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Aloe Vera as an Antagonist for TNF-A: In-Silico Study

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Abstract:- Rheumatoid arthritis is a chronic inflammatory disease that affects multiple joints. Tumor necrosis factor (TNF-α) is believed to be the major culprit involved in the progression of inflammation. In the present study, we investigated the phytochemicals of *Aloe vera* as antagonist for TNF-α using *in-silico* approach. Firstly the 3D structure of human TNF-α (PDB ID: 2az5) and ligands were retrieved from RCSB PDB and Pubchem database respectively. The molecular docking was done using Hex 8.0.0 CUDA server followed by pharmacological analysis using SwissADME. The study revealed that Quercetin, Kampferol, Aloe-emodin, and Emodin have higher affinity for tumor necrosis factor (TNF-α) satisfying the pharamacological parameter. Thus, these phytochemicals have therapeutic prospective to be developed into drug for managing RA.

Keywords: *Rheumatoid Arthritis, Aloe Vera, TNF-A, Molecular Docking, Flavanoids, Anthraquinones, Pharmacology.*

I. INTRODUCTION

Rheumatoid arthritis (RA) is a systemic autoimmune disease affecting the joints resulting in intense pain, swelling and in severe cases deformity. Multiple factors believed to be associated with RA are genetic, epigenetic, smoking, obesity and environmental [1]. Tumor necrosis factor (TNF-α) is believed to be a major culprit involved in the inflammation process in RA. Currently there are no biomarkers or effective treatment for curing RA. The current available treatment for curing rheumatoid arthritis includes the administration of NSAIDs and DMARDs. These drugs exhibit various side effects such as viral infections, bacterial infections including tuberculosis and in certain cases malignancy [2].

Aloe vera belongs to the Asphodelaceae family is known for its multiple benefits. *Aloe vera* leaf contains vitamins, minerals, enzymes, sugars, anthraquinones or phenolic compounds, lignin, tannic acids, polysaccharide, glycoprotein, saponins, sterols, amino acids and salicylic acid [3]. Aloe contains two major active components aloe polysaccharides presenting in aloe filet and anthraquinone derivatives present in the leaves of different aloe plants. It is useful in various diseases such as type II diabetes, arthritis, eye disease, tumor, spleen enlargement, liver complaints, vomiting, bronchitis, asthma, jaundice and ulcers. Relieves constipation, maintains a good gastric pH, help in inflammatory bowel diseases, non-ulcer dyspepsia, gastric and duodenal ulcers [3, 4].

The current investigation aimed at assessing the inhibitory effects of *Aloe vera* phytochemicals against TNF-α using *in-silico* approach as it is rapid and cost effective. Molecular docking using Hex CUDA 8.0 server and pharmacokinetic analysis using SwissADME has been carried out for the phytochemicals which included flavonoids, steroids and anthroquinones present in *Aloe vera.*

II. METHODOLOGY

➢ *Preparation of Target Protein and Ligands*

The crystallized 3D structure of human TNF-α (PDB ID: 2az5) was retrieved from Research Collaboratory for Structural Bioinformatics (RCSB) protein data bank. The retrieved TNF- α (PDB ID: 2az5) of resolution 2.1Å consists of four chains (A, B, C, and D) with 148 amino acids.

The structure of the ligands of *Aloe vera* which included the flavonoids, steroids and anthraquinones were retrieved from the PubChem database from NCBI [5]. The retrieved structures of the ligands were minimized for their potential interactions with the target protein TNF-α for molecular simulations.

➢ *Molecular Docking*

Molecular docking analysis for the retrieved ligands with their corresponding target protein (PDB: 2az5) was carried out using Hex 8.0.0 CUDA server [6]. The 3D crystal structure of human TNF-α (PDB ID: 2az5) was complexed with an inhibitor molecule (307) [7] which was eliminated and docking with TNF-α was performed.

