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IL-6: A Potent Target for the Treatment of Rheumatoid Arthritis by *In-silico* Approach

Sneha Bharti[1], Prachi Agnihotri[1], Archana Tiwari[2], Sagarika Biswas[1*]
[1*]Department of Genomics & Molecular Medicine, CSIR- Institute of Genomics & Integrative Biology, Mall Road, Delhi-110007
[2]School of biotechnology, Rajiv Gandhi Technological University, Bhopal- 462033, Madhya Pradesh, India

Abstract:- Rheumatoid Arthritis (RA) is an autoimmune disease affecting life globally. It is characterized by synovial inflammation and joint destruction, eventually inducing severe disability and joint deformity. Many of the research have been done towards the treatment of RA. Since it is inflammatory disease, verities of inflammatory cytokines are involved in RA. IL-6 and TNF-α are most prominently causing RA. In this study we are looking for an inhibitory compound of natural origin which can be used to target the above two inflammatory factors. Two plants namely *Aloevera* and *Murraya koenigii* were selected to find out the activity of their active compounds to inhibit the target interleukin 6 (IL-6). The protein-ligand docking plays an important role in structural based drug designing. Various molecular docking tools namely SwissDock, PatchDock, Argus Lab 4.0.1, UCSF Chimera-Autodock Vina and Hex 8.0 were used to find the best scoring function of protein ligand interaction. Molecular docking followed by ADME/Tox to limit the study of molecular docking was used on the basis of Lipinski rule of five. The results of these tools showed that out of 10 active components of both the plants only two have potential to be an inhibitor of the IL-6. The aloe emodin of *Aloevera* and *phebalosin* of *Murraya koenigii* have spontaneous binding with IL-6 protein. These active compounds have potential which can be used as drug to treat the inflammatory condition of RA.

Keywords:- *Autoimmune Disease, Molecular Docking, TNF -α, SwissADME, Lipinski Rule.*

I. INTRODUCTION

RA is a chronic inflammatory autoimmune disease resulting into mortality, severe disability and bones deformity globally [1, 2]. It is characterized by the pannus formation in synovial membrane, deformity in affected joints, leading to dysfunction of the interior milieu of joints [3]. Women are largely affected then men that degrade the quality of health and increase the difficulties because it increases vividly around the time of menopause [4]. Since 1998, lot of advancement has been achieved for the treatment of RA such as Disease Modifying Anti-Rheumatic Drugs (DMARDS) [5], NSAIDS and steroids to control the disease activity and they have become anchor for the treatment of RA [6]. The high cost and adverse effects of drugs makes it unaffordable and less popular. Therefore, there is a necessity of alternative biological agent to overcome the related health issues of these drugs. The early diagnosis and treatment of RA is still giving challenges to the medical field [7].

The plant comprises of various bioactive compounds that can be considered as a therapeutic agent for RA [8]. In this study, we are approaching towards the bioactive compounds of *Aloevera* and *Murraya koenigii* (commonly known as curry leaves) as these plants possess different medicinal properties such as antioxidant, anti-microbial and anti-inflammatory that accelerates wound healing and burn, immunity, itching, blood disorders, improves digestion and skin eruptions [9, 10, 11]. IL-6 is the key cytokine in the pathogenesis of RA, its role in synovitis and joint damage makes it the potential target. The bioavailability of the bioactive compounds of these two plants used to target the IL-6 by molecular docking and was checked with ADME/Tox tool.

The various pro-inflammatory cytokines entailed in articular cartilage destruction during disease. The present research targeted the IL-6, to obtain a compound of the natural origin which can be further used as predictive drug treatment. To achieve this, different steps and bioinformatics tools were used for the validation and screening of the compounds on the basis of their properties.

II. MATERIAL AND METHODS

A. Protein Preparation

The crystallographic structure of IL-6 bearing PDB (Protein Data Bank) ID: 1ALU retrieved from the RCSB (Research Collaboratory for Structural Bioinformatics) protein databank (www.rcsb.org). The ribbon model of IL-6 shown in figure 1 and the stereochemistry of the protein structure was analysed by the PROCHECK tool (http://services.mbi.ucla.edu/PROCHECK/) for assessing the quality on the basis of Ramachandran Plot[12].

