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Original Research Article

Inhibition of TNF-α and IL-1 by compounds from selected plants for rheumatoid arthritis therapy: *In vivo* and *in silico* studies

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Abstract

Purpose: *To investigate the inhibitory activities of herbal compounds from Curcuma longa, Sophora japonica and Camellia sinensis against tumor necrosis factor alpha (TNF-α) and interleukin-1 (IL-1) using in vivo and in silico tools.*
Methods: *The extracts of the medicinal herbs (Curcuma longa, Sophora japonica and Camellia sinensis) were evaluated for immune-modulatory activities based using neutrophil oxidative burst assay. The compounds present in the medicinal herbs were screened for their inhibitory effects against TNF-α (PDB ID: 2AZ5) and IL-1 (PDB ID: 2L5X) using Molegro Virtual Docker 6.0 (MVD). The stabilities of the top docking poses were confirmed by Molecular Dynamics (MD) simulation run for 20 nanoseconds (ns).*
Results: *The herbal compounds exerted strong inhibitory effects against TNF-α (PDB ID: 2AZ5) and IL-1 (PDB ID: 2L5X), implying their therapeutic potential for use in rheumatoid arthritis (RA). Of the compounds, curcumin diglucoside and curcumin monoglucoside showed the strongest inhibitory effects on monocytes, with inhibitory levels of 82.75 and 81.34 %, respectively, while eugenin had the weakest inhibitory activity (11.12 %). In addition, molecular docking scores were consistent with the in vivo results, and revealed strong inhibitory effects of curcumin diglucoside and curcumin monoglucoside against TNF-α and IL-1.*
Conclusion: *Herbal compounds present in Curcuma longa, Sophora japonica and Camellia sinensis possess strong inhibitory effects against the pro-inflammatory cytokines TNF-α and IL-1. Thus, these compounds have therapeutic potentials that can be exploited for the treatment of RA.*

Keywords: *Curcuma longa, Sophora japonica, Camellia sinensis, Rheumatoid arthritis, Cytokines, TNF-α, IL-1, Immuno-modulation, Molecular docking*

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INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disorder in which the immune system targets the joints thereby causing pain and swelling [1]. In some cases, the disease also affects other parts of the body resulting in a low red blood cell count and lung inflammation [2,3]. The exact cause of

RA is not yet fully understood, although some researchers reported that multiple factors such as obesity, smoking, genetic factors and environmental factors may be involved [4,5]. The ultimate goal of treatment of RA is to reduce the pain and inflammation associated with the disease which is usually characterized by cellular activation that leads to autoimmunity.

The presence of immune complexes in the joints lead to swelling and congestion due to immune cell infiltration [6]. The chronic phase of the disease manifests in tissue injury and inflammation due to the release of cytokines such as TNF-α and IL–1 [6].These cytokines are produced by mononuclear leukocytes in response to numerous agents such as endotoxin and lipopolysaccharide [7,8]. Over-expression of TNF-α and IL-1 is a hallmark of many inflammatory diseases such as RA and inflammatory bowel disease. Thus, the two cytokines are potential therapeutic targets for these diseases. Furthermore, in several RA cases, the innate immune cells become activated and release a spectrum of pro-inflammatory mediators such as the TNF-α and IL-1 [9]. Indeed, TNF-α andIL-1 are involved in tissue damage and joint inflammation [10,11].

In patients with RA, the concentrations of TNF-α and IL-1 are high in the plasma and synovial fluid. Thus, inhibiting the effects of these cytokines may be beneficial for treating a variety of inflammatory diseases including RA [12]. The current investigation was aimed at assessing the inhibitory effects of herbal compounds which possess strong medicinal properties against TNF-α and IL-1 using *in vivo and in silico* tools and techniques.

EXPERIMENTAL

Herbal compounds

Compounds present in *Aloe vera*, *Arctiumlappa*, *Camellia sinensis*, *Capsicum annuum*, *Chamaemelum nobile*, *Curcuma longa*, *Curcuma longa*, *Matricari achamomilla*, *Matricaria recutita*, *Myrica cerifera*, *Senegalia catechu*, *Sophora japonica*, *Syzygium aromaticum*, *Tagetes lucida* and *Wikstroemia indica* were purchased from ABI Chem, Germany and Sigma-Aldrich China Inc.

Subjects

Healthy human donors aged 20 - 30 years were used. The procedure of obtaining blood samples was carried out according to the guidelines of the ethics committee of Renmin Hospital of Wuhan University. Twenty milliliters of blood samples were collected from each subject through veno-puncture. The samples were then transferred to EDTA-potassium-monovettes.

