle-ester***	-8.8	0.349	-7.6	2.468	-8.4	0.685	-8.5	0.579
11	***Mexicanolide***	-9.0	0.249	-8.0	1.347	-8.4	0.685	-8.6	0.489
12	***Odoratone***	-9.5	0.107	-8.1	1.138	-9.4		-8.4	0.685
13	***3-beta-hydroxystigmast-5-en-7-one***	-10.3	0.028	-6.6	14.34	-8.2	0.961	-7.1	6.164
14	***17-epiazadiradione***	-9.7	0.076	-7.1	6.164	-8.5	0.579	-7.6	2.468
15	***Geranyl acetate***	-6.8	10.23	-4.4	590.4	-5.6	77.73	-4.8	300.3
16	***Kulactone***	-9.6	0.090	-8.3	0.812	-8.0	1.347	-8.1	1.138
17	***Kulinone***	-9.2	0.177	-8.6	0.489	-8.2	0.961	-8.6	0.489
18	***Linalyl-acetate***	-5.1	180.9	-5.0	214.2	-5.2	152.8	-4.4	590.4
19	***Meldenin***	-8.8	0.349	-6.0	39.54	-9.0	0.249	-8.6	0.489
20	***Melianone***	-8.2	0.961	-7.0	7.299	-7.0	7.299	-7.4	3.713
21	***N-heptanol***	-4.0	1160	-3.0	6288	-4.0	1160	-4.4	590.4
22	***N-hexanol***	-4.0	1160	-3.2	4485	-3.8	1627	-3.5	2701
23	***Nimbinin***	-9.0	0.249	-7.5	3.136	-8.0	1.347	-7.2	5.206
24	***Nimbiol***	-8.5	0.579	-6.5	16.98	-8.4	0.685	-8.0	1.347
25	***Nimbolidin-A***	-8.0	1.347	-6.5	16.98	-6.9	8.643	-7.1	6.164
26	***Ohchinin***	-8.7	0.413	-6.9	8.643	-8.1	1.138	-8.6	0.489
27	***Ohchinolide-b***	-9.1	0.210	-6.1	33.39	-7.5	3.136	-6.1	33.39
28	***Quercetin-3-o-l-rhamnoside***	-8.9	0.294	-7.7	2.237	-8.3	0.812	-8.8	0.349
29	***Stigmast-4-en-3-one***	-7.7	2.237	-7.3	4.397	-6.8	10.23	-6.2	28.20
30	***Triacontanol***	-4.5	498.6	-3.9	1374	-4.0	1160	-3.4	3199

Bovine viral diarrhea virus (BVDV)

Hepatitis C virus subtype 3a (HCV 3a)

3-beta-hydroxystigmast-5-en-7-one showed highest binding affinity against RdRp of HCV and made conventional hydrogen bond with ASN_{142} ,Pi-sigma bond with TYR_{162} ,Pi-Anion bond with GLU_{398}, Alkyl and Pi-Alkyl bond with ALA_{97}, MET_{139}, LYS_{141}, VAL_{405},PRO_{404}, and ILE_{160} (Figure 3). Other than that, 19 compounds displayed the binding affinity equal of more than the threshold value (Table 4).

Yellow Fever Virus (YFV)

Quercetin-3-o-l-rhamnoside made conventional hydrogen bonds with GLY148, LYS_{182}, GLU_{218} and CYS_{82}, and Pi-Anion bond with ASP_{146} with the highest binding affinity against RdRp of YFV (Figure 3). Not only this but twelve more compounds were found to have binding affinity ≥ -8.0kcal/mol (Table 4).

