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IN SILICO INVESTIGATION OF PHYTOCONSTITUENTS OF MEDICINAL HERB 'ADHATODA VASICA' AGAINST ASTHMA BY MOLECULAR DOCKING AND ADMET PREDICTION

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ABSTRACT: Asthma is chronic airway inflammatory disease that is raising highest prevalence in industrialized countries worldwide. There is a high incidence of usage of complementary medicine for asthma. In traditional systems of medicine, many plants have been documented to be useful for the treatment of various respiratory disorders including asthma. *Adhatoda vasica* (Family: Acanthaceae) is a small evergreen shrub found many regions of India and throughout the world. It is well known medicine for bronchitis, asthma and other pulmonary infections. Cysteinyl leukotriene receptor type1 (CysLT$_1$R) mediates inflammatory processes and plays a major role in numerous disorders, including asthma, allergic rhinitis, cardiovascular disease, and cancer. The objective of the present study is to investigate the anti-asthmatic activity of phytoconstituents from the ethanolic root extract of *Adhatoda vasica* against CysLT$_1$R *insilico* docking by using Schrodinger Maestro version 2021-4 *Insilco* ADMET screening also performed by qikprop module of Schrodinger suit. The results showed the interactions between CysLT$_1$R with 6 phytocompounds, a natural compound vitamin E showed the best glide docking XP score -7.52453 kcal/mol and the binding energy value of -73.71167776 kcal/mol. The ADMET screening of the designed compounds have almost all the properties of the compounds are within the recommended values. Based on the result, Vitamin E and target were run on MD simulations stable at 10 ns. Finally, this study concludes that the Vitamin E is a suitable drug candidate for reduces alleviates asthmatic features.

Key words: Anti-asthmatic activity, CysLT$_1$R, Phytoconstituents, Molecular docking, ADMET property, MD simulations.

INTRODUCTION

Asthma is a heterogeneous infection, typically portrayed by constant aviation route irritation. It is characterized by the historical backdrop of respiratory side effects viz., wheeze, windedness, chest tightness, and cough that shift after some time and in power, along with variable expiratory wind stream limitation [1]. Although rates of deaths due to asthma worldwide have increased greatly over the past 25 years, no available therapeutic regimens can cure asthma, and the burden of asthma will continue to be driven by increasing prevalence [2]. Cysteinyl leukotriene receptor type1 (CysLT$_1$R) along with Cysteinyl leukotriene receptor type2 (CysLT$_2$R) are two G Protein coupled receptors (GPCRs) activated by the endogenous leukotrienes LTC4, LTD4, and LTE4, produced from arachidonic acid. CysLT$_1$R is a key player in allergic and inflammatory disorders, such as asthma, allergic rhinitis, atopic dermatitis, and urticaria.. Drugs derived from natural sources play a major role in the prevention and treatment of human diseases [3]. Many countries follow the traditional medicine as one of the prime health care systems [4] Phytoconstituents from herbal plants are particularly advantageous due to their easy availability and also preferred over synthetic drugs[5] ***Adhatoda vasica*** (L) Nees (Family: Acanthaceae) is a shrub widespread throughout Southeast Asia. It is commonly known as Vasaca or Malabar nut [6]. The leaves, roots, flowers and bark of Adhatoda are used for medicinal purposes. It is an official drug and is mentioned in the Pharmacopoeia of India. It is well known for preparation of medicine for bronchitis, asthma and other pulmonary infections. It has been used by European herbal practitioners as an antispasmodic, expectorant and febrifuge. It is also known for its antiarthritis, antiseptic, antimicrobial, expectorant, sedative and antituberculosis properties [7] Qualitative analysis of this plant extract showed the presence of terpenoids, flavonoids, tannins, cardiac glycosides, alkaloids, reducing sugars, and saponins. Eleven different phytoconstituents have been identified from ethanolic root extract of *Adhatoda vasica* by gas chromatography mass spectrographic analysis [8]. Molecular docking is a key tool in structural molecular biology and computer-assisted drug design. Docking can be used to perform virtual screening on large libraries of compounds, rank the results, and propose structural hypotheses of how the ligands inhibit the target, which is invaluable in lead optimization [9]. This study, therefore investigated the molecular interaction between the phytoconstituents in the ethanol extract of *Adhatoda vasica* roots and the CysLT$_1$R for preventing asthma through *insilico* docking by using Schrodinger Maestro version 2021-4. In addition to these, the drug likeness, absorption, distribution, metabolism, excretion and toxicity (ADMET) properties were also assessed based on Lipinski’s rule of five by employing “QikProp” module of Maestro version.