The affinity of the ligands was compared with the approved biologic DMARD's that are specific for TNF-α using docking Hex 8.0 CUDA server. Structure of standard biologic DMARD's adalimumab (PDB ID:3wd5), golimumab (PDB ID:5yoy) and infliximab (PDB ID: 5vh3) were retrieved from the RCSB protein data bank and were selected and docked against TNF-α followed by its comparison with the docking results of various components of *Aloe vera.*

➢ *Pharmacology Analysis*

The pharmacokinetic profile prediction for the ligands were conducted using SwissADME server (http://www.swissadme.ch/) which involves the absorption,

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metabolism, distribution, excretion, and toxicity prediction of the potential compounds. Further it analyzes the bioavailability score, drugability and synthetic accessibility score of the potential compounds [8].

III. RESULTS & DISCUSSION

Inflammatory autoimmune disease rheumatoid arthritis affects nearly 1% of the population worldwide resulting in socio-economic burden. The current treatment for RA possesses various drawbacks [9]. Therefore, there is a need to look for an alternate therapeutic intervention in managing the disease with negligible side effects.

The objective of the present study is to investigate the potential of phytochemicals to target the cytokine TNF-α, thus exhibiting there anti-inflammatory activity. The retrieved 3D structure of the target protein TNF-α from PDB database at RCSB was visualized (Figure 1) and stereochemical properties were evaluated. Based on the literature review, 11 phytochemicals were selected for the study. Table 1 enlists the phytochemical of *Aloe vera* which included the anthraquinones, flavonoids and steroids with their corresponding molecular weight, molecular formula, hydrogen acceptor, hydrogen donor and their corresponding structures.