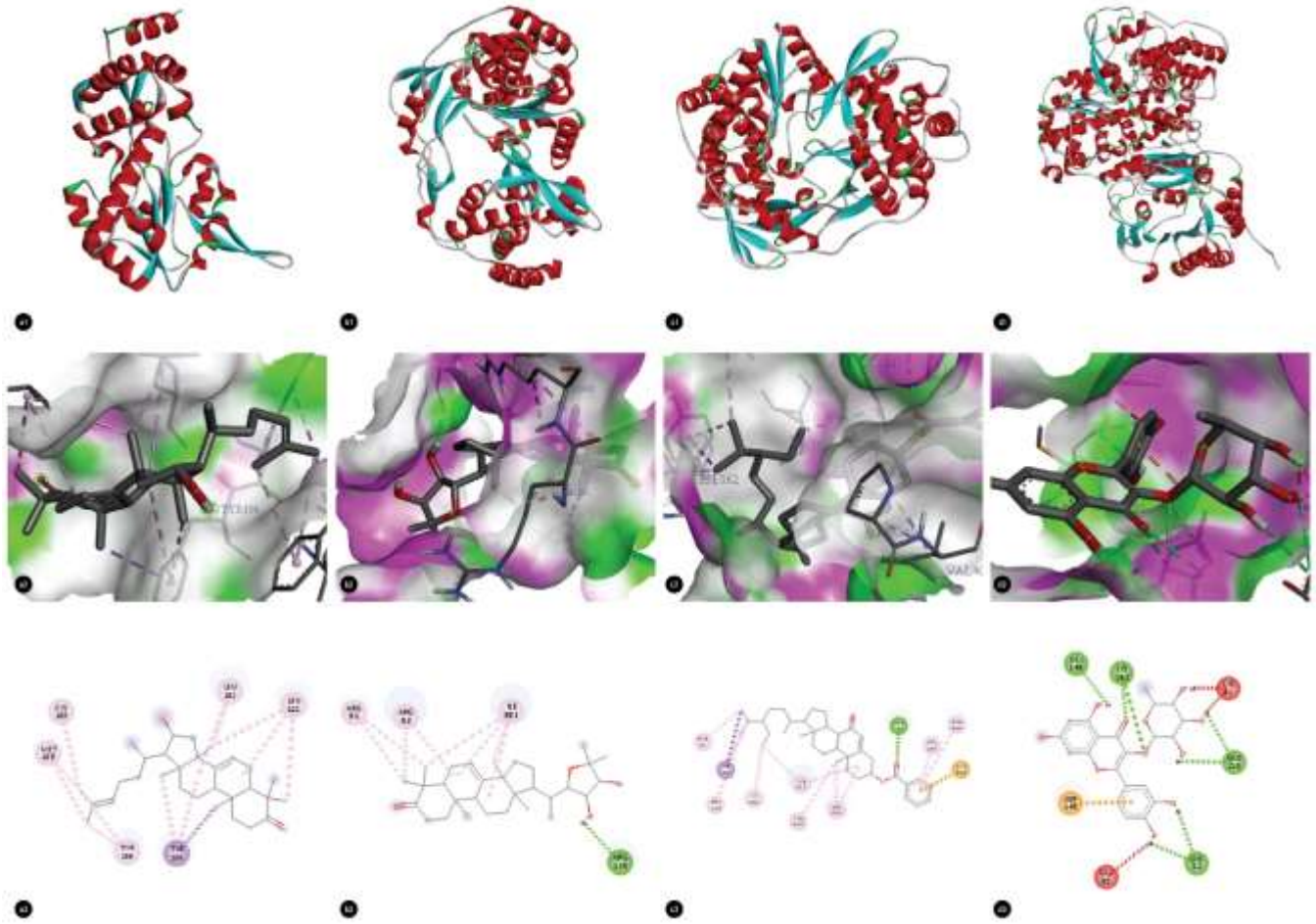

Figure 3: Phytochemicals displaying promising results against targeted receptor: RNA Dependent RNA Polymerase with highest binding affinities ≥− 8.0 kcal/mol. (a1) BVDV (a2) 3D representation of BVDV complexed with *Kulinon* (a2) 3D representation of BVDV complexed with *Kulinon* (b1) PGV (b2) 3D representation of PGV complexed with *Odoratone* (b3) 2D structure PGV complexed with *Odoratone* (c1) HCV (c2) 3D representation of HCV complexed with *3-beta-hydroxystigmast-5-en-7-one* (3c) 2D representation of HCV complexed with *3-beta-hydroxystigmast-5-en-7-one* (d1) YFV (d2) 3D representation of YFV complexed with *Quercetin-3-o-l-rhamnoside* (d3) 2D representation of YFV complexed with *Quercetin-3-o-l-rhamnoside.*

DFT analysis and reactivity of screened complexes

HOMO-LUMO analysis, and energy gap (ΔE) of the potential phytochemicals was carried out through DFT calculation. In the DFT analysis, gradient corrected correlation function of Lee, Yang and Parr (LYP) combined with the Becke's three parameter exchange functional (B3), considers the computation of molecular structure optimization , and energy gaps by implementation of the split-valence polarized 6-31G(d,p) and 6-31++G (d,p) basis sets. Energy gap (ΔE) is estimated by noting down the difference between HOMO and LUMO energies (Table 5). The information provided in the table points toward the considerable transfer of charge from electron donor to electron acceptor groups and consequently highlights these phytochemicals as compounds with high chemical reactivity and better bioactivity against the targeted proteins.

