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MOLECULAR DOCKING AND ADMET ANALYSIS OF NATURAL PHYTOCONSTITUENTS ON TOLL-LIKE RECEPTOR - 4 AND NOD-LIKE RECEPTOR - 3 INHIBITORS USED FOR THE TREATMENT OF RHEUMATOID ARTHRITIS

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ABSTRACT: Rheumatoid arthritis (RA) causes joint disability due to pannus formation, synovitis, joint erosion, joint destruction and cartilage damage. Disease-modifying antirheumatic drugs (DMARDs) and nonsteroidal anti-inflammatory drugs (NSAIDs) prevent the disease progression but suffer the disadvantage of severe adverse drug reactions. An extensive literature search revealed reports of 176 phytoconstituents having anti-inflammatory and/or anti-arthritic activity. Their binding affinities to TLR-4 and NLRP3 receptors were predicted by molecular docking using target proteins of TLR4-MD2 (PDB ID: 3FXI) and chain A and chain B of NLRP3 (PDB ID: 6NPY). Docking score results revealed that 11 phytoconstituents with promising inhibition of TLR4-MD2 along with 27 phytoconstituents inhibiting binding to Chain A of NLRP3 and 65 phytoconstituents binding to Chain B of NLRP3 proteins were identified through an extra precision (XP) docking study by comparing standard antagonists of TLR4 such as Resatorvid and standard antagonists of NLRP3, *i.e.,* MCC950 and Anakinra. Further, we analyzed the pharmacokinetic properties (absorption, distribution, metabolism, excretion, and toxicity) of selected phytoconstituents using pKCSM. Thus, this study reveals that the selected phytoconstituents could have promising anti-inflammatory and antiarthritic activity in rheumatoid arthritis.

INTRODUCTION: Rheumatoid arthritis (RA) is a systemic, chronic autoimmune disease that indicates nonspecific inflammation of peripheral joints and the destruction of articular tissue leads to joint deformities [1]. The causes for the progression of Rheumatoid arthritis is unknown [2]. It affects about 0.8% of the world population. The prevalence of rheumatoid arthritis is 0.7% in the adult Indian population and 0.06% in Gujarat [3].

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Apart from the elderly population and women, adults are equally affected, which is a major concern as the disease causes disability, progression of mortality and comorbidity. The pathophysiology involves the role of a combination of various factors such as age, environment, genetic factors, hormonal levels, endotoxins from pathogens and imbalance in gut microbiota.

All of these results in autoimmune reactions followed by inflammatory cascades leading to bone destruction, pannus formation, bone erosion and synovitis [4]. Pathophysiology of RA is still unclear due to the involvement and cross-bridging of different cellular pathways related to chronic inflammation, metabolic syndrome, autoimmunity,

genetic and environmental factors. The drug therapy is mainly focused on disease-modifying agents (DMARDs) which include small molecules such as methotrexate, leflunomide, chloroquine, hydroxychloroquine, sulfasalazine and monoclonal antibodies targeting inflammatory mediators such as infliximab, adalimumab, rituximab, tocilizumab *etc* [5]. Innate immunity activation leads to inflammation due to monocytes (macrophages), dendritic cells and osteoclast release. Macrophage activation in an inflamed synovial membrane stimulates the expression of chemokine and cytokines, which acts as a chemoattractant protein at the site of inflammation [6].

Therapeutic drug action supports this theory because the treatment given for RA decreases cytokine levels in the initial stages. Antigen-presenting cells called dendritic cells mainly involves in both adaptive and innate immunity. It guides the newly formed T cells from other antigen-presenting cells (APC), which release pro-inflammatory mediators, NK cells infiltrate synovial fluid and tissues [7]. Osteoclasts are formed on activation of NF-κB ligand (RANKL), regulated by pro-inflammatory cytokine abundant in the synovial membrane. A pro-osteoclastogenic factor stimulates T cells that produce IL-17, a major cytokine in osteogenesis [8]. Natural killer cells are lymphocyte that is neither B nor T cell, found in normal individuals. They are known as natural killers of tumor or virus-infected cells. In RA, NK cells play a role in cell-cell interaction, which is a common factor for persistent inflammation; being an immunological component, these cells help in the secretion of cytokines and stimulate the release of T cell and B cells, which progress towards erosion and Pannus formation in the synovial membrane [9].

Toll-like Receptor (TLR) activation has a pivotal role in the progression of RA by causing Inflammation [10, 11]. Immunological components such as Damage associated molecular patterns (DAMPs) and pathogen-associated molecular patterns (PAMPs) activate the innate immune system by modulating the pattern recognition receptors (PRRs), *i.e.,* Toll-like receptor-4 and NOD-like receptor-3 as well as the danger signals released from damaged cells in infectious and inflammatory conditions [10]. Both types of PRRs (TLR 4 and NLRP3) are widely expressed on various immune cells and recognize distinct microbial products such as lipopolysaccharides nucleic acids, heat shock protein (by TLR), uric acid and MDP (by NLRP3). Additionally, injury to various cells (damaged cells) produces several mediators, such as reactive oxygen species and fibrogenic cytokines [12]. TLR4 subfamily is a trigger factor known as highly expressed proteins in the synovial lining and sub-lining layer of RA synovium. Stimulation by LPS for activation of TLR4 to the endosomal surface, which internalized and triggered the TIRAP/MyD88 pathway, leads to TLR4 signaling by TRAM/TRIF from endosome to activate NF-κB and transcription factor IFN-3.