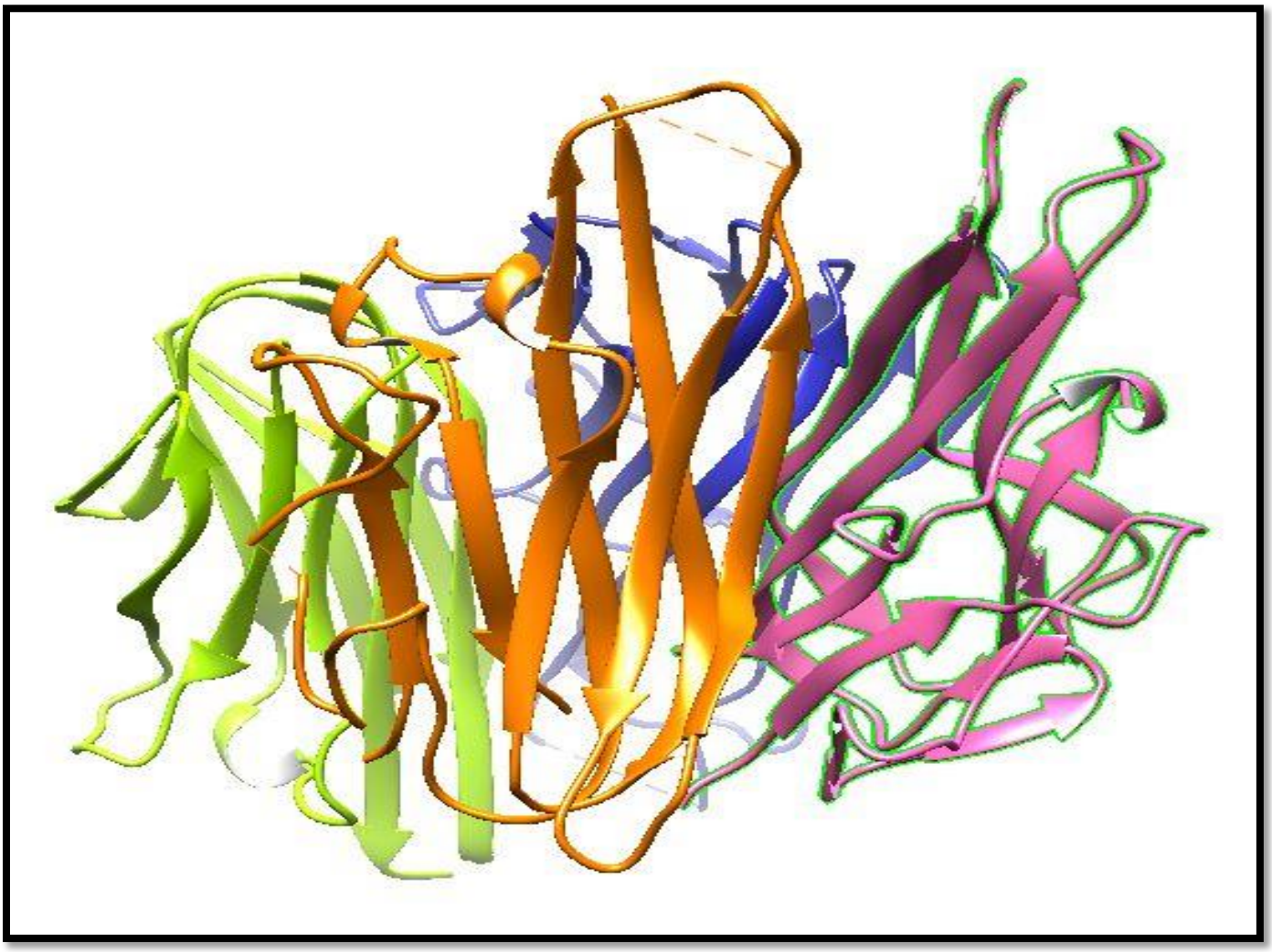

Fig 1:- 3D structure of TNF- (PDB ID: 2az5) with four chains A (orange), B (pink), C (yellow) & D (blue).

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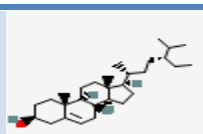
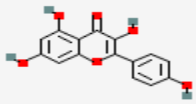
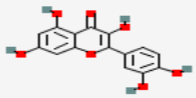
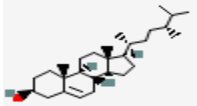
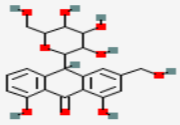
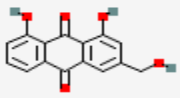
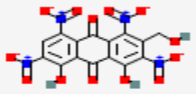
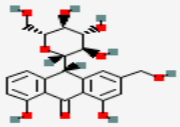
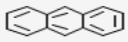
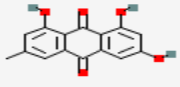
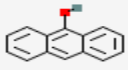
Ligands	Pubchem CID	Molecular formula	Molecular weight (g/mol)	Hydrogen Bond Donor Count	Hydrogen Bond Acceptor Count	2D Structure
β-sitosterol	222284	$C_{29}H_{50}O$	414.718	1	1	
Kampferol	5280863	$C_{15}H_{10}O_6$	286.239	4	6	
Quercitin	5280343	$C_{15}H_{10}O_7$	302.238	5	7	
Campesterol	173183	$C_{28}H_{48}O$	400.691	1	1	
Aloin	313325	$C_{21}H_{22}O_9$	418.398	7	9	
Aloe-emodin	10207	$C_{15}H_{10}O_5$	270.24	3	5	
Aloetic acid	5464178	$C_{15}H_6N_4O_{13}$	450.228	3	13	
Barbaloin	12305761	$C_{21}H_{22}O_9$	418.398	7	9	
Anthracin	8418	$C_{14}H_{10}$	178.234	0	0	
Emodin	3220	$C_{15}H_{10}O_5$	270.24	3	5	
Anthranol	10731	$C_{14}H_{10}O$	194.23	1	1	

Table 1:- Properties of phytochemicals of *Aloe vera* ligand molecules.

The molecular binding score of the phytochemicals of Aloe vera and the standard biologics against target protein TNF-α is presented in Table 2. As it is obtained from the results that the hex score of Adalimumab is highest with -654.41 Kcal/mol followed by Infliximab with score -572.75 Kcal/mol, while amongst the screened phytochemicals Campesterol, β-sitosterol of *Aloe vera* have exhibited highest score -303Kcal/mol and -318.98 Kcal/mol score.