Evaluation of immune-modulatory activities of herbal compounds

The immuno-modulatory activities of the procured chemical compounds were evaluated based on chemiluminescence neutrophil oxidative burst assay [13]. The anti-coagulated blood from the healthy donors was used to purify the neutrophils by Percoll density gradient centrifugation and dextran sedimentation according to a slight modification of the procedure described by Luo *et al* [14]. Cells from the purified neutrophils were adjusted to required concentrations using Hank's balance salt solution containing Ca^{2+} and Mg^{2+} (HBSS ++). Thereafter, 25 µL of the neutrophil cells were incubated with 25 µL of serially-diluted 10µM solution of each herbal compound. After 30 min of incubation, the cells were washed with the serially diluted herbal compounds and HBSS++, and thereafter activated by adding 25 µL of zymosan-A, followed by opsonisation with further addition of 25 µL HBSS ++. The oxidative burst results were monitored as chemiluminescence RLU (relative light unit), with peak and total integral values set through repeated scans at 30s intervals and one-second point measuring time [15].

Molecular docking studies

Protein retrieval

Molecular docking simulation studies of the herbal compounds against TNF-αand IL-1were carried out to understand the molecular interactions of these compounds at the active sites of the enzymes and their binding conformations. The crystal structures of TNF-α (PDB ID: 2AZ5) and interleukin 1 (PDB ID: 2L5X) were retrieved from the Protein Data Bank. Structurally, 2AZ5 consists of four chains (A, B, C, and D) with 148 amino acids, and has a resolution of 2.1Å; 2L5X also consists of four chains with (A, B, C, and D) but with resolution of 2.3Å. The A and D chains contain 151 amino acids while the C and D chains contain 98 amino acids.

Cavity prediction

The binding cavities of TNF-α (PDB ID: 2AZ5) and IL-1(PDB ID: 2L5X) were predicted using Molegro Virtual Docker 6.0 (MVD) [16]. A binding site of volume 2432.45$Å^3$ and surface area 5204.48$Å^2$ was predicted for 2AZ5, while a

binding site volume of 33.28 $Å^3$ and a surface area of 143.36$Å^2$was predicted for 2L5X. The binding site was set inside a restricted sphere of X: -15.92, Y: 67.52, and Z: 26.91 for 2AZ5. For 2L5X, the corresponding values were X: 36.43, Y: 2.84, and Z: 12.99. The two binding sites had a radius 17Å and a grid resolution of 0.30Å.

Chemical data set

The 2D structures of the herbal compounds were retrieved from the NCBI PubChem database [17] and their structures were optimized and converted to the 3D format for molecular docking simulations.

Docking set-up

For the molecular docking simulation, only the A chains of both enzymes were considered. The herbal compounds and the protein structures were imported in MVD. The bond flexibility for the side chains of the amino acids was set for the assigned sphere within the restriction of 17 Å of the binding cavity. The flexibility was set with a value of 1.10 tolerance and 0.90 strengths. The RMSD threshold for the multiple cluster poses was set at 2.00 Å with energy penalty value of 100.00. The docking algorithm was with a maximum iteration of 1500 and a simplex evolution size of 50. The docking simulation was run at least 100 times for 10 poses and the best poses were chosen based on the Rerank score, MolDock score and interaction energy [18].

Molecular dynamics (MD) simulation

The MD simulations for the protein-ligand docked complexes were carried out using GROMACS 5.0 installed in Ubuntu Linux 15.0 LTS powered with Intel i7 processor, 8GB RAM [19]. The simulations were processed with standard GROMOS96 43a1 force field (25). The MD simulation was carried out for the top docking hit protein-ligand complexes of TNF-α (PDB ID: 2AZ5) and IL-1 (PDB ID: 2L5X). Initially, the system was immersed in a cubic water box and the energy of the complexes was minimized using the approach of steepest descent energy minimization [20]. Furthermore, the systems were equilibrated for 100ps with NVT-canonical (number of particles, volume, and temperature) and NPT-isothermal–isobaric (number of particles, pressure, and temperature) after energy minimization and ensemble with the equilibration protocol for another 5000 steps. The equilibrated system was programmed for 20-nanosecond (ns) production of MD simulation. During the MD production, the system was held under a constant number of particles at 310K and a pressure of 1 bar. The generated trajectory during the 20 ns run was analyzed based on the RMSD backbone of the docked-ligand complexes and the protein system.