B. Ligand Preparation

The bioactive compound structures of *Aloevera* are aloin, campesterol, β-sitosterol, lupeol and aloe emodin and *Murraya koenigii* are quercitin, catechin, epicatechin, phebalosin and mukonicine. The structures were taken from the NCBI PubChem compound database (www.pubchem.ncbi.nlm.nih.gov/), drawn with the help of Marvin Sketch and were saved as mol2 format and

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PDB format. The pharmacokinetics evaluation was performed using the Swiss ADME/Tox based on the Lipinski rule of 5[13]. The prepared ligand structures were analysed by SwissADME on the basis of Lipinski rule of five. Lipinski rule of five helped to determine the physicochemical, lipophilicity, water solubility, medicinal chemistry, pharmacokinetics and drug likeliness of the selected compounds. For drug likeliness molecular weight, number of hydrogen bond donor, number of hydrogen bond acceptor and total polar surface should come under Lipinski rule of five.

C. Molecular Docking

Protein–ligand docking plays significant role in predicting the orientation of the ligand on binding with the protein. The docking of bioactive compounds with IL-6 protein were performed using 5 different tools namely SwissDock, PatchDock, Argus Lab 4.0.1, UCSF Chimera-AutoDock Vina and Hex 8.0. SwissDock and PatchDock are online servers [14, 15] whereas, Argus Lab 4.0.1, UCSF Chimera-AutoDock Vina and Hex8.0 are offline tools used for flexible docking [16, 17, 18].

D. Protein-Protein Interaction

The interaction within the proteins is helpful to know about the novel therapeutic approaches. STRING Database (Search Tool for Retrieval of Interacting Genes/Proteins) (https://string-db.org/) has been used to identify the interaction between IL-6 and other proteins. The study will enable us to analyse metabolic and signal transduction and also the pharmacogenetics research [19].

III. RESULT

The bioactive compound structures of *Aloevera* are aloin, campesterol, β-sitosterol, lupeol and aloe emodin and *Murraya koenigii* are quercitin, catechin, epicatechin, phebalosin and mukonicine properties were analysed on the basis of Lipinski rule of five (Table 1). Lipinski rule of five helped to determine the physicochemical, lipophilicity, water solubility, medicinal chemistry, pharmacokinetics and drug likeliness of the selected compounds. For drug likeliness molecular weight, number of hydrogen bond donor, number of hydrogen bond acceptor and total polar surface should come under Lipinski rule of five using SwissADME and their structures were drawn using Marvin sketch (Table 2).

The IL-6 protein was validated, using PROCHECK tool. According to the standard percentage, the most favoured region should consist of more than 90%. The Ramachandran plot of IL-6 interprets that the total number of residues are 157 and residues under most favoured region are 139 i.e. 95.2% (Figure 2).

Ligands, aloe emodin and phebalosin and the receptor 1ALU were subjected to the docking procedure with the SwissDock, PatchDock, Argus Lab 4.0.1, UCSF Chimera-Autodock Vina and Hex 8.0 (Figure 4). On the basis of obtained binding energies, the aloe emodin of *Aloevera* and phebalosin of *Murraya koenigii* attained the satisfactory binding energies (Table 3) and graphically represented in figure 3. Binding energy is the energy utilised by the ligand to dock with the receptor. The more negative the energy the more stable the conformation. The ligands ranked according to the binding energy they used to reach the stable conformation. Aloe emodin and Phebalosin has been proved to be the appropriate ligand that binds to the protein molecule with maximum binding energy.

Interactions among IL-6 and other proteins using STRING database provides the description of various interacting partner of IL-6 such as interleukins like IL-4 which participates in the B-cell activation process, IL-10 inhibits the synthesis of a number of cytokines like IFN-gamma, IL-2, IL-3, TNF and GM-CSF pathway, IL-13 inhibits inflammatory cytokine production and SOCS (Suppressor of cytokine signalling 3) which is involved in negative regulation of cytokines that signal through the JAK/STAT pathway.