DISSCUSION

In order to meet primary health needs, medicinal plants are considered an effective source of both traditional as well as modern medicines and are being used since decades to cure human diseases. On the other hand problems associated with the use of anti-biotic, has revived the use of plant based, bioactive compounds (Meresaa, 2020).

Although a large amount of flora has been extensively surveyed to explore the plant extracts with pharmacological and medicinal potential but this study was focused upon Family Meliaceae, as more than seventeen genera are endemic to Asia. Seventy species from these genera are reported to have a large number of phytochemicals with potential activities against various infectious diseases. Out of these seventy species, three species: *C. ordata*, *A. indica*, and *M. azedarach* were selected to investigate their phytochemicals against the targeted protein. *A. indica* and *M. azedarach* were selected because these species are found abundantly in Pakistan. However, *C. ordata* was selected because, as per our survey this species hasn't yet been explored much for the bioactivity of its phytochemicals.

A. indica and *M. azedarach* are reported to have similar and closely relative properties. *A.indica*, commonly known as Neem, is reported to be the most useful plant in Asian countries, especially in Pakistan, where almost all parts of it are used as traditional medicine and household remedies, and can be exploited commercially for its biological activities against different ailments. The stem bark of *A.indica* is found to have certain compounds which treat malarial fever, general debility, fatigue, thirst, bad taste, cough, ulcers, leprosy, urinary discharge and several corneous diseases *Kulinone* is one of the most important constituent found in the stem bark of *A. indica* (Meresaa, 2020).

Although, as mentioned above, all of the NS proteins display properties that make them a potential drug target, our target protein was NS5B protein which has the RdRp activity and plays an important role in the replication of viral genome. The NS5 region of polyprotein is located on the C terminal which is processed through NS3} NS4A and makes up the two viral polypeptides named as NS5A and NS5B. Based upon previous finding, the NS5B is responsible for RdRp activity (Steffens *et al.*, 1999). RNA polymerases encoded by flavivirids are the largest viral proteins being which vary in length genera to genera, yet the reason why we selected the RdRp

proteins of each type species are the common structural features among RdRp from each genus. For example, large thumb domain and a c-terminal motif which forms a template binding channel (Choi and Rossmann, 2009).

Discovery and advancement of novel compounds is a parlous process which typically cost from 0.8 to 1.0 billion USD. Moreover, various steps to analyze the drug-likeliness, affectivity and safety, make it a tie consuming process. Typically one project typically takes more than 14 years to finally introduce a dependable drug in the market. Although the investment in the development of drugs has been increased since past decade yet the failure rates and low efficiency contributed to the loss of time, money and efforts altogether(Lobanov, 2004).

Several approaches such as; the analysis of already existing antiviral molecules, advancement of novel against and High through put screening of compounds to analyze their affectivity against replication and packaging of targeted cell lines, are commonly practiced and utilized by scientific community to combat with viral infections. However, High throughput screening is taken as the more promising approach as it assist scientist to analyze and evaluate large amount of compounds for their drug likeliness. Moreover, already known drugs can also be analyzed and screened for drug repurposing(Shekhar, 2008).