Another activation of the TRAF6 pathway releases inflammatory mediators NF-κB and AP-1. Activation of TLR4 increases the production of reactive oxygen species and causes chronic inflammation. These receptors produce endotoxemia, infiltrate the cell and stimulate cytokines to release like TNF-α, IL-6, and NF-κB [11]. Another intracellular receptor NLRP3 belonging to the NOD-like receptor family present on various cells like macrophages and neutrophils [7]. It has a role in causing inflammation by cleaving and proliferating pro-inflammatory cytokines via sensing the danger signals. There are two processes for activating inflammation: (1) upregulation of pro-inflammatory cytokine *via* TLR4 induced by LPS and (2) PAMPs, DAMPs and MAMPs modulating NLRP3 signal and activate inflammation by releasing inflammatory cytokine *i.e.,* NF-κB [13].

Other target protein NLRP3 inflammasome activation is a key factor for the innate immune system by diverse stimulation, mitochondria dysfunction, production of reactive oxygen species, lysosome damage, multiple molecular and cellular events including ionic flux activate caspase-1 and secrete pro-inflammatory mediators IL-1β/IL-18 cause cellular damage [13]. Activated NLRP3 inflammasomes have a role in various inflammatory diseases, including Rheumatoid arthritis, Alzheimer's disease, diabetes, and atherosclerosis. But these signaling pathway is not fully understood in Pathological condition associated with RA [14]. All of the current treatments used for RA suffer from the possibility of several

adverse effects. Also, most agents are quite costly and not affordable by many. Several herbal drugs have shown efficacy in experimental studies. However, due to lack of systematic and mechanism-based studies has resulted in poor acceptability for long-term therapy. There is a need to do specific target-based studies with well-known phytochemicals to apply them in therapeutics. Out of the various targets, it is observed that TLR-4 and NLRP3 play a central role in the pathophysiology. Modifying them can prevent autoimmune reactions and significantly reduce the inflammatory reactions [10].

Therefore, we intend to undergo a systematic approach in searching out a total of 176 phytoconstituents which can alter the effects of TLR4 and NLRP3. The approach involved *in-silico* screening of phytoconstituents followed by cell-line-based studies to shortlist the phytoconstituents which can target specifically the TLR4 and NLRP3. The combinations of these shortlisted phytoconstituents will be further tested on animal models of rheumatoid arthritis to develop a rational combination of phytoconstituents which has high efficacy and less toxicity. This rational combination will be developed into a suitable formulation.

EXPERIMENTAL:

Preparation of Proteins: The selected proteins TLR4-MD2 complex (PDB ID: 3FXI) and NLRP3 (PDB ID: 6NPY) 3D structure was downloaded from RCSB Protein Data Bank (https://www.rcsb.org/structure/3FXI) and (https://www.rcsb.org/structure/6NPY) from the X-ray crystal of selected proteins TLR4-MD2 complex and NLRP3 having atomic resolution of 3.10 Å and 3.80 Å respectively [15, 16]. Protein preprocessing was done by importing these selected proteins and process by using the protein preparation wizard (Prep Wizard) of Schrodinger suite (Maestro 11.6). The protein structures were refined by précising stearic interactions, hydrogen bond consistency, removal of a water molecule, orders of bond, ionization state, optimization and minimization of energy by using OPLS3e force field [17]. The more stable minimized proteins were selected for molecular docking study.

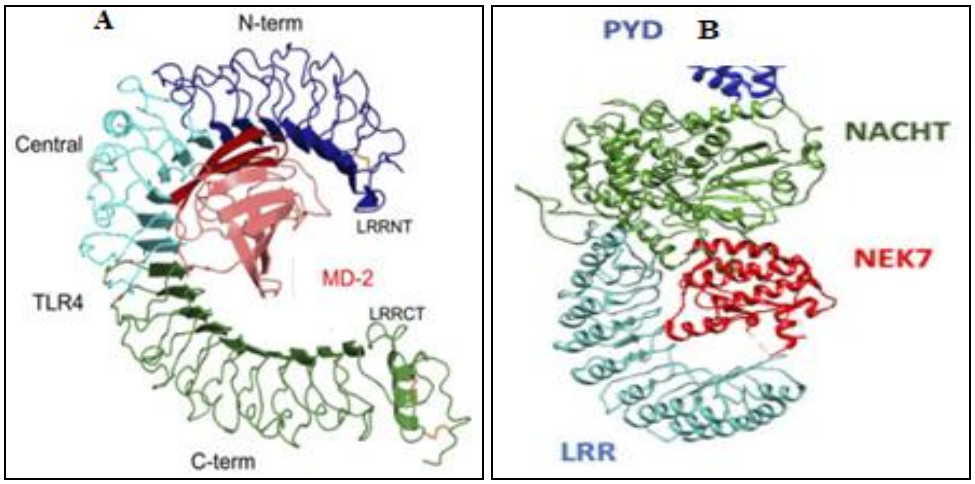

FIG. 1: (A) THE 3D STRUCTURE OF TLR4-MD-2 COMPLEX (PDB ID: 3FXI) (B) THE 3D STRUCTURE OF NLRP3 PROTEIN (PDB ID: 6NPY CONTAINS NACHT, LRR DOMAINS AND PYD DOMAIN)