RESULTS

The evaluation of the immune-modulatory activities of the herbal compounds and their medicinal potential for the treatment of RA revealed that these compounds possessed strong inhibitory effects on monocytes. Curcumin diglucoside and curcumin monoglucoside had the strongest inhibitory effects, with inhibitory levels of 82.75 and 81.34 %, respectively. On the other hand, eugenin produced the weakest immuno-modulatory and inhibitory effect of 11.12 %. These results are are detailed in Table 1.

The molecular docking scores of the herbal compounds against TNF-α (PDB ID: 2AZ5) and IL-1 (PDB ID: 2L5X) are presented in Table 2, which depicts the scores and results based on ascending order of the re-rank score, and the MolDock scores and interaction energies. *Curcuma longa*, *Camellia sinensis* and *Sophora japonica* had strong molecular interactions at the potential ligand binding sites of TNF-α (PDB ID: 2AZ5) and IL-1 (PDB ID: 2L5X). The ligand-protein interaction analyses of the top docked herbal compounds with TNF-α (PDB ID: 2AZ5) and IL-1 (PDB ID: 2L5X) are presented in Table 3 and Table 4, respectively. The ligand-protein interaction analysis was carried out to understand the in-depth molecular interaction of these docked herbal compounds at the active site of TNF-α(2AZ5) and IL-1 (2L5X), and its binding mechanism. Curcumin diglucoside, curcumin monoglucoside and sophoricoside docked at the binding cavity of TNF-α (2AZ5) with rerank scores of -84.50 kJ-mol^{-1}, -83.45 kJ-mol^{-1} and -75.55 kJ-mol^{-1}, respectively. The binding modes of the docked compounds with TNF-α (PDB ID: 2AZ5) and IL-1 (PDB ID: 2L5X) are illustrated in Figures 1-6. The trajectory profiles which depict the RMSD backbones of the 2AZ5ligand-docked complexes and 2L5Xligand-docked complexes from production of MD simulation are shown in Figure 7 and Figure 8, respectively.

DISCUSSION

The pro-inflammatory cytokines TNF-αandIL-1 are major catabolic factors in cartilage metabolism. There are several reports on the targeting of these enzymes for potential therapeutic applications [21].

Table 1: Immuno-modulating and inhibitory properties of the herbal compounds studied

Compound	Plant source	RLU*	Inhibition (%)
Curcumin Diglucoside	*Curcuma longa*	104.2 ± 0.2	82.8
Curcumin Monoglucoside	*Curcuma longa*	107.7 ± 0.1	81.3
Epigallocatechin gallate	*Camellia sinensis*	124.1 ± 0.4	79.9
Sophoricoside	*Sophora japonica*	129.2 ± 0.7	79.7
Arctiin	*Arctium lappa*	156.7 ± 1.0	75.5
Capsaicin	*Capsicum annuum*	166.7 ± 1.2	74.2
Daphnoretin	*Wikstroemia indica*	309.5 ± 1.1	63.1
Isorhamnetin	*Tagetes lucida*	330.5 ± 2.1	61.3
Catechin	*Senegalia catechu*	380.9 ± 2.3	53.2
Myricetin	*Myrica cerifera*	390.6 ± 2.7	50.3
Bisabolol	*Matricaria recutita*	401.4 ± 3.9	48.1
Kaempferol	*Aloe vera*	580.1 ± 3.2	21.5
Chamazulene	*Matricaria chamomilla*	680.1 ± 5.2	19.1
Apigenin	*Chamaemelum nobile*	731.1 ± 7.6	15.2
Eugenin	*Syzygium aromaticum*	877.3 ± 9.7	11.1