Great advantages has been gained through in-silico methods to determine chemical ADMET properties as various computational models has been designed and put forward for the prediction of various thresholds such as acute oral toxicity. ADMET predictions such as admetSAR, SwissADME, pre ADMET server, Way2Drug and many other tools has been designed and updated to find out the quantitative regression models, ADME properties and toxicity, through the use of such tools one can screen potential molecules in fast, reliable and easy way (Cheng *et al*, 2012; Daina *et al.*, 2017; Li *et al*, 2014; Li *et al*, 2015; Lipinski, 2016; Xu *et al.*, 2019; Xu *et al*, 2015; Yang *et al*, 2017; Yang *et al.*, 2018; Yang *et al*, 2018). For the screening of potential drug, Lipinski's rule of five, algorithm consisting of four rules is used that uses number 5 as a threshold value to predicts the poor oral absorption and describes that if the: 1) Molecular weight of a drug is above 500, 2) lipophilicity of the drug is over 5 ,3) presence of more than five hydrogen bond donors and 4) presence of more than ten hydrogen bond acceptors (Lipinski, 2016).

The target for the drug designing can be the proteins that have their significant role in disease progression through various interactions leading to signaling that are involved in the disease proliferation. In Receptor based *in silico* screening, known 3D structures and their active sites that are usually determined by the X-ray crystallography and NMR of target protein is essential for the initial steps of drug discovery. Also for the generation of testable hypotheses proteins structures are valuable (Baker and Sali, 2001; Schwede *et al*, 2009).

However if these structures aren't available then homology model is the important method to determine the three-dimensional structure of the targeted proteins. One of the computer based program for comparative protein structure modeling is MODELLER ,that needs the input of alignment of sequences needed to be modeled and script files with coordinates of templates after which it automatically design and calculate the model having all the non-

hydrogen atoms within minutes. From fold assignment to alignment of target sequences and templates and from building a model based upon these alignments to predicting the most accurate model, four main steps are basically followed during the comparative modeling. (Fiser *et al.,* 2000; Marti-Renom *et al.*,, 2004; Marti-Renom *et al*, 2000; Sali and Blundell, 2002) The results showed that the protein models generated were reliable structures as 89.6-93% of dihedral angles fall within the most favored region (Table 2). This showed that the protein models have low steric hindrances.

Different people have different meaning when it comes to the function of protein and in our case it is to find out the nitty-gritty of active site and enzymatic activity of modeled proteins. Consequently the most essential step "finding the binding pocket of the protein". For this purpose, we searched out the homologous proteins, since similar sequences tend to have similar structures and in turn similar functions. And that's why homologues proteins are the reliable source to provide information about the newly modeled proteins. This means the higher the sequence similarity, higher are the chance of getting appropriate active site based upon conserved regions in both proteins. Here it is also important to mention the ortholougs and parologous, where the functions of proteins tend to be more conserved in the former than later. Popular sequence alignment methods included are HMMER, SWISS-PROT, SAM and PSI BLAST. (Baker and Sali, 2001; Schwede *et al*, 2009).

Defined as the determination of binding affinities between two molecules, Molecular docking is very important computational tool which uses either receptor base methods as well as ligand based methods to search the potential candidate to achiever certain goals including successful drug designing of more quick and less costly drugs, to transform biological information into workable information and to facilitate and help access the enormous amount of data. Various steps such as receptor preparation that includes selection of structure and sites, addition and removal of charges, cofactors and hydrogen , and ligand preparation that may include conversion of one form to another form such as conversion of SMILES and SDF forms to PDB forms and addition of various charges as well as removal , minimization of these structures based upon different docking tools and their sensitivity (Gangrade, Sawant, & Mehta, 2016; Mandal, Moudgil, & Mandal, 2009).

The aim of virtual screening is to search hits and lead compounds, chiefly on the basis of molecular descriptions and physiochemical properties. Through three dimensional representation of ligand interactions, molecular docking provides rather a broad idea about the affectivity of the ligand interactions with the target (Sousa, Fernandes, & Ramos, 2006). Molecular docking tools, such as AutoDock, FlexX, ICM, GOLD *etc*. are available (Morris *et al*, 1998). Although docking tools also provide idea about the ligand interactions but also predict the binding energies, complementarities associated with the shape. Yet critical observation and investigations are required to finalize a compound as an effective and potent drug. This can't be done merely by molecular docking and points out toward the need of more efficient tools to solve the problems associated with optimization algorithm (Jain *et al.*, 2016). In this regard, the phytochemicals with highest binding affinity against each targeted protein were selected for Density Functional Theory analysis as it

has assisted investigators to predict the potential of a drug based upon the laws which chiefly govern the behavior of electrons through quantum mechanics. Moreover, DFT analysis provides the investigators with the accurate results such as the true minimum potential energy surfaces, co-efficient of highest occupied molecular orbital (HOMO), co-efficient of lowest unoccupied molecular orbital (LUMO) and band gap simulated at simulated at B3LYP/6-31 G (d, p) (Rasool *et al*., 2021).