Preparation of Ligands: A total of 176 natural phytoconstituents reporting anti-inflammatory as well as antiarthritic activity were selected through a literature survey and their 3D structures were downloaded from different databases *i.e.*, PubChem, SciFinder *etc* [18, 19]. From the ligand preparation wizard of Schrödinger module, LigPrep was used to prepared by generating stable states of ligands with pH 7.0 ± 2.0 using Epik, by eliminating bias of bond length and its angle, adding metal binding states, de-saltation, tautomer's generation and stereoisomers by applying OPLS3e (Optimized Potentials for Liquid Simulations) force field [20].

Site Map Analysis: This analysis is mostly useful for the selected protein PDB IDs without co-crystallized ligands or inhibitors for binding sites with ligands and their functionality [21]. from the analysis we get a site score known as druggability

score (D-score) which indicates the binding of ligands to the selected proteins. The highest D-score indicates the accurate binding towards the selected proteins.

Molecular Docking:

Receptor Grid Generation: From site map analysis grid generates for protein by using GLIDE (Grid-based Ligand Docking with Energetics) size of 10 Å to determine the binding site and size of the active site of protein [22].

Ligand Docking: GLIDE module used for docking the ligands to the selected active site of proteins by extra precision (XP) for generating 10 poses per ligand. The obtained docking score ≥ 8 is selected for the most effective binding with the active site of a protein.

ADMET Analysis: pKCSM web tool was used for predicting the absorption, distribution, metabolism, excretion and toxicity (ADMET) analysis of all the top ligands [23, 24].

RESULTS AND DISCUSSION:

Sitemap Analysis: From sitemap analysis for selected protein obtained binding site for chain A of TLR4-MD2 the D score and site score was obtained 1.065 and of 1.047. The NLRP3 obtained D score was 1.146 and Site score was 1.117.

TABLE 1: OBTAINED D-SCORE AND SITE SCORE OF TLR4-MD2 AND NLRP-3 FROM SCHRODINGER SUITE

Entry	TLR4-MD2 (PDB ID:3FXI)		NLRP3 (PDB ID: 6NPY)	
	D-score	Site score	D-score	Site score
1	1.065	1.047	1.146	1.117
2	1.054	1.030	1.064	1.078
3	1.000	1.029	1.017	1.065
4	0.983	1.022	1.013	1.051
5	0.946	0.990	0.996	1.019

Molecular Docking: It is mainly used for predicting the accurate binding site of protein with ligands for getting the best phytoconstituent with highest docking score by using Schrödinger software through module of GLIDE having protein size 10 Å by extra precision (XP) mode [22]. According to the literature survey, we select a docking score ≥ 8 with good binding affinity towards both target proteins. Here there are two target proteins, TLR4-MD2 as well as the NLRP3 binds with ligands which shows favorable docking score indicates may have potential to inhibit the disease. Here we enumerate those selected ligands having highest docking score with its interaction of target protein *i.e.,* TLR4-MD2 and NLRP3.

6-p-coumaroyl sucrose binds active site chain A of target protein TLR4-MD2 form hydrogen bond by Interacting Glu 154, Asn156, Asp84, Asp99, Asp181 and Arg106 residues possessing highest docking score -8.998. **Table 2**, Entry 1, **Fig. 2I**. The binding of 6-transcaffeoyl ajugol with chain A of TLR4-MD2 obsess docking score -8.87 initiating hydrogen bond accompanied by Asn156, Asp84, Asp99, Asp100, Arg106 and Asp181. **Fig. 2II** Delphinidin 3, 5-di-O-(6-O-malonyl-β-D-glucoside) formed HB with Asn156, Lys130, Arg106, His179, Asp100 and Asp209, Lys230 form salt bridging with docking score of -10.291 **Fig. 2III**. Cyanidin 3-O-p-(6-O-malonyl)-D-glucopyranoside interact by forming HB with Arg106, Asn156, Hie179, Arg 134 and salt bridging form with Lys230, Asp209 and Lys130 binding site of TLR4-MD2. The literature review suggested that chains A and B of TLR4 having residues of Cys29, Cys40, Glu24 to Lys47 antagonize the binding of TLR4 to MD-2 [25]. On the other side, MD2 residues also consist of Cys95, Cys105, Asp99, Asp100 and Asp101 residues responsible for retarding the binding of TLR4 [26]. These residues are present in various phytoconstituents of plants *i.e.,* 6-p-coumaroyl sucrose, 6-transcaffeoyl ajugol *etc*. which could be responsible for the inhibitory activity of TLR4.