IV. DISCUSSION

RA is an autoimmune disease affecting joints and leads to disability. The disease can be targeted by different available drugs targeting the various biological pathways. It is reported that IL-6 is the most significant cytokines causing synovitis deformities in the joints of the RA patients [2]. Present research aims to target the prominent cytokine IL-6 by the naturally occurring compound through *in-silico* approach. Earlier, *Aloevera* has been reported for the treatment of RA by not extracting the bioactive compounds but by preparing the extract of leaves for inhibiting the inflammatory factors; but this study focuses basically on the bioactive compounds present in plant, to find out the basically which bioactive compound responsible for this inhibitory action [20]. In this study two plants *Murraya koenigii* and *Aloevera* having anti-inflammatory properties, makes them more suitable to target the IL-6 [8, 10]. The in-silico approach gives the prediction of the analysis before approaching towards laboratory experiments. The bioactive compound of *Aloevera* are aloin, campesterol, β-sitosterol, lupeol and aloe emodin and *Murraya koenigii* are quercitin, catechin, epicatechin, phebalosin and mukonicine were selected for the molecular docking followed up by the Procheck and swissADME analysis. It enables to validate the protein and the compound for the flexible molecular docking [11]. The docking study with the different tools results the scoring function of ligand with the receptor binding site. Two bioactive compounds aloe emodin and phebalosin have the potential to act as a drug for inhibition of IL-6. The binding energy of the Aloe emodin resulted in 5 molecular docking tools namely SwissDock, PatchDock, Argus Lab 4.0.1, UCSF Chimera- AutoDock Vina and Hex 8.0 were -7.73, -3.748, 8.478, -8.98 and -192.16, and of phebalosin was -6.98, -3.87, -7.884, -6.48 and -223.35 Kcal/mole. The binding energy of these two compounds was higher among all the 10 selected

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compounds. The more negative the energy, the more stable the conformation of the docked compound. Furthermore, the protein protein interaction analysis performed using STRING Database, provides the base to study about pharmacogenetics research, metabolic and signal transduction pathway. Above study suggests aloe emodin and phebalosin as the potential compound out of 10 selected compounds to target the protein for anti- inflammation which further needs to be validated by *in vitro* and *in vivo* approaches.

V. CONCLUSION

Compounds derived from natural origin are capable to treat RA as novel therapeutic agents. Formulations of these compounds are mostly preferred by the traditional system of medicine. This study shows that the aloe emodin and phebalosin are potential inhibitor of IL-6 and have better activity than other compounds as these compounds shows more stable conformation. The anti-inflammatory property of these two compounds further needs to be validated by *in vitro* and *in vivo* approaches.

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FIGURE LEGENDS:

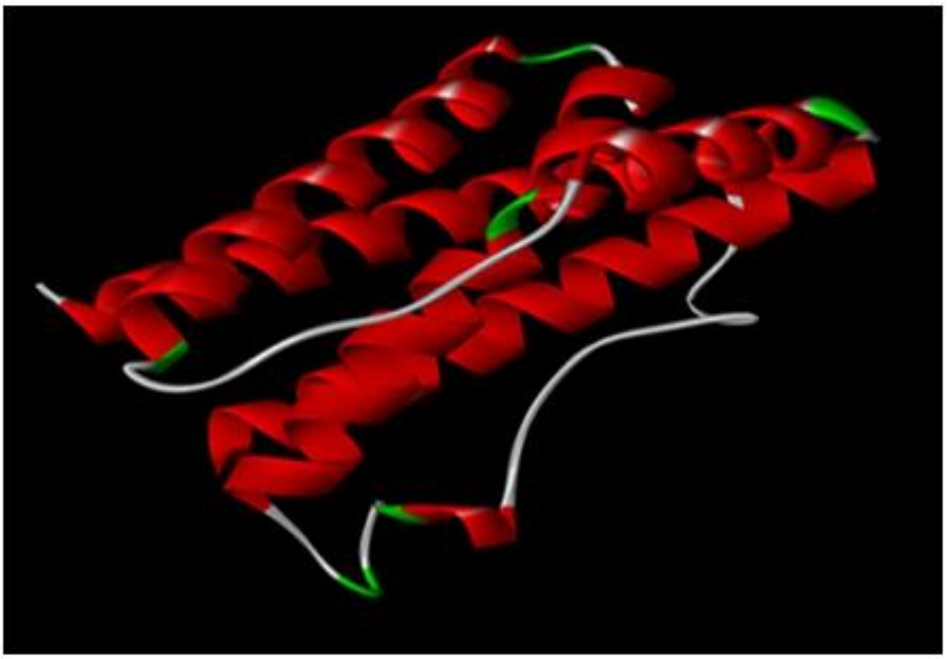

Fig 1:- Ribbon model view of IL-6 protein using Discovery studio 4.0.

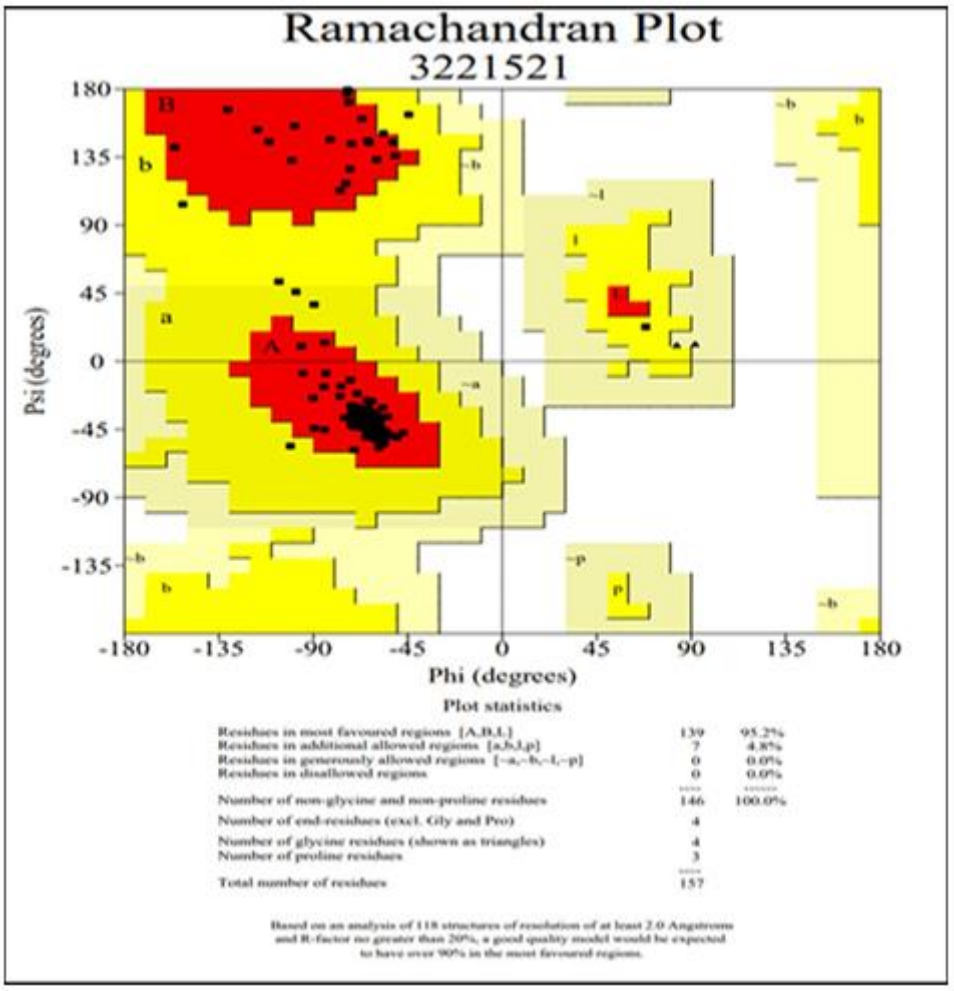

Fig 2:- Ramachandran plot of IL-6 protein.