CONCLUSION

Secondary metabolites derived from the plant family Meliaceae were used to identify potential inhibitors of RdRp from flavivirids isolates. At least four phytochemicals: *3-beta-hydroxystigmast-5-en-7-one*, *Kulinone*, *Odoratone*, and *Quercetin-3-O-L-Rhamnoside* have potential to inhibit the targeted protein, as they displayed the binding affinities equal or above the threshold values. Whereas, *Quercetin-3-O-L-Rhamnoside* was found to be the most reactive phytochemical. The phytochemicals identified can further be assessed *in vitro* and *in vivo* to determine their efficacy and safety.

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Table 4: Complexes of screened phytochemicals and their binding residues.

Phytochemical Complexes with *Bovine viral Diarrhea Virus, 1 NADL* (BVDV) affinity ≥-8.0kcal/mol.		
No	**Compound Name**	**Bond types and interaction Residues**
1	***Kulactone***	Alkyl and Pi-Alkyl bonds: LYS_{118}, TYR186, LEU_{122}, $LEU_{182.}$
2	***Odoratone***	Alkyl and Pi-Alkyl bonds: LEU122, TYR_{196}, TYR_{186}, $LEU_{182.}$
3	***Mexicanolide***	Pi-sulfur bond: $MET_{185;}$ Carbon-Hydrogen bonds: ALA_{235}, ASP_{119}; Alkyl and Pi-Alkyl bonds: LEU_{122}, TYR_{186}, LEU_{17} and $LEU_{182.}$
Phytochemical Complexes with *Hepatitis GB Virus* (PGV) with affinity ≥-8.0kcal/mol.		
1	***Meldenin***	Conventional hydrogen bond: ARG_{21}, Alkyl bonds: VAL_{137}, PRO_{18}, VAL_{36} and $ARG_{13;}$Carbon-hydrogen bond: SER_{375}
2	***17-epiazadiradione***	Conventional hydrogen bonds:ARG_{398};Alkyl and Pi Alkyl Bonds:ALA_{481}, ARG_{48}, PRO_{408}, ILE_{402}, PRO_{237}, LEU_{436}
3	***Nimbiol***	Conventional hydrogen bonds: ARG_{135}, SER_{375};Alkyl and Pi-Alkyl bonds: VAL_{137}, VAL_{36} and ARG_{372};Pi-Anion bond: ASP_{379}
4	***Mexicanolide***	Conventional hydrogen bonds:GLN_{425} ,ARG_{372};Carbon-hydrogen bonds: VAL_{242} and THR_{364}; Alkyl and Pi-Alkyl bonds:TYR_{291}, PRO_{269}, LYS_{368}
5	***Angolensic-acid-methyle-ester***	Conventional hydrogen bonds: ARG_{148}, GLN_{423}, GLY_{426};Alkyl and Pi-Alkyl bonds: MET_{390}, LYS_{368}, and PRO_{369};Pi-Cation bond: ARG_{372}
6	***Gedunin***	Conventional hydrogen bonds: ARG_{372}, ARG_{148}, LYS_{134};Alkyl and Pi-Alkyl bonds: LYS_{368} and PRO_{369};Carbon-hydrogen bond: SER_{383}; Pi- Sulfur bond: MET_{390}
7	***3-beta-hydroxystigmast-5-en-7-one***	Alkyl and Pi-Alkyl bonds:ARG_{135}, VAL_{36}, PRO_{18}, ILE_{381}
8	***Kulinone***	Alkyl Bonds: LEU_{406}, ARG_{398}, ILE_{402}, ALA_{481}, LEU_{488}, LEU_{433}, PRO_{437}, LEU_{449}, LEU_{440}
9	***Ohchinin***	Conventional hydrogen bonds:ARG_{189}, ARG_{148};Alkyl and pi-Alkyl bonds: ALA_{301},CYS_{345}; Carbon-hydrogen bond: SER_{346}; Pi-sulfur bond: MET_{390}
10	***Azadiradione***	Alkyl bonds: PRO_{18},VAL_{36};Carbon-hydrogen bonds: PRO_{380}, ASP_{379}; Amide-Pi Stacked bond: ARG_{135}