*Values are mean ± SD

Table 2: Molecular docking scores of the investigated compounds

Compound	TNF-α (2AZ5)			Compound	IL-1 (2L5X)		
	MolDockscore	***Rerankscore***	***H-Bond***		***MolDockscore***	***Rerankscore***	***H-Bond***
Curcumin Diglucoside	-91.24	-84.50	-8.42	Curcumin Diglucoside	-138.50	-115.43	-9.34
Curcumin Monoglucoside	-99.61	-83.45	-2.70	curcumin monoglucoside	-124.33	-101.02	-8.15
Sophoricoside	-82.78	-75.55	-9.49	Epigallocate-chin gallate	-120.75	-96.58	-11.65
Arctiin	-96.59	-71.98	-8.78	Sophoricoside	-94.70	-80.09	-13.48
Capsaicin	-86.48	-66.85	-1.80	Arctiin	-135.02	-79.70	-4.00
Epigallocat-echingallate	-90.90	-65.98	-14.72	Capsaicin	-104.21	-75.82	-2.24
Daphnoretin	-75.14	-59.53	-4.79	Daphnoretin	-87.19	-70.87	-3.14
Isorhamnetin	-66.50	-59.45	-5.78	Bisabolol	-84.00	-65.64	-1.40
CID 9064	-70.51	-59.18	-8.73	CID 9064	-76.16	-63.67	-12.94
Myricetin	-62.50	-59.03	-7.34	Isorhamnetin	-71.16	-59.85	-5.19
Bisabolol	-71.25	-58.00	-3.90	Ibuprofen	-68.61	-57.93	-2.24
Kaempferol	-64.93	-56.15	-7.98	Myricetin	-64.84	-55.04	-9.09
Chamazulene	-65.06	-54.22	0.00	Chamazulene	-64.85	-54.61	0.00
Apigenin	-62.25	-52.21	-5.55	Kaempferol	-61.00	-54.40	-4.02
Ibuprofen	-58.55	-50.77	-6.12	Apigenin	-68.16	-53.97	-4.30
Eugenin	-55.12	-42.42	-2.86	Eugenin	-53.75	-46.95	-2.50

Table 3: Molecular interaction analysis of the top hits with TNF-α (2AZ5)

Compound	Interacting amino acid	Interaction energy (kJ-mol^{-1})	Interaction distance
Curcumin diglucoside	Asn39(ND)	-2.50	3.08 Å
	Lys11(N)	-0.66	3.23 Å
	Lys11(O)	-2.50	2.78 Å
	Ala156(O)	-2.50	2.67 Å
	Gly121(N)	-0.32	3.02 Å
	Gly121(O)	-2.5	2.66 Å
Curcumin monoglucoside	His15(O)	-2.0	3.20 Å
	Leu36(N)	-2.2	2.56 Å
	Gly121(O)	-2.5	2.74 Å
Sophoricoside	Tyr59(OH)	-0.47	2.36 Å
	Tyr151(OH)	-1.71	3.26 Å
	Tyr151(OH)	-2.5	3.06 Å
	Gln61(OE)	-2.36	3.13 Å
	Ser60(O)	-2.5	2.61 Å
	Leu120(N)	-1.36	2.87 Å
	Leu120(O)	-2.5	2.70 Å
	Leu120(O)	-2.15	3.17 Å

ND: Nitrogen δ atom; N: Nitrogen; O: Oxygen; OE: Oxygen ε atom; OH: Hydroxyl group

Table 4: Molecular interaction analysis of the top hits with IL-1 (2L5X)

Compound	Interacting amino acid	Interaction energy (kJ-mol^{-1})	Interaction distance (Å)
Curcumin diglucoside	Asp49(OD)	-0.15	3.56
	Ser75(O)	-1.25	3.35
	Glu21(OE)	-2.5	2.68
	His46(NE)	-2.29	3.09
	Glu50(OE)	-2.37	3.12
	Glu50(OE)	-2.50	3.00
	Asn47(OD)	-1.40	3.05
	Arg34(O)	-0.28	3.54
Curcumin monoglucoside	Asp49(OD)	-2.11	3.18
	Asp49(O)	-2.50	2.66
	Asn47(OD)	-1.50	3.29
	Asn47(OD)	-2.50	2.82
	Glu50(O)	-2.50	2.62
	Arg34(O)	-2.50	2.79
Epigallocatechin gallate	Glu50(OE)	-0.14	3.57
	Glu50(O)	-1.63	3.27
	Asp49(O)	-1.69	3.26
	Asp49(OD)	-2.46	3.11
	Asp49(OD)	-0.13	3.42
	Arg34(O)	-2.27	2.57
	Asn47(OD)	-2.50	2.64
	Thr77(OG)	-1.95	3.21

O: Oxygen; OE: Oxygen ε atom; OD: Oxygen δ atom; OG: Oxygen γ atom; NE: Nitrogen ε atom