We also bind all phytoconstituents to other target protein NLRP3 having active binding site of chain A and chain B. kigelinone enact hydrogen bond with Ile475, Lys550 and π– π cation with Tyr563 residue of Chain A of NLRP3 obsess docking score -10.113 **Fig. 3I**. Verminoside got bound chain A of NLRP3 forming π cation with Arg643 and hydrogen bond with Ser624, Lys550, and Tyr532 residues possess -10.112 docking score. **Fig. 3II** 5-caffeoyl quinic acid form a hydrogen bond with Gly564, Ser624, Val544, and Tyr532 residues and

π cation with Arg643 residue on chain A of NLRP3 with docking score -9.573. **Fig. 3III** 3-o-p-coumaroyl quinic acid form hydrogen bond with Ser624, Lys550 and Tyr532 residues and π cation with Arg64 of NLRP3 binding site of chain A possess docking score-8.676 **Fig. 3IV** Wilkstromal (Ser547, Ile475, Ser624 and **Fig. 3V** and α-bisabolol (Tyr532, **Fig. 3VI** accompanied hydrogen bond with docking score -10.412 and-8.741 respectively. Wilkstromal also forms π cationic interactions with Arg643 of chain A binding to the target site of NLRP3 protein.

TABLE 2: DOCKING SCORE (kcal/mol) ≥ 8 OF SELECTED PHYTOCONSTITUENT IN XP MODE

Entry	Name of phytoconstituents	Docking score (kcal/mol)		
		TLR 4-MD2 (PDB ID: 3FXI)	NLRP3 (PDB ID: 6NPY) Bınding to chain A	NLRP3 (PDB ID: 6NPY) Binding to chain B-NEK7
1	6-p-coumaroyl-sucrose	-8.998	-8.514	-13.262
2	6-transcaffeoyl ajugol	-8.87	-9.105	-12.479
3	kigelinone	-	-10.113	-9.485
4	lapachol	-	-7.38	-9.004
5	7-hydroxyeucommic acid	-	-8.555	-8.036
6	verminoside	-	-10.112	-13.642
7	quercetin	-	-7.368	-11.86
8	ethylgallic acid	-	-7.443	-9.097
9	chlorgenic acid	-	- 8.834	-11.057
10	β-sitosterol	-	-	-9.47
11	ϒ-sitosterol	-	-	-9.47
12	Kigelinol	-	-	-10.232
13	Isokigelinol	-	-	-10.232
14	Isopinnatal	-	-	-9.681
15	7-hydroxyviteoid-II	-	-	-9.59
16	Ajugol	-	-	-10.872
17	Catalpol	-	-	-12.041
18	Specioside	-	-	-11.166
19	1-dehydroxy 3,4 dihydro-aucubigenin	-	-	-9.056
20	cyanidin-3-O-galactoside	-7.170	-	-9.135
21	kaempferol-7-O-glucuronide	-7.047	-	-13.455
22	kaempferol-3-O-glucuronide	-7.047	-	-13.455
23	4,5-dicaffeoylquinic acid	-8.526	-7.781	-11.02
24	Cyanidin 3-O-p-(6-O-malonyl) D-glucopyranoside	-8.768	-	-9.337
25	crepidiaside-B	-7.108	-	-9.904
26	cichorioside B	-8.187	-	-11.397
27	sonchuside C	-7.185	-	-9.379
28	Delphinidin 3,5-di-O-(6-O-malonyl-β-D-glucoside)	-10.291	-	-
29	5-caffeoyl quinic acid	-	-9.573	-11.223
30	5-caffeyl shikimic acid	-	-7.106	-12.467
31	4-O-feruloyl quinic acid	-	-7.364	-11.117
32	1,4-dicaffeoylquinic acid	-	-7.823	-11.353
33	8-deoxylactucin	-	-7.967	-10.586
34	Jacquilenin	-	-7.063	-9.333
35	Delphinidin	-	-8.274	-9.368
36	3-O-p-coumaroyl quinic acid	-	-8.676	-
37	n-heneicosane	-	-8.047	-
38	3,5-dicaffeoylquinic acid	-	-7.907	-10.115
39	Campesterol	-	-	-9.375
40	oxalic acid	-	-	-11.777
41	shikimic acid	-	-	-8.029
42	crepidiaside A	-	-	-9.828
43	Lactucin	-	-	-8.872
44	Lactucopicrin	-	-	-9.473
45	11β,13-dihydrolactucin	-	-	-8.093