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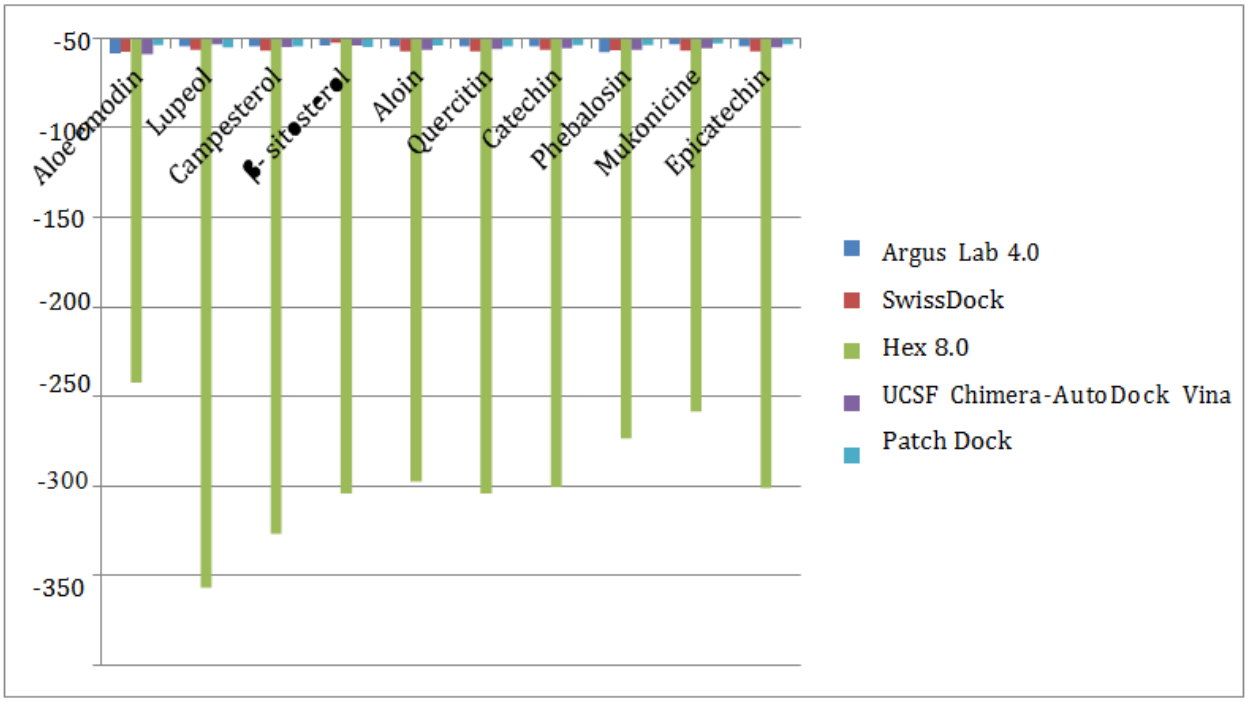
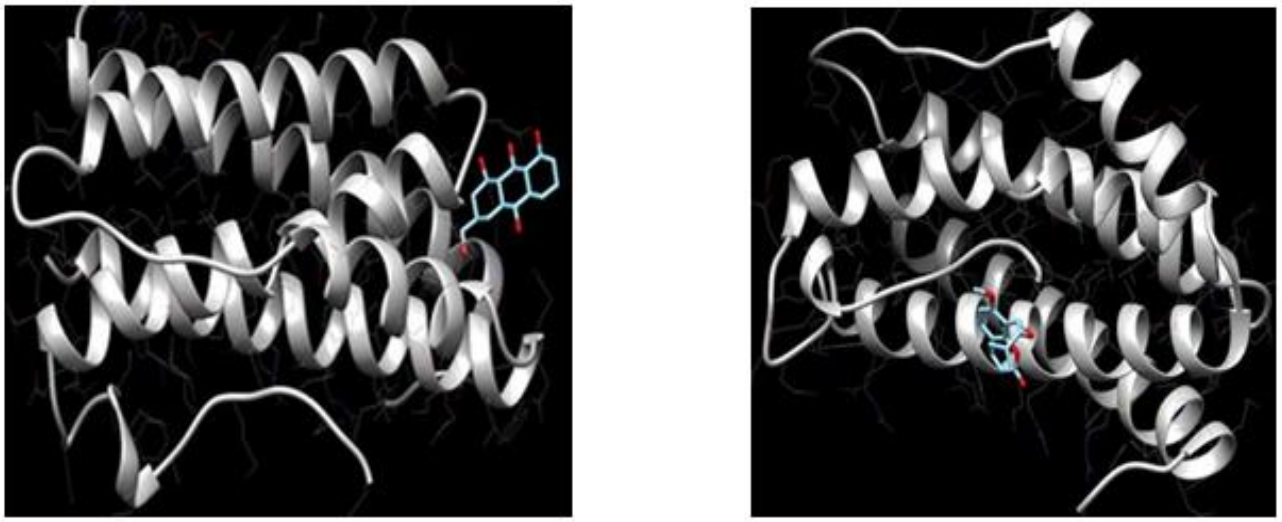