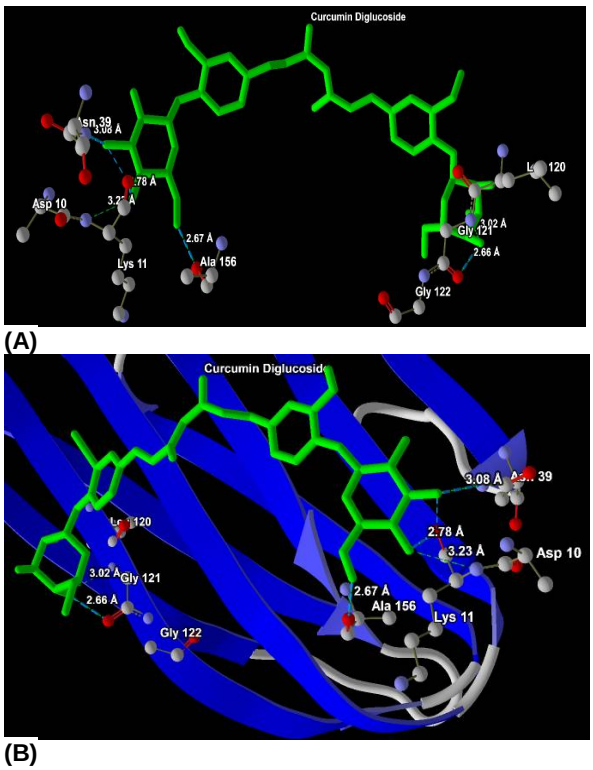


Figure 1: (A) Binding mode of curcumin diglucoside (green color) at the active site of TNF-α (PDB ID: 2AZ5); (B) Secondary structure depicting the molecular interactions between curcumin diglucoside and Lys11, Asn39, Gly121 andAla156 residues

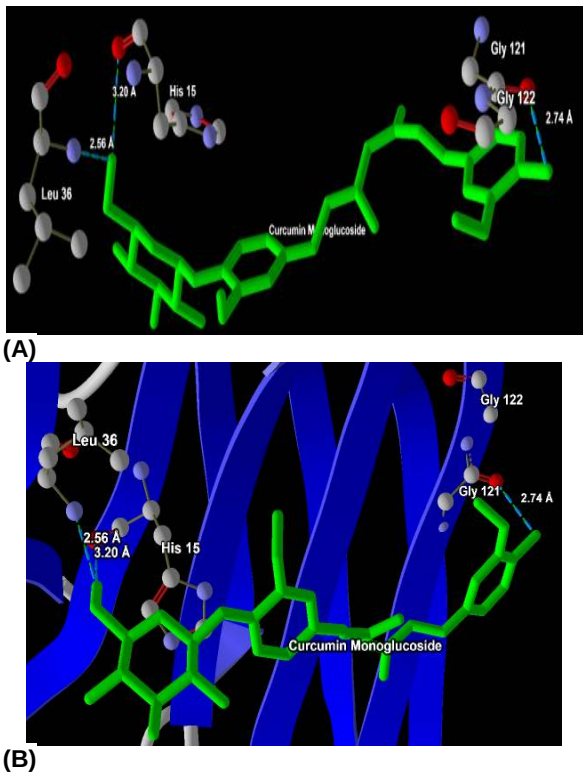
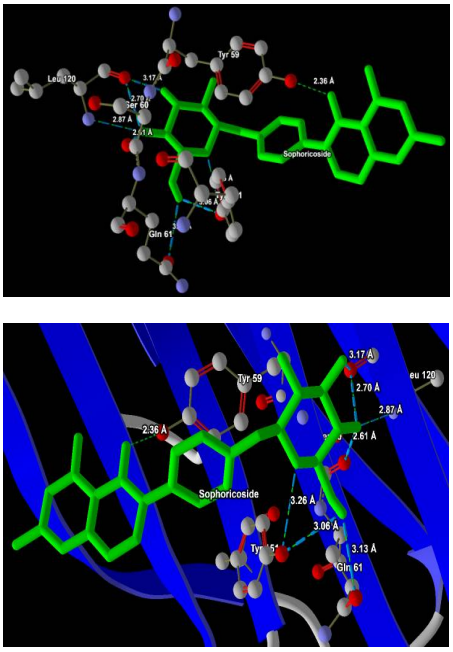