46	Magnolialide	-	-	-8.658
47	sonchuside A	-	-	-9.769
48	Artesin	-	-	-8.658
49	3-O-p-coumaroyl quinic acid	-	-	-10.275
50	3-caffeoyl quinic acid	-	-	-8.197
51	4-caffeoyl quinic acid	-	-	-12.252
52	cis-5-caffeoyl quinic acid	-	-	-11.06
53	cis-caftaric acid	-	-	-9.568
54	trans-caftaric acid	-	-	-9.568
55	Cyanidin	-	-	-9.783
56	malvidin-3-O-glucoside	-	-	-9.125
57	1,3-dicaffeoylquinic acid	-	-	-10.761
58	3,4-dicaffeoylquinic acid	-	-	-10.237
59	kaempferol-7-O-rutinoside	-	-	-10.529
60	kaempferol-3-O-glucoside	-	-	-8.947
61	kaempferol-7-O-neohesperidoside	-	-	-17.313
62	Cichoralexin	-	-	-9.533
63	β-sitesterol	-	-	-9.167
64	Myricetin	-	-7.864	-11.325
65	Quercetin	-	-7.569	- 9.116
66	Wikstromal	-	-10.412	-10.806
67	α-bisabolol	-	-8.741	-
68	β-copaene	-	-8.442	-
69	α-atlantones	-	-8.652	-
70	β-atlantones	-	-8.447	-
71	isorhamnetin	-	-	-8.957
72	matairesinol	-	-	-10.026
73	Resatorvid	-2.587	-	-
74	MCC950	-	-	-5.162
75	Anakinra	-	-5.066	-

We represent dock poses of selected phytoconstituents by using PyMol software, whereas ligands are represented by ball stick models (yellow colored), and protein is represented by colored cartoons, respectively [27]. Verminoside **Fig. 4A** makes a hydrogen bond with Thr167, Arg165, Gly229 and Lys230 along with π cationic interaction with His520, π-π cationic interactions with Trp414, Phe506 and salt bridge forming with His520, Lys230 residues of chain B of NLRP3.

A total five HB with residues His520, Gly227 (2HB), Arg165, Glu150 and one π-π cationic interaction with His520 **Fig. 4B** were formed by 6-p-coumaroyl sucrose binds chain B of NLRP3. The docked complex of kaempferol-7-O-neohesperidoside with chain B of NLRP3 manifests a hydrogen bond with Thr167, Glu150, π-π cationic interactions with Tyr379, salt bridge with Arg 165, Lys230 and Arg235 residues **Fig. 4C**. The kaempferol-7-O-glucuronide was found to bind at the junction of chain B of NLRP3 forming HB interactions with residues of Thr167, Arg165, Asp151, salt bridge with His520 and Arg235 **Fig. 4D**. Myricetin construct hydrogen bond with Thr167, Asn151 and Arg165 residues **Fig. 4E**. Whereas, wilkstromal was bound at the active site of chain B NLRP3 with residues Thr167 and Asp151 forming HB, π cationic interactions with Trp414. **Fig. 4F**. According to the literature review Gly231, Lys232 and Thr233 to Ala residues link with reduction of ATP binding thus, impairment of NLRP3 inflammasome-dependent signaling pathway occur [28, 29].

Here, the standard antagonist of TLR4-MD2 Resatorvid and the NLRP3 standard antagonist MCC950 were selected to inhibit Rheumatoid arthritis. Resatorvid **Fig. 3E** formed HB with residues Asp70, Arg69, Ser141, one salt bridge and π– cation with Lys130 having docked score -2.587 while another NLRP3 antagonist MCC950 **Fig. 4G** possess docking score -5.162 interact with residues Thr167 and Arg165 forming HB, π-π cationic interactions with residues of His520 and salt bridge with Arg165 NLRP3 target protein site of chain B.

