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Virtual Screening and Molecular Dynamic Simulation Studies of Anti-TNF-α Phytochemicals from Major Spices

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Abstract: Inflammation is a vital response to an injury or infection. During inflammation, many cytokines are over-expressed to regulate the inflammation, and it is found that Tumor necrosis factor-alpha (TNF-α) is one of them. To inhibit TNF-α pro-inflammatory cytokine, the present study focuses on some major spices with anti-inflammatory properties. The major spices such as turmeric, cinnamon, cayenne pepper, ginger, and garlic were selected, and the study's phytochemicals were retrieved from the PubChem database. TNF-α's 3D structure was fetched from the PDB and validated the structure by SAVES. PDBsum was used to predict active sites of a target protein, and docking parameters were studied by Autodock. Further, the ADMET properties of the selected spices were predicted. Insilico screening and evaluation study reveals that Bisdemethoxycurcumin and Zerumbone were found to show significant inhibitory effects on TNF-α. Importantly, no toxicity was found for the compounds. The study also analyzed the Protein-Ligand structure stability by molecular dynamic simulations, and structure stability was found within the range. These molecules are considered potent inhibitors of TNF-α, and further validation *in vitro* and *in vivo* may explore the herbal-based therapeutic modality for managing inflammatory diseases.

Keywords: TNF- α; inflammatory disease; spices; ADMET; in silico; molecular dynamic simulation.

1. Introduction

Inflammation is a vital response originated by bacterial infection, chemical injury, and environmental pollution, which ends in cell injury or cell death and releases inflammatory mediators such as TNF-α [1], IL-1 [2], IL-6 [3], COX-1, COX-2 [4], 5-LOX [5], PDE4 [6] etc. These inflammatory mediators are reported in several inflammatory diseases like asthma, psoriasis, inflammatory bowel disease, rheumatoid arthritis, and systemic sclerosis [7]. Many cytokines are over-expressed during inflammation to regulate the inflammation process. One such critical pro-inflammatory cytokine is tumor necrosis factor-alpha (TNF-α). TNF-α is produced by monocytes and macrophages in response to various injuries or infections. TNF-α has been found to be a significant therapeutic target for a variety of diseases, particularly inflammatory diseases [8]. We aim to identify small molecules which can inhibit TNF-α and thereby reduce the manifestation of inflammation. Various anti-TNF-α therapeutic modalities were available such as infliximab, adalimumab, golimumab, etanercept, and certolizumab.

However, these therapeutic regimes showed noted side effects such as the risk of infection, cost, and the need for intravenous injections. Therefore, there is a constant hunt for new molecule/s that can become an alternative to existing anti-inflammatory regimes. Since ancient times spices have been used as folk medicines to manage inflammation; therefore, our study aims to identify the precise phytoconstituents from the spices to manage inflammatory diseases with no or