MATERIALS AND METHODS

The *In-silico* fragment-based drug design study was carried out using a Schrodinger Maestro suit using different modules such as Protein and ligand preparation, glide XP docking, Molecular docking analysis, Molecular Dynamic simulation, MM-GBSA, and ADMET.

Biological data

In this study, 10 bioactive molecules from ethanolic root extract of *Adhatoda vasica* were selected against the target of Cysteinyl leukotriene receptor type1 (CysLT1R). These bioactive molecules names were listed in **Table 1**. Later, these collected 11 bioactive molecules were retrieved from the chemical database [10]. CysLT$_1$R was obtained from Protein Data Bank PDB ID: 6RZ5

Preprocessing and preparation of protein target structure

Protein X-ray crystal structures of CysLT$_1$R was identified and imported from the protein data bank (PDB.) The features of the protein structure from the protein data bank are not appropriate to carry out molecular docking study. The 3D structure of the protein was prepared using protein preparation wizard module of Schrodinger suite Maestro version 2021-4.

Preprocessing and preparation of ligands

The ligand molecules imported from the PubChem data bank were saved in SDF format for computational calculations and placed into Maestro workspace. All the ligand molecules are prepared by the tool ligprep in Schrodinger 2021-4. The 2D structure of the ligand was converted into 3D with its likely tautomers and ionization states at pH 7.0 ± 2.0. The ligand was further generated and geometrically minimized by means of optimized potential liquid simulations force field using "ligprep" module for further docking analysis [11]

Molecular Docking Analysis

The Maestro suite 2021-4 was used to perform molecular docking and utilized to prepare the input PDB file CYSLT$_1$R (PDB ID: 6RZ5). Molecular docking uses the computational simultion predicts the ligand preferred orientation to a receptor when interact each other to form a higher stability complex. Molecular docking was performed by docking the prepared ligand on the selective site of the protein using the Glide molecule. The result was analyzed based on the docking score and molecular interaction formed between the ligand and the protein molecule [12].

Binding free energy calculation by using prime MM-GBSA approach

The best ligands molecules were selected. The ligand binding energy of total 6 phytocompounds to inhibit CysLT$_1$R were estimated using Prime MM-GBSA module in Schrodinger Suite 2021-4 the total free energy of binding, dGbind (kcal/mol) is estimated by the software as:

$$\Delta G \text{ bind} = G \text{ complex} - (G \text{ protein} + G \text{ ligand})$$

We then used this score to rank the ligand-protein glide XP docked complex. [13]

Molecular Dynamic Simulation

Molecular Dynamic simulation was performed using Macromodel Version 9.0 (a Schrodinger module). The OPLS_2005 force field was used for the energy calculation. The constant temperature was 300 K and in the integration step, 1.0 fs was given. Run the MD simulations for complex structures. MD simulation with position restraints was carried out for a period of 4000 PS to allow the accommodation of the water molecules in the system. Finally, Root Mean Square Deviation (RMSD) was calculated for checking the stability of 6RZ5 protein with their native motion. All coordinate file was saved every 1000ps up to 10 ns and the result was analyzed by Scatter Plot [14-16].

ADMET Analysis

ADMET (absorption, distribution, metabolism, excretion and toxicity) predictions for the top docking hits (6 natural bioactive compounds) were calculated by using the QikProp module of Schrodinger suite program (Schrödinger software) running in normal mode. QikProp generates physically relevant descriptors, the toxicity a ligand is considered important for the ligand to act as an effectual drug discovery of new drug development. These entire processes were used by Schrodinger software. Among these, Lipinski's rule of five or rule of thumb is also a tool, which was used in the present study to evaluate the drug-likeness as well as certain pharmacological or biological activity of a new Ligand/chemical entity. [17]

RESULT AND DISCUSSION:

Table 1: Phytochemicals of *Adhatoda vasica* root

S.NO	COMPOUND NAME
1	Ethanethioic acfid, S[2dimethylamino)ethyl] ester
2	2-Aminononadecane
3	3-Eicosyne
4	trans-Z-à-Bisabolene epoxide
5	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl ester)
6	Vitamin E
7	β-Sitosterol
8	Spiro[androst-5-ene-17,1'- cyclobutan]-2'-one,3-hydroxy- ,(3á,17á)
9	1,6,10-Dodecatrien-3-ol, 3,7,11- trimethyl-,[S-(Z)]-
10	Lupeol