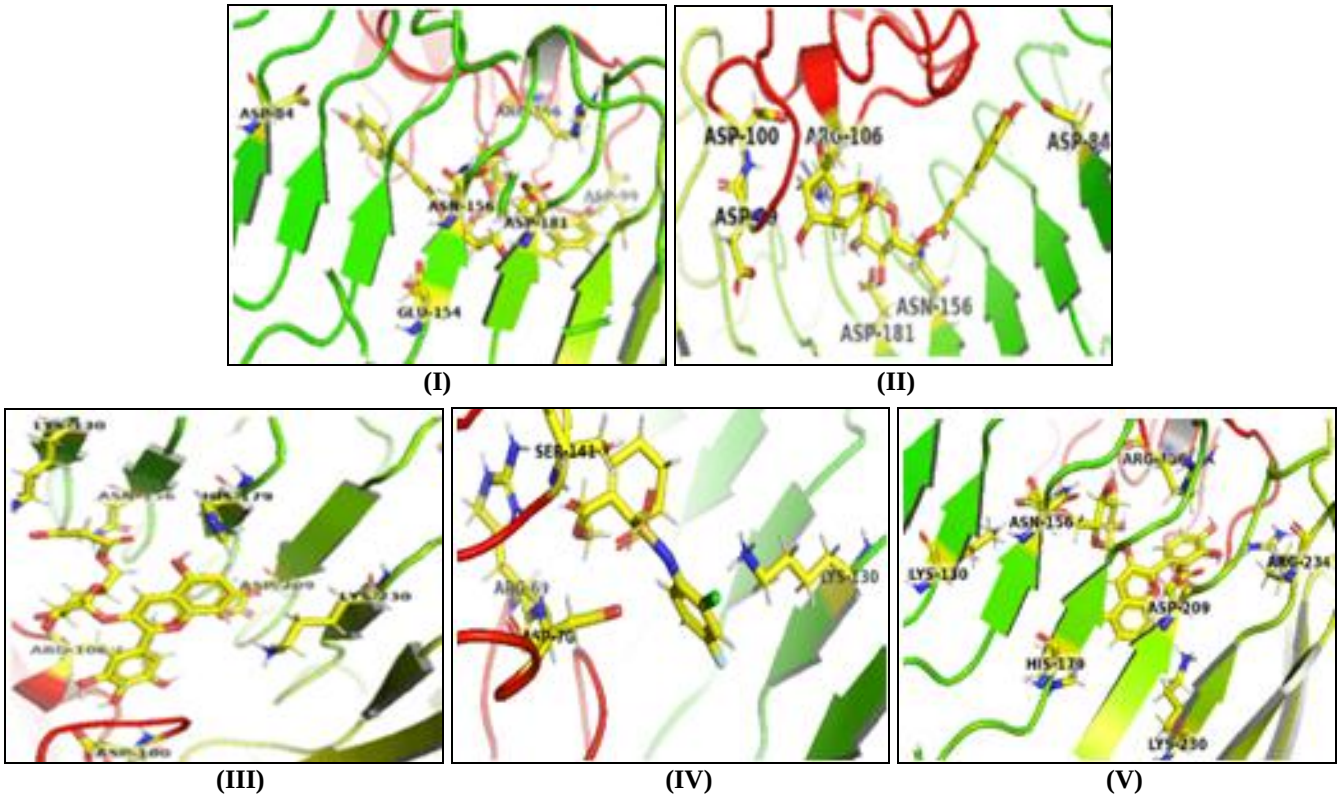

FIG. 2: DOCKED 3D POSES OF SELECTED PHYTOCONSTITUENTS ON TLR4-MD2 PROTEIN: 6-P-COUMAROYL SUCROSE (I), 6-TRANSCAFFEOYL AJUGOL (II), DELPHINIDIN 3, 5 DI-O-(6-O-MALONYL- B-D-GLUCOSIDE) (III), CYANIDIN 3-O-P- (6-O-MALONYL)-D-GLUCOPYRANOSIDE (IV) AND STANDARD ANTAGONIST OF TLR4-MD2-RESATORVID (V)