11	***Nimbinin***	Conventional hydrogen bond: LEU_{339}, ASP_{340}; Alkyl and Pi-Alkyl bonds: LEU_{349},TRP_{360}, TRP_{197}; Pi-cation and Anion bonds: , LYS_{457}
12	***Kulactone***	Conventional hydrogen bond:THR_{138}; Alkyl and Pi-Alkyl bonds: VAL_{424}, PHE_{431}, LEU_{443}, LEU_{389}, MET_{390}, ILE_{170}; Pi-Sigma bond: TYR_{429}
Phytochemical Complexes with *Hepatitis C Virus Subtype 3a* (HCV) with affinity ≥-8.0kcal/mol.		
1	***Gedunin***	Alkyl and Pi-Alkyl bonding: CYS_{366}, LEU_{384}, PRO_{197}, TYR_{415}, MET_{414}, PHE_{445}; Conventional hydrogen bond:ARG_{394}, GLY_{449}
2	***17-epiazadiradione***	Conventional hydrogen bond: ASN_{142}, Pi-anion bond: GLU_{398};Pi-sigma bond: $TYR_{162;}$Alkyl and Pi-alkyl bonds:ALA_{97}, MET_{139}, ILE_{160}, LYS_{141}, PRO_{404}, VAL_{405}, VAL_{144}, ARG_{394}
3	***Kulactone***	Conventional hydrogen bond: GLY_{557};Alkyl and Pi-Alkyl bonds: PRO_{93}, HIS_{95}, ALA_{97}, VAL_{405}, PRO_{404}, LYS_{14}
4	***Azadiradione***	Alkyl and Pi Alkyl bonds: ILE_{160}, MET_{139}, CYS_{14}, TYR_{160};Amide-Pi Stacked bond: GLY_{557} ; Conventional hydrogen bond: ASP_{559}
5	***Odoratone***	Alkyl and Pi-alkyl bonds CYS_{366}, TYR_{415}, CYS_{316}, and PHE_{193}
6	***Kulinone***	Pi-Sigma bond: TYR_{415};Alkyl and Pi-alkyl bonds: LEU_{384}, MET_{414}, PRO_{197}, TYR_{448}, CYS_{366}, PHE_{193}
7	**Ohchinolide-B**	Conventional hydrogen bonds: $ARG_{200;}$Pi-cation bond: ARG_{394}; Alkyl and Pi-Alkyl bonds: PHE_{193}, CYS_{366}, MET_{414};Pi-cation bond: ARG_{394}; Pi-sigma bond with TYR448
8	***Epoxyazadiradion***	Carbon hydrogen bond: GLY_{556};Pi-Alkyl and Alkyl bonds: TYR_{448}, MET_{414} and CYS_{366}
9	***Nimbinin***	Carbon hydrogen bond:GLY_{556};Alkyl and Pi-Alkyl bonds: TYR_{448},MET_{414}, CYS_{366}
10	***Mexicanolide***	Conventional hydrogen bond: ARG_{158}; Carbon hydrogen bond: GLY_{317};Alkyl and pi-Alkyl bonds with PHE_{193}, CYS_{366}, MET_{414}, TYR_{448}
11	***Nimbaflavone***	Pi-Pi Stacked and Amide-Pi Stacked bonds: TYR_{448};Carbon hydrogen bonds: ARG_{200}; Alkyl and Pi-Alkyl bonds: MET_{414}, PHE_{445}, CYS_{366}
12	**Quercetin-3-o-l-rhamnoside**	Conventional hydrogen bond: TYR_{448}, ASN_{411}; Pi-Pi Stacked and Pi-Pi T-shaped bonds: TYR_{415}, TYR_{448};Pi-Alkyl bond: $CYS_{366;}$ Un-favorable bond: SER368
13	***Angolensic-acid-methyle-ester***	Conventional hydrogen bond: GLY_{556};Alkyl and Pi Alkyl bonds: PHE_{193}, CYS_{316}, CYS_{366}, TYR_{448}, MET_{414}
14	***Meldenin***	Conventional hydrogen bonds: GLY_{449}, $ARG_{386;}$Alkyl and Pi-alkyl bonds: CYS_{366} ,PHE_{193};Carbon hydrogen bond: ASP_{318}
15	***Ohchinin***	Pi-anion bond: GLU_{446};Pi Donor bond: $GLY_{557;}$ Alkyl and Pi-Alkyl bonds: TYR_{162}, ALA_{92}, PRO_{404},HIS_{95}, VAL_{405}, LYS_{141}