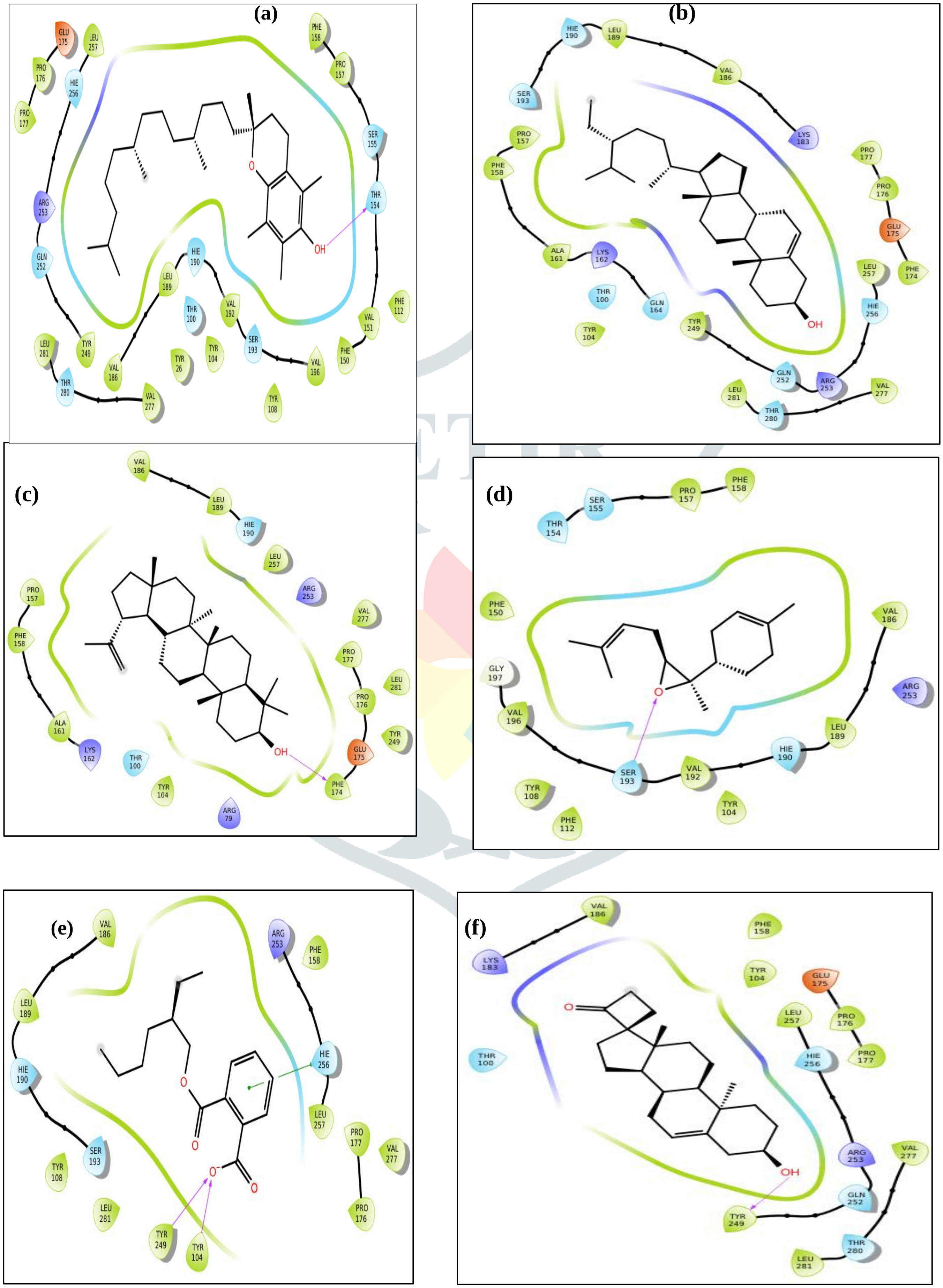
Molecular docking

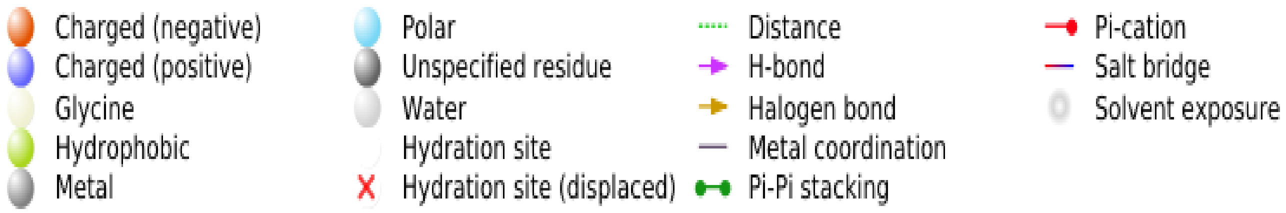
Prediction of interaction energies between ligand and receptor has been a major challenge for molecular docking. Six Phytoconstituents from the ethanolic extract of *Adhatoda vasica* root were performed docking analysis with CYSLT$_1$R primarily by using Schrodinger software. The Schrodinger software resulted in identifying the best compound that interacts with the receptor. The results were evaluated based on binding compatibility that is docked energy in kcal/mol. The docking score and binding energies for the phytochemicals are given below in **Table 2.** The results indicate that compound vitamin E has the highest affinity to bind with cysLT$_1$R with a docking score of -7.52453. The β-Sitosterol, Lupeol, trans-Z-à-Bisabolene epoxide,, 1,2-Benzenedicarboxylic acid, mono(2-ethyl hexyl ester), Spiro[androst-5-ene-17,1'- cyclobutan]-2'-one,3-hydroxy-,(3á,17á)-, has showed relatively good binding affinity with docking score of -7.00215,-5.76163,-5.50326,-5.49791,-5.17799 respectively. The 2D-ligand interaction diagram of phytochemicals of *Adhatoda vasica* root with CysLT$_1$R is given in **Figure 1** (a–e).

Table 2 Docking score of phytochemicals present in *Adhatoda Vasica* root

S. No	PubChem ID	Compound Name	Docking Score	MMGBSA dG Bind (kcal/mol)	H Bond
1	14985	Vitamin E	-7.52453	-73.71167776	0
2.	175268335	β-Sitosterol	-7.00215	-54.28960627	0
3.	259846	Lupeol	-5.76163	-30.31791051	-0.368582
4.	91753504	trans-Z-à-Bisabolene epoxide	-5.50326	-12.90008487	-0.690394
5.	20393	1,2-Benzenedicarboxylic acid,mono(2-ethylhexyl ester)	-5.49791	-14.20927502	-0.760675