Fig 3:- Representation of the binding energy (Kcal/mole) of the ligands resulted from the docking tools; Aloe emodin and Phebalosin are the two potent compounds.



a) Aloe emodin **b) Phebalosin**

Fig 4:- Docking images of a) Aloe emodin and b) Phebalosin using SwissDock server.

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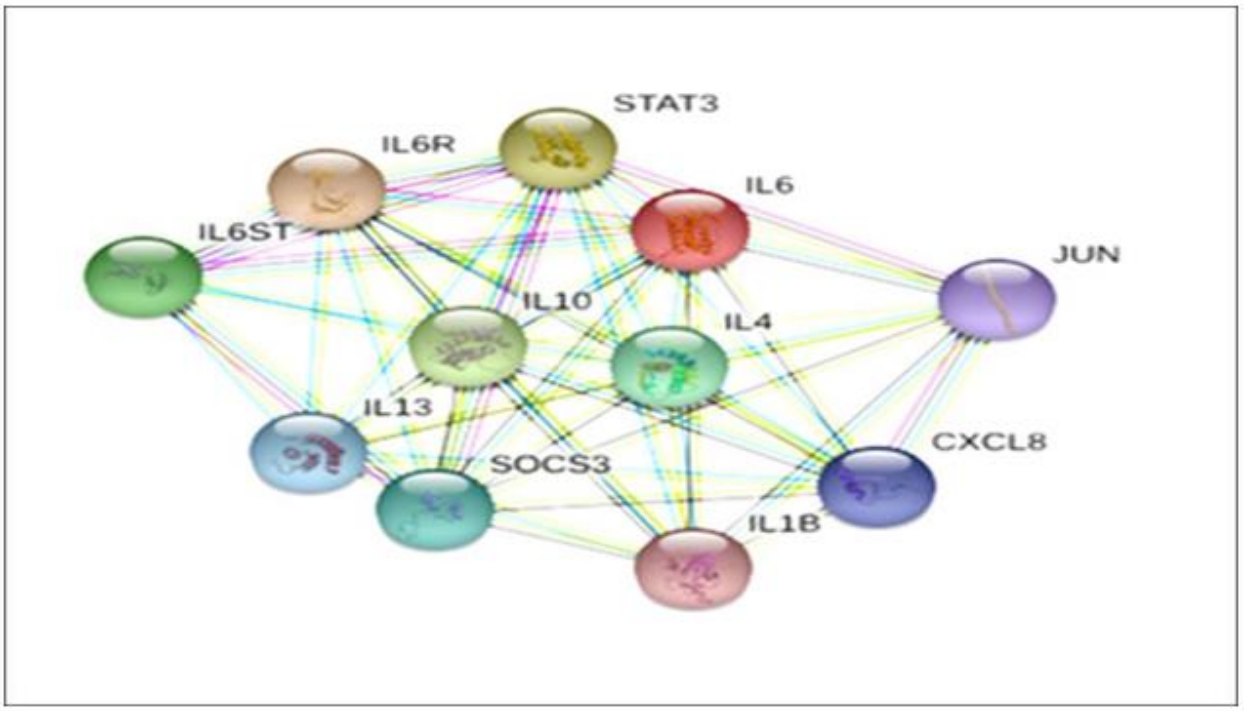

Figure 5: IL-6 interaction with other proteins using STRING Database.