16	***Nimbiol***	Alkyl bonds: LEU_{547}, VAL $_{564}$, VAL_{454}, ILE_{462}
17	***Melianone***	Pi-sigma bond:TYR_{162}; Alkyl and pi-Alkyl bond:RO_{93}, ILE_{160}, ALA_{97}, MET_{139}
18	***Sugiol***	Pi Alkyl and Alkyl bonds: MET_{414}, LWU_{384}, PRO_{197}, PHE_{193}, $CYS_{366;}$ Pi-Pi Stacked bond:TYR_{448}
19	***Nimbolidin-A***	Conventional hydrogen bond:GLY_{556}, GLY_{449},TYR_{448},TYR_{415};Pi Sulfur bond: MET_{414}, Pi-cation bond: ARG_{394}; Alkyl and Pi-Alkyl bonds: PHE_{193}, TYR_{448}
Phytochemical Complexes with *Yellow Fever Virus* (YFV) with affinity ≥-8.0kcal/mol.		
1	***Epoxyazadiradion***	Conventional hydrogen bond:HIS_{110};Pi-sigma bond: LEU_{105};Alkyl and Pi Alkyl bond: ILE_{147}
2	***Meldenin***	Pi-sigma bond: TRP_{799};Pi-Anion bond:ASP_{540};Pi-Alkyl bond: PHE_{466};Carbon hydrogen bonding: GLU_{604};Conventional hydrogen bond: TYR_{413}
3	***Mexicanolide***	Conventional hydrogen bond:GLU111;Carbon hydrogen bond:ASP_{146},Alkyl and Pi alkyl ILE_{147}, HIS_{110}
4	***Beta-sitosterol***	Pi-alkyl bonds: TRP_{799}, LYS_{413}, TYR_{609}, PHE_{466}
5	***Kulinone***	Conventional hydrogen bond: ASN_{612}, SER_{800}, SER_{714} and Pi-alkyl TRP $_{799}$
6	***Ohchinin***	Conventional hydrogen bond: ASP_{540}, TRP_{799}; Pi-Pi T Shaped bond: TRP_{799};Pi alkyl bond PHE_{466}
7	***Angolensic-acid-methyle-ester***	Conventional hydrogen bonds:ASP_{667}, Tyr_{413}, TRP_{199}; Pi Alkyl bond: PHE_{466}
8	***Odoratone***	Conventional hydrogen bond:ARG_{243}; Alkyl and Pi alkyl bonds: PRO_{210}, ARG_{208}, LEU_{239} and ILE_{207}
9	***Gedunin***	Conventional hydrogen bonds: TYR_{413}, ASN_{612}, SER_{03}; Pi alkyl bond: TRP_{199}, Carbon hydrogen bond: CYS_{713}
10	***Nimbaflavone***	Conventional hydrogen bonds: ASP_{667}, TYR_{609}, GLU_{415}; Carbon hydrogen bond: THR_{608}
11	***Kulactone***	Conventional hydrogen bond: LYS_{286};Alkyl and pi Alkyl bonds:VAL_{282}, TRP_{293} and Pi-Sigma bond: TYR_{295}
12	***Nimbiol***	Conventional hydrogen bond: LEU_{16};Pi-Pi stacked bond: PHE_{24},Alkyl and Pi alkyl bond: LYS_{21}

Table 5: Density functional theory based analysis**.**

Phytochemicals	**E_{LUMO} (Kcal/mol)**	**E_{HOMO} (Kcal/mol)**	**Band energy gap (ΔE) (Kcal/mol)**
3-beta-hydroxystigmast-5-en-7-one	-0.05063	-0.23251	-0.18188
Kulinone	-0.02048	-0.22509	-0.20461
Odoratone	-0.01697	-0.22174	-0.20477
Quercetin-3-O-L-Rhamnoside	-0.05939	-0.20916	-0.14977