6.	534435	Spiro[androst-5-ene-17,1'-cyclobutan]-2'-one,3-hydroxy-,(3á,17á)-	-5.17799	-24.87988958	-0.7

Figure 1: 2D ligand interaction for phytoconstituents of Adhatoda *vasica* root with CysLT$_1$R





Binding free energy calculation by using prime MM-GBSA approach

Molecular docking was furthermore assessed with MM-GBSA free restricting vitality, which is identified with the post scoring approach for CYSLT$_1$R (PDB ID: 6RZ5) target and the values are shown in **table 2.** From the results of MM-GB/SA studies, the ΔG bind values were observed in the range of -73.71 to -24.87 kcal/mol) for significantly active compounds and also dGvdw values, dG lipophilic values and the energies are positively contributing towards total binding energy. The stability of docking complex will be predicted by MMGBSA scoring function. The Glide score and MM-GBSA free energy are obtained by the docking of ligands into the coupling pocket, which are more stable.

MD simulation

The molecular dynamics simulation was carried out for the phytocompounds vitamin E and protein CysLT$_1$R. For evaluate the structural constancy of those molecules with the help of Desmond. The final trajectory files were taken for calculating the RMSD of the complex structures. At the same time as running MD simulation for CysLT$_1$R protein and vitamin E for 10 ns, the RMSD (Root Mean Square Deviation) plot shows the stability of the complex structures. The period and the constant potential energy stable at 1.2 ns to 10 ns. In addition, when performing the simulation for 10 ns, and it makes the stability of the complex structure during the entire simulation time up to 10 ns **Figure 2**.