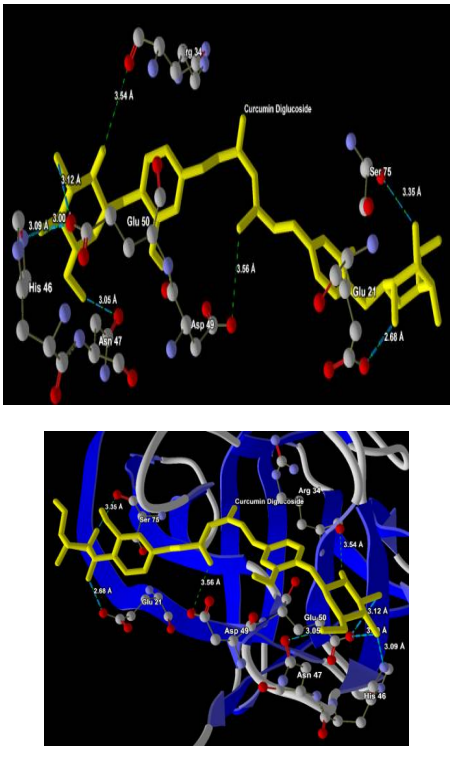
Figure 2: (A) Binding mode of curcumin monoglucoside (green color) at the active site of TNF-α (PDB ID: 2AZ5). (B) Secondary structure depicting the molecular interactions between curcumin monoglucoside and His15, Leu36 and Gly121 residues



(A)

(B)

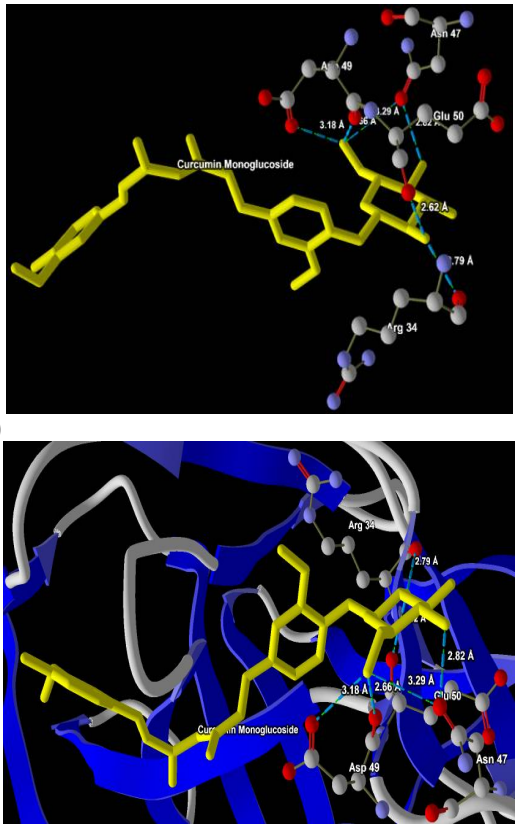
Figure 3: (A) Binding mode of sophoricoside (green color) at the active site of TNF-α (PDB ID: 2AZ5). (B) Secondary structure depicting the molecular interactions between sophoricoside and Tyr59, Ser60, Gln61, Leu120 and Tyr151 residues



(A)

(B)

Figure 4: (A) Binding mode of curcumin diglucoside (yellow color) at the active site of ofIL-1 (PDB ID: 2L5X). (B) Secondary structure depicting the molecular interactions between curcumin diglucoside and Glu21, Arg34, His46, Asn47, Asp49, Glu50 and Ser75 residues



(A)

(B)

Figure 5: (A) Binding mode of curcumin monoglucoside (yellow color) at the active site of IL-1 (PDB ID: 2L5X).(B) Secondary structure depicting the molecular interactions between curcumin monoglucoside and Arg34, Asn47, Asp49 and Glu50 residues

In this study, drug targets for these proteins were identified and virtual screening was carried out. Molecular docking analysis of binding is an essential step in the drug discovery process. It gives a picture of the binding mode of a compound under investigation. The results of the present study show that the herbal compounds present in *Curcuma longa* produced the strongest inhibitory effects on monocytes. Curcumin diglucoside and curcumin monoglucoside which are constituents of *Curcuma longa* elicited strong immune-modulatory and monocyte-inhibitory effects based on the neutrophil oxidative burst assay. The strong inhibitory effects on monocytes are bound to have significant impact on the production of inflammatory cytokines, TNF-α and IL-1.

Epigallocatechin gallate, sophoroside and arctin also showed good immune-modulating and inhibitory effects. This study also demonstrated the effectiveness of combining molecular docking studies with functional assays for the discovery

and development of novel small-molecule drugs that target TNF-α and IL-1pathways.