Table Legends:

Compound Name	Molecular Weight(g/mol)	H-bond Acceptor	H-bond Donor	Rotatable Bonds
Aloin	418.39	9	6	3
Aloe emodin	270.24	5	3	1
β-Sitosterol	414.71	1	1	6
Lupeol	426.72	1	1	1
Campesterol	400.68	1	1	5
Quercitin	302.24	7	5	1
Phebalosin	258.27	4	0	3
Mukonicine	323.39	3	1	2
Catechin	290.27	6	5	1
Epicatechin	289.14	6	5	2

Table 1:- Molecular weight, hydrogen bond donor, hydrogen bond acceptor and rotational bond of different ligands using SwissADME software.

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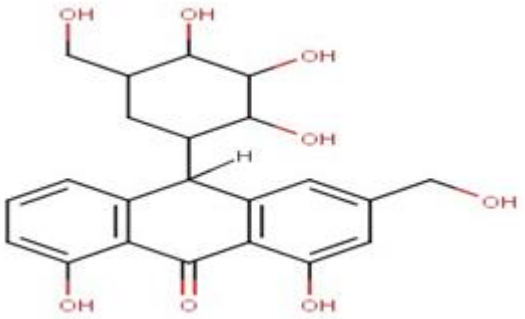
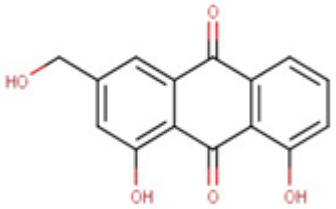
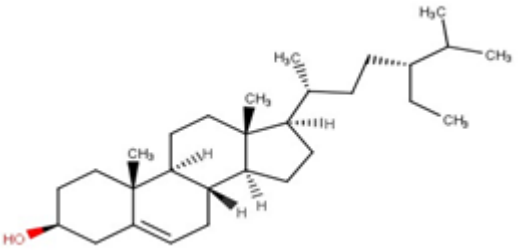
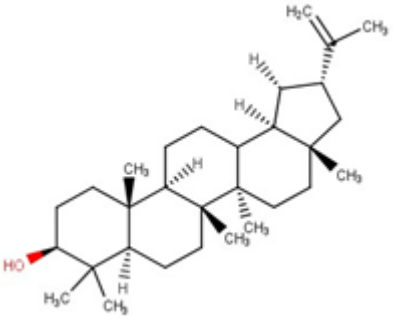
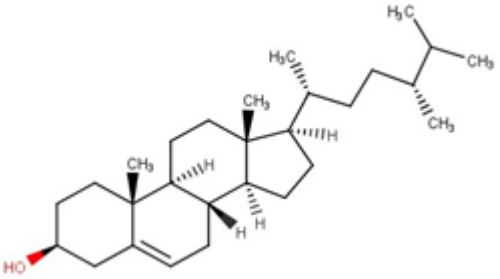
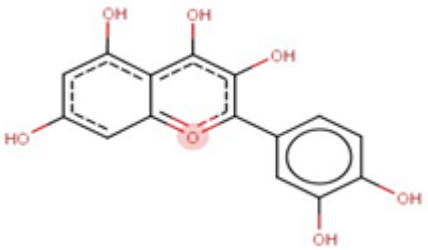
Ligand Name	Ligand Structure
Aloin (10S)-1,8-dihydroxy-(hydroxymethyl)-10- [(2S,3R,4R,5S,6R)-3,4,5-trihydroxy-6- (hydroxymethyl) oxan-2-yl]-10H-anthracen-9- one	
Aloe emodin 1,8-dihydroxy-3-(hydroxymethyl)anthracene-9,10-dione	
β-Sitosterol (3S,8S,9S,10R,13R,14S,17R)-17-[(2R,5R)-5- ethyl-6-methylheptan-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol	
Lupeol (1R,3aR,5aR,5bR,7aR,9S,11aR,11bR,13aR,13 bR)-3a,5a,5b,8,8,11a-hexamethyl-1-prop-1-en-2-yl 1,2,3,4,5,6,7,7a,9,10,11,11b,12,13,13a,13b-hexadecahydrocyclopenta[a]chrysen-9-ol	
Campesterol (3S,8S,9S,10R,13R,14S,17R)-17-[(2R,5R)-5,6-dimethylheptan-2-yl]-10,13-dimethyl-2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a] phenanthren-3-ol	
Quercitin 2-(3,4-dihydroxyphenyl)-3,5,7- trihydroxychromen-4-one	