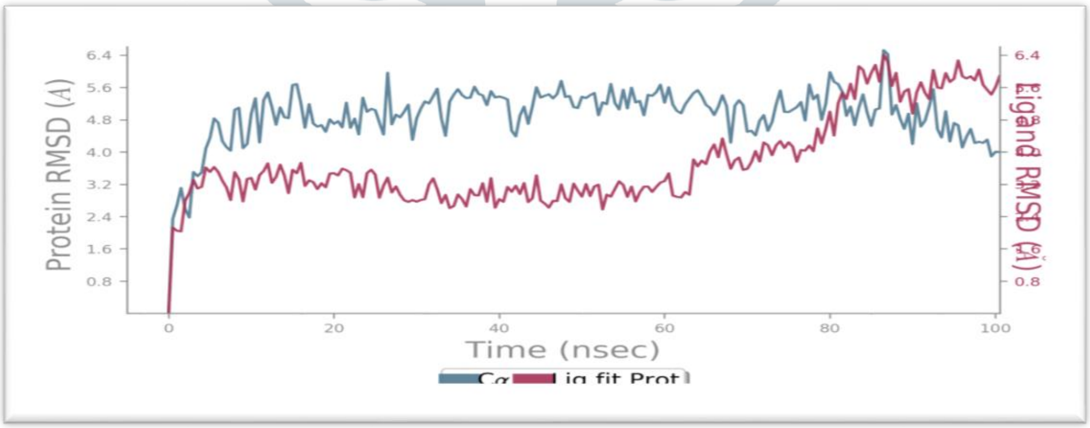


Figure 3: The root mean square deviation (RMSD)

ADMET Analysis

Physico-chemical indicators identify the crucial elements influencing biological processes (ADME). But the concept of ADME has been expanded by toxicity.[18-19] In this analysis, the significant pharmacokinetic parameters such as aqueous-solubility (QPlogS), IC50 value for blockage of HERG K+ channels (QPlogHERG), blood/brain partition coefficient (QPlogBB), predicted human oral absorption (PHOA), and QPPCaco-gut-blood barrier/cell permeability in nm/s were also identified. Hence, we revealed that six Phytoconstituents from the ethanolic extract of *Adhatoda vasica* root showed a better pharmacokinetic and toxicity profile. The result of pharmacokinetics properties are given in the **table-3.** Among these, Lipinski's rule of five or rule of thumb is also a tool, which was used in the present study to evaluate the drug-likeness as well as certain pharmacological or biological activity of a new Ligand/chemical entity. The rule portrays the molecular properties and ADME characteristics of a drug in the human body. In addition to these, Lipkinski's rule is playing an active role in lead structure design, step-wise increase in the activity and selectivity of a compound to ensure drug-like physicochemical properties. Lipinski's rule includes, (i) no more than 5 hydrogen bond donors, (ii) no more than 10 hydrogen bond acceptors, (iii) a molecular mass less than 500 daltons, and (iv) octanol-water partition coefficient (QPlogP) not greater than 5. All compounds meet the drug-likeness criteria in the Lipinski rule. The result of Lipinski's rule is given in the **table-4**.

CONCLUSION

In conclusion, the insilico docking study revealed that all six phytoconstituents in ethanolic root extract of *Adhatoda vasica* showed efficient docking score and effective binding affinities. It was observed that the all the compounds have good physicochemical profiles with several other ADMET properties. The drug-like property predictions showed that most of the compounds comply with Ro5. From this study, best compound (vitamin E) has been identified for treatment and management of asthma and, this study will be addressed to further drug processing analysis

Table 3: ADME values of Ligand molecules using a QikProp module of Schrodinger

Pharmacokinetic properties					
S.No	**QPlogS**	**QPlogHERG**	**QPlogBB**	**PHOA**	**QPPCaco**
1	-10.341	-5.786	-0.649	3	4539.648
2.	-8.638	-4.684	-0.354	3	3381.652
3.	-7.998	-3.788	0.11	3	4367.727
4.	-4.939	-3.13	0.162	3	9906.038
5.	-4.574	-3.489	-1.3	3	127.567
6.	-5.257	-3.558	-0.178	3	1740.02
Recommended values	–6.5 – 0.5	<–5	–3.0 – 1.2	1-low 2-medium 3-high	<25 poor, >500 great

Where, QPlogS denotes aqueous solubility, QpHERG-predicted IC50 value for blockage of HERG K+ channels, QPlogBB-Brain/blood partition coefficient, PHOA- Predicted human oral absorption and QPPCaco-gut-blood barrier/cell permeability in nm/s

Table 4: Qikprop Property of natural phytocompounds representatives based on Lipinski's rule

Factors of Lipinski's rule of 5					
S.No	**MW**	**HB-Donor**	**HB-Acceptor**	**QPLogP o/w**	**Rule of Five**
1	430.713	1	1.5	8.97	1
2	414.713	1	1.7	7.623	1
3	426.724	1	1.7	7.087	1
4	220.354	0	2	3.02	0
5	278.347	1	4	3.785	0
6	328.494	1	3.7	3.976	0
Recommended values	130.0 – 725.0	0-6	2-20	-2-6.5	Max 4

Where, MW - molecular weight; HB - Hydrogen bond; QPLogP (O/W) octanol/water partition coefficient

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