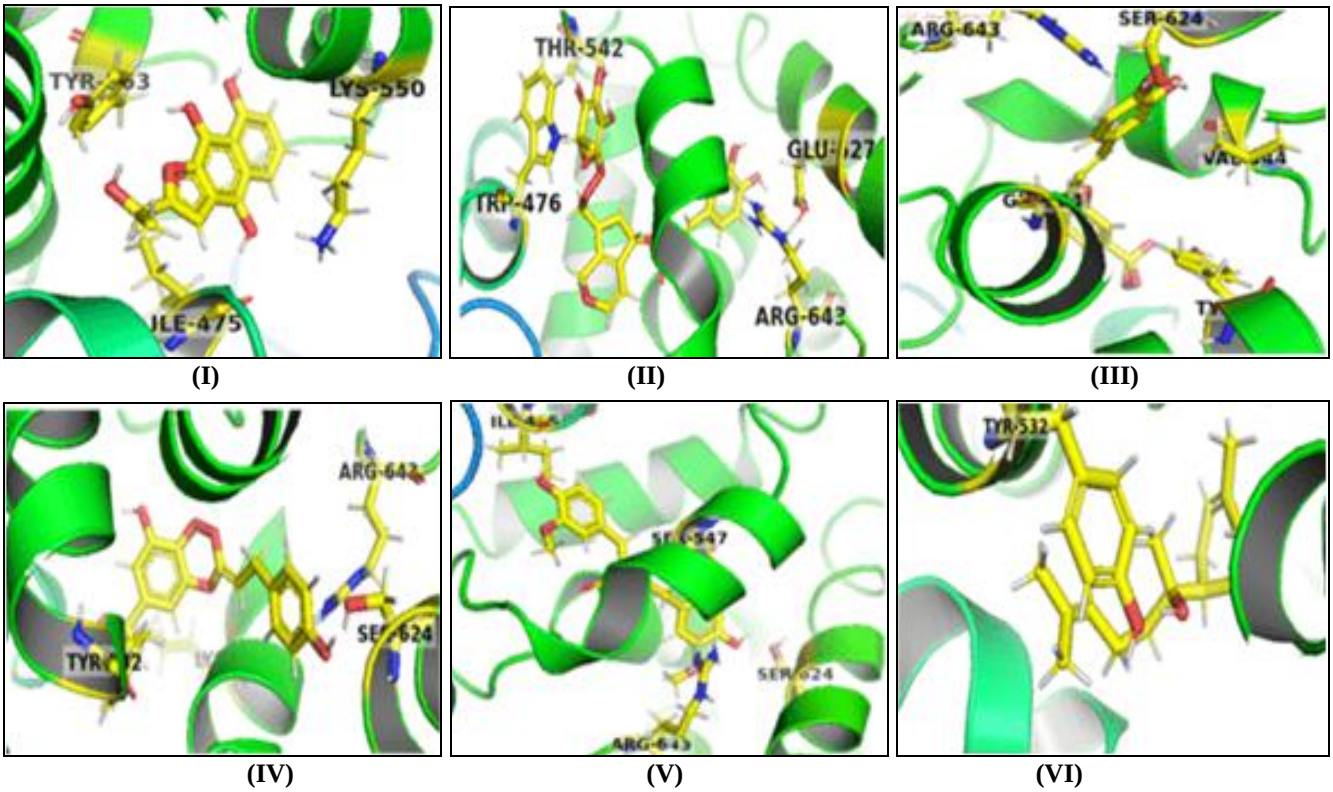

FIG. 3: DOCKED 3D POSES OF SELECTED PHYTOCONSTITUENTS BINDING ON CHAIN-A OF NLRP-3 KIGELINONE (I), VERMINOSIDE (II), 5-CAFFEOYL QUINIC ACID (III), 3-O-P-COUMAROYL QUINIC ACID (IV), WILKSTROMAL (V), α-BISABOLOL

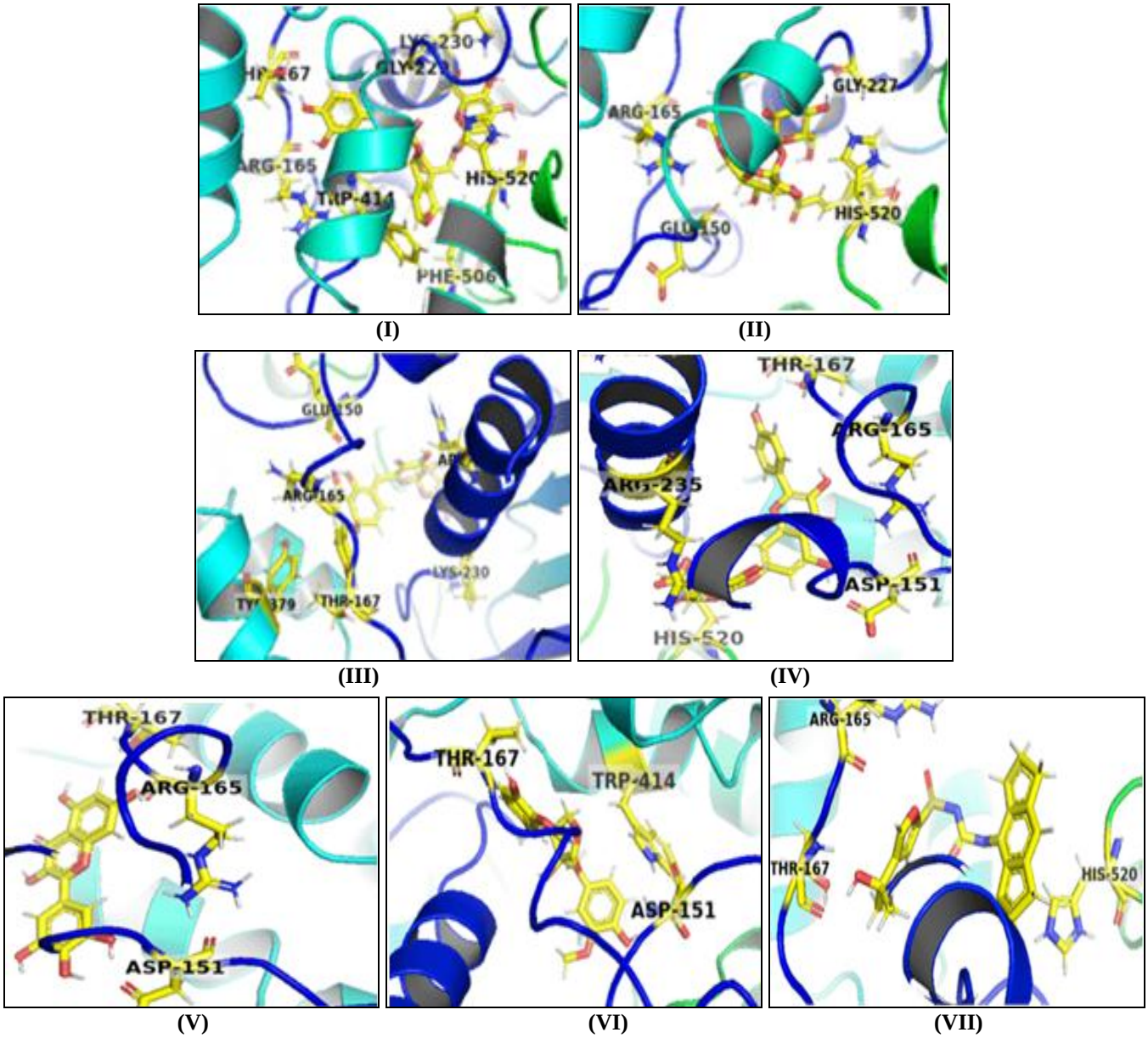

FIG. 4: DOCKED 3D POSE OF SELECTED PHYTOCONSTITUENT ON CHAIN-B OF NLRP3 VERMINOSIDE (I), 6-P-COUMAROYL SUCROSE (II), KAMPFEROL-7-O-NEOHESPERIDOSIDE (III), KAEMPFEROL-7-O-GLUCURONIDE (IV), MYRICETIN (V), WILKSTROMAL (VI), MCC950 (NLRP3 ANTAGONIST) (VII)

ADMET Analysis: The results indicates that all the selected phytoconstituents had good lipophilicity (Log P), water solubility, intestinal absorption, BBB permeability (Log BB), volume of distribution (VD), total clearance, maximum tolerated dose and oral acute Toxicity (LD_{50}) within the recommended range given by pKCSM **Table 3** except compound no: 1, 3, 4 and 10 have not fitted with the recommended ranges for parameter of lipophilicity. As natural products, we can enhance lipophilicity using various methods during formulation-based studies.