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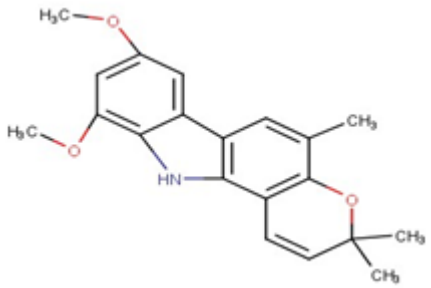
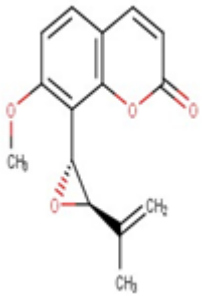
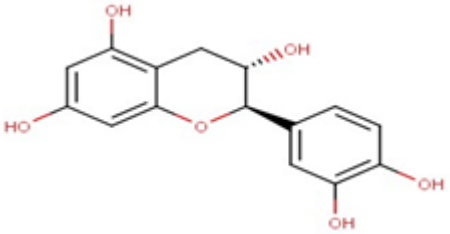
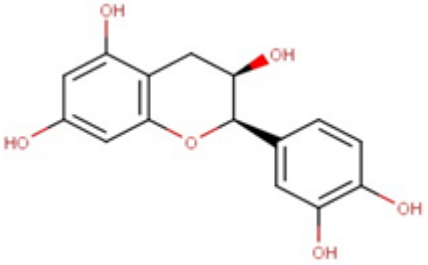
Mukonicine 8,10-dimethoxy-3,3,5-trimethyl-11H- pyrano[3,2-a] carbazole	
Phebalosin 7-methoxy-8-[(2R,3R)-3-prop-1-en-2- yloxiran-2-yl]chromen-2-one	
Catechin (2R,3S)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol	
Epicatechin (2R,3R)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol	

Table 2:- Ligands structure drawn using Marvin Sketch 19.8.

Ligands		Binding Energy (Kcal/Mole) with 1ALU				
Selected	Active	Argus	SwissDock	Hex8.0	UCSF	PatchDock
Plants *Aloevera*	Compounds Aloe emodin	-8.478	-7.73	-192.16	-8.98	-3.748
Murrraya	Lupeol	-4.656	-6.68	-306.47	-3.45	-5.458
	Campesterol	-4.805	-7.18	-276.46	-5.1	-4.72
	β- sitosterol	-4.267	-2.56	-254.22	-4.23	-5.074
	Aloin	-4.696	-7.31	-247.31	-6.56	-4.232
	Quercitin	-4.556	-7.49	-254.18	-6.2	-4.56
koeinigii	Catechin	-4.615	-6.73	-250.66	-5.9	-3.674
	Phebalosin	-7.884	-6.98	-223.25	-6.48	-3.87
	Mukonicine	-3.592	-6.91	-208.54	-5.9	-2.904
	Epicatechin	-4.600	-7.32	-250.82	-5.6	-3.564

Table 3:- Binding energy (Kcal/mole) of different ligands docked with IL-6 protein (PDB ID:1ALU).