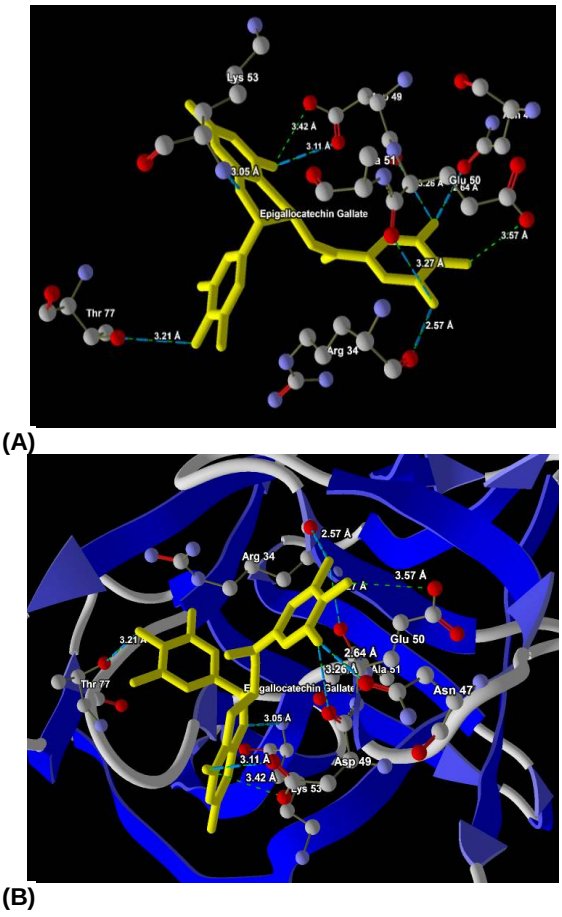


Figure 6: (A) Binding mode of epigallocatechin gallate (yellow color) at the active site of IL-1 (PDB ID: 2L5X).(B) Secondary structure depicting the molecular interactions between epigallocatechin gallate and Arg34, Asn47, Asp49 and Glu50 residues

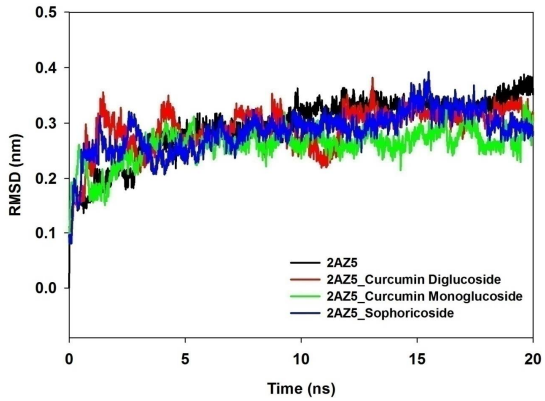


Figure 7: RMSD backbone of 2AZ5ligand-docked complexes during the 20ns MD simulation run

The results from molecular docking analyses of the herbal compounds against TNF-α (PDB ID: 2AZ5) and IL-1 (PDB ID: 2L5X) correlate with results from assays of immuno-modulatory activities. The herbal compounds present in *Curcuma longa*, *Camellia sinensis* and *Sophora japonica* had strong molecular interactions at the potential ligand binding sites of TNF-α (PDB ID: 2AZ5) and IL-1 (PDB ID: 2L5X). The key interactions (bonded and non-bonded) were formed between the docked compounds and the binding cavities of these enzymes.

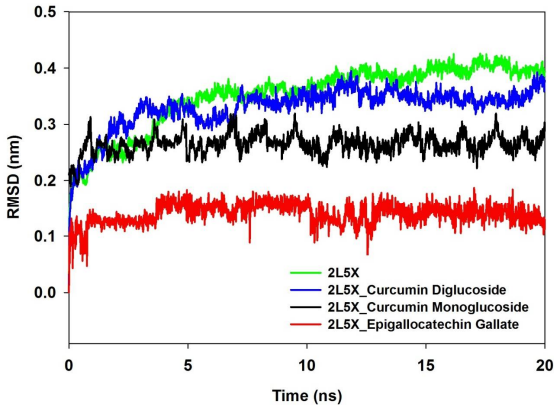


Figure 8: RMSD backbone of 2L5Xligand-docked complexes during the 20ns MD simulation run.