TABLE 3: ADMET PROPERTIES OF THE SELECTED PHYTOCONSTITUENTS

No.	Name of phytoconstituents	LipoPhilicity (LogP)[a]	Solubility in water (mol/L)[b]	Intestinal absorption (% absorbed)[c]	BBB permeability (log BB)[d]	VDss (L/kg)[e]	Total clearance (ml/min/kg)[f]	Max tolerated dose (human) log (mg/kg/ day)[g]	Acute Oral Toxicity (LD50) (mol/kg)	AMES Toxicity (Yes/No)[h]
1	6-p-coumaroyl-sucrose	-1.4558	0.0536	29.914	-1.918	4.051	2.1148	1.3579	15.799	No
2	6-transcaffeoyl ajugol	-0.9036	0.03967	37.416	-1.704	1.356	2.2456	0.9231	25.128	No
3	delphinidin 3,5-di-O-(6-O-malonyl-β-D-glucoside)	0.1132	0.05607	5.923	-2.436	2.7787	2.0319	1.4433	2.513	No

4	cyanidin 3-O-p-(6-O-malonyl) D-glucopyranoside	0.4076	0.05254	14.425	-2.608	1.0746	2.5651	1.9798	2.57	No
5	kigelinone	1.8139	0.07863	95.681	0.07	1.548	1.0366	1.2250	8.036	No
6	verminoside	-1.9148	0.07397	35.347	-1.713	1.648	1.9837	0.6563	20.800	No
7	5-caffeoyl quinic acid	1.1922	0.04646	71.359	-1.734	2.866237	1.6454	1.2930	2.373	No
8	3-O-p-coumaroyl quinic acid	1.9491	0.04586	83.333	-1.018	1.680346	1.0140	0.8187	1.786	No
9	Kaempferol-7-O-neohesperidoside	3.1585	0.06911	35.579	-2.489	2.2773	0.5035	1.1571	2.487	No
10	kaempferol-7-O-glucuronide	-0.1522	0.05996	8.361	-1.378	2.5960	1.6454	1.6753	2.549	No
11	Wikstromal	1.8043	0.03445	70.196	-0.861	1.025	1.3034	1.0040	6.930	No
12	α-bisabolol	4.2302	0.01253	93.014	0.605	1.521	3.9078	1.5952	5.691	No
13	Myricetin	1.6936	0.05420	65.93	-1.493	3.732	1.5250	1.5250	12.146	No

Parameters calculated using pKCSM: a. Lipophilicity- log P (≤ 5), b. Descriptor of aqueous solubility (≤ 0), c.Intestinal absorption (human): less than 30% is considered poorly absorbed, and > 30% is considered highly absorbed. d.BBB permeability: Log BB > 0.3 is considered to readily cross the BBB; if Log BB<-1 is poorly distributed to the brain, e. Volume of distribution (human, L/kg): (VDss is considered low if below 0.71 L/kg and high if above 2.81 L/kg.) f. total renal clearance is high (>1 mL/min/kg), medium (>0.1 to 1 mL/min/kg), or low (0.1 mL/min/kg), g. Max. tolerated dose (human): ≤ 0.477 logs (mg/kg/day) is considered low, and > 0.477 logs (mg/kg/day) is considered high, h. AMES toxicity: positive test indicates mutagenic may act as a carcinogen, the negative test indicates non-mutagenic and act as non-carcinogenic.

CONCLUSION: In search of potent inhibitors for rheumatoid arthritis, the selected 176 phytoconstituents obtained from plants of medicinal importance were tested for binding to the active site of target receptors TLR4-MD2 and NLRP3 using molecular docking.

Through extra precision (XP) docking, 11 phytoconstituents with promising inhibition of TLR4-MD2 were identified, as well as 27 phytoconstituents inhibiting binding to chain A of NLRP3 and 65 phytoconstituents inhibiting binding to chain B of the NLRP3 protein with significant docking scores and binding interactions indicating their candidacy as promising rheumatoid arthritis inhibitors. pKCSM results for analysing pharmacokinetic properties show that 6-p-coumaroyl-sucrose, 6-transcaffeoyl-ajugol, verminoside and 3-o-p-coumaroyl-quinic acid would be more suitable drug candidates among other phytoconstituents as they are more selective, potent, non-carcinogenic and non-tumorigenic. This computational study may hold some promise for the design of new antirheumatic drugs that reduce the side effects of current treatments and are more target-oriented.

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