	Ligands	Hex score (Kcal/mol)
Biologics DMARD's	Adalimumab	-654.41
	Infliximab	-572.75
	Golimumab	-94.11
Aloe vera	Quercetin	-251.63
	Kampferol	-251.86
	Campesterol	-303.33
	β-sitosterol	-318.98
	Aloin	-293.40
	Aloe-emodin	-253.46
	Barbaloin	-295.24
	Emodin	-253.57
	Anthracin	-211.47
	Anthranol	-223.42
	Aloetic acid	-338.64

Table 2:- Docking results of the phytochemicals and the standard biologics using Hex 8.0 server.

Further, for a molecule to be developed as drug the pharmacological analysis is needed which was done using online server SwissADME. Based on the pharmacological parameters the drug molecule should be hydrophobic, lipophilic. Also it should have higher adsorption and must satisfy the lipinkis rule of drug likeliness. According to the Lipinski rule the molecule should have molecular weight < 500, log P < 5, hydrogen bond donor <5 and hydrogen bond acceptor <10 (10). Based on the SwissADME results (as shown in Table 3) the absorption of phytochemicals is represented by Topological polar surface area (TPSA) while logP indicates the partition coefficient. The results showed that the phytochemicals Quercetin, Kampferol, Aloe-emodin and Emodin have high GI absorption, bioavailability of 0.55 and synthetic accessibility of 3.23 for Quercetin, 3.14 for Kampferol, 2.57 and 2.60 for Aloe-emodin. They also obeys Lipinski rule for druglikeliness.

Name of Ligands	TPSA (Å)	Bioavailability score	Synthetic accessibility	logP	GI absorption
β-sitosterol	20.23	0.55	6.30	4.79	Low
Kampferol	111.13	0.55	3.14	1.70	High
Quercetin	131.36	0.55	3.23	1.63	High
Campesterol	20.23	0.55	6.17	4.92	Low
Aloin	167.91	0.55	4.97	1.86	Low
Aloe-emodin	94.83	0.55	2.60	1.95	High
Aloetic acid	278.11	0.55	3.36	-2.17	Low
Barbaloin	167.91	0.55	4.97	1.86	Low
Anthracin	61.45	0.55	1.00	2.41	Low
Emodin	94.83	0.55	2.57	1.81	High
Anthranol	20.23	0.55	1.00	2.14	High

Table 3:- Absorption, Distribution, Metabolism and Excretion properties of phytochemicals present in *Aloe vera*

The steroids Campasterol and β-sitosterol has demonstrated the highest affinity for TNF-α but fails to obey the pharmacological parameters and violates the Lipinski rule of druglikeliness. Also synthetic accessibility for Campasterol and β-sitosterol was the highest among all other phytochemicals indicating their synthesis is difficult. While the flavonoid Quercetin and Kampferol, anthraquinone Aloe-emodin and Emodin have emerged out as the potential TNF-α inhibitors, satisfying all the pharmacological parameters to be developed as an effective drug. The binding energy exhibited by Quercetin, Kampferol, Aloe-emodin and Emodin is -251.63, -251.86 -253.46 and -253.57Kcal/mol respectively as shown in Figure 2. In addition to this, they also satisfy all rules of pharmacology and have leadlikeness. Therefore, these phytochemicals can be flourished into drug molecules targeting cytokine TNF-α.