Molecular docking simulation analysis revealed that curcumin diglucoside, curcumin monoglucoside and sophoricoside docked at the binding cavity of TNF-α (2AZ5) with rerank scores of -84.50 kJ-mol^{-1}, -83.45 kJ-mol^{-1} and -75.55 kJ-mol^{-1}, respectively. Interestingly, curcumin diglucoside and curcumin monoglucoside also docked at the binding cavity of IL-1 (2L5X) with rerank scores of -115.43 kJ-mol^{-1} and -101.02 kJ-mol$^{-1,}$ respectively. These results are consistent with data from the assay of immuno-modulatory activities. On the other hand, eugenin showed the weakest docking score with a rerank score of -42.42 kJ-mol^{-1}forTNF-α (2AZ5), and -46.95 kJ-mol^{-1}for IL-1(2L5X). The rerank scoring function is actually more expensive in computational terms than the scoring function used during the docking simulation but it is generally better than the docking score function.

Results from the introduction of ibuprofen during the docking simulation validated the docking protocol used. Ibuprofen is a known inhibitor of TNF-α (2AZ5) and IL-1 (2L5X). The investigated herbal compounds produced more favorable docking scores (Rerank and MolDock) for 2AZ5 and 2L5X than ibuprofen. The molecular docking simulation also revealed that the top docking poses were docked into binding cavities exhibiting both bonded as well as non-bonded interactions. The ligand–protein interaction analyses using ligand energy inspector revealed the interaction distances and interaction energies of the docked compounds with the corresponding amino acid residues. In case of TNF-α (2AZ5), curcumin diglucoside interacted with Lys11,

Asn39, Gly121, and Ala156; curcumin monoglucoside with His15, Leu36 and Gly121 and sophoricoside with Tyr59, Ser60, Gln61, Leu120, and Tyr151.

Earlier, Takasaki *et al* proposed a similar molecular docking approach used for successful identification of a small molecule which inhibited TNF-α at the same binding site that formed hydrogen bonds with Asn39, Tyr59, Gln61 and Gly121 [22].Similar results have also been obtained by Ma *et al* [23], which are in agreement with the results obtained in the present study.

With respect to IL-1 (2L5X), curcumin diglucoside showed interactions with Glu21, Arg34, His46, Asn47, Asp49, Glu50 and Ser75; curcumin monoglucoside interacted with Arg34, Asn47, Asp49 and Glu50 while epigallocatechin gallate showed interaction with Arg34, Asn47, Asp49, Glu50 and Thr77.

The molecular interactions with Arg34, Asn47, Asp49, and Glu50 of IL-1 are also in agreement with data reported by Wu *et al* which specifically implicated these amino acid residues in the binding mode of resveratrol docked against IL-1 [15]. Moreover, the molecular interaction distance of the docked herbal compounds confirmed strong binding affinities as were evident from energy scores. The interaction energies also revealed that majority of the interactions were within 3.5Å, close enough to elicit strong inter- molecular forces.

Since the biological activity of any drug is based on the binding between a receptor (protein) and a ligand (drug), the results of the present investigation indicate that the investigated herbal compounds have strong binding affinities for the pro-inflammatory cytokines studied. This is strong evidence of their inhibitory potential. Furthermore, the backbone root mean square deviation (RMSD) values of protein and protein-ligand complex during 20ns of MD simulations for TNF-α(2AZ5) ligand-docked complex and IL-1 (2L5X) ligand-docked complex indicate the stability of the protein-ligand complexes. This is confirmation of conformational flexibility and stability in dynamic behavior. In both cases, the apo enzymes (2AZ5 and 2L5X) and the docked ligand complexes showed stability right from the launch of the MD run with stable conformations throughout the simulation period, except for 2L5X-epigallocatechin gallate-docked complex which drifted at 7ns and 13ns, probably as a result of the chemical nature of the compound.

CONCLUSION

The herbal compounds present in *Curcuma longa*, *Sophora japonica*, and *Camellia sinensis* possess strong inhibitory potentials against TNF-α and IL–1. The immune-modulatory activities of the herbal compounds also reveal the therapeutic potential of these herbal compounds for use in RA. In addition, molecular docking simulation results show that curcumin diglucoside, curcumin monoglucoside, sophoricoside and epigallocatechin gallate inhibit TNF-α and IL–1 with strong molecular interactions as evidenced by their molecular docking scores and ligand-protein interaction energies. Thus, curcumin diglucoside, curcumin monoglucoside, sophoricoside and epigallocatechin gallate are bioactive compounds with strong potentials for the treatment of RA.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work

Authors' contribution

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Shengkang Xu prepared this manuscript, Na Wang collected blood samples, Meng Zhao gave suggestions in interpreting the data, Hao Peng designed this study. The authors' named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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