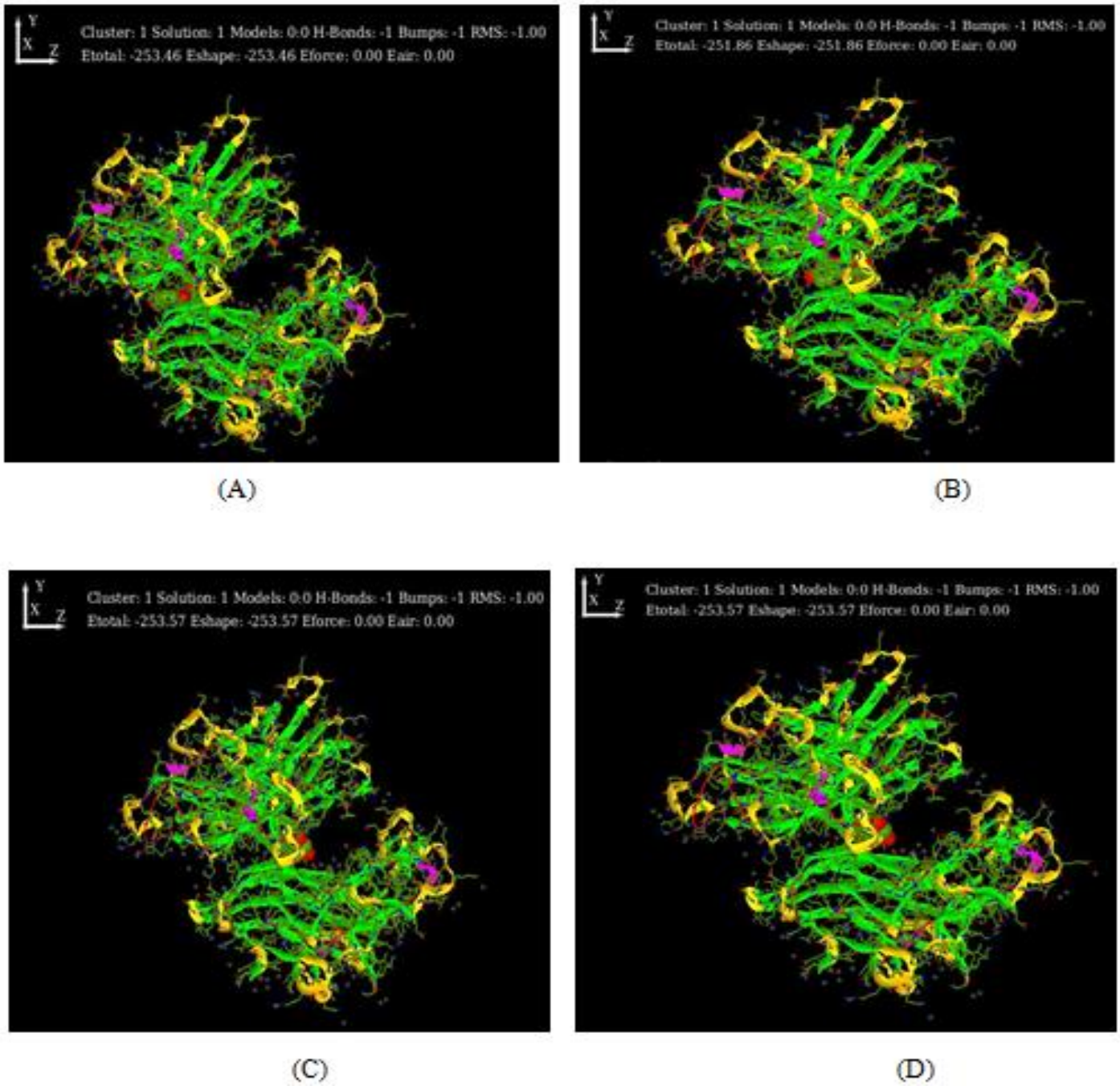


Fig 2:- Molecular docking result of (A) Quercetin, (B) Kampferol, (C) Aloe-emodin and (D) Emodin using Hex 8.0.0 CUDA server.

IV. CONCLUSION

The present study focuses on the anti-inflammatory potential of *Aloe vera* by targeting the cytokine TNF-α. Molecular docking and further pharmacological analysis are fruitful methodologies in finding novel and effective drugs that show great impact for the future studies. Our study demonstrated that the phytochemicals Quercetin, Kampferol, Aloe-emodin and Emodin present in *Aloe vera* possess strong inhibitory activity against TNF-α. These can be useful in managing RA and other inflammatory diseases. Therefore, they can be used as a dietary supplement or can be formulated into novel drug after further in-vitro and in-vivo validation.

CONFLICT OF INTEREST

All authors declare no potential conflict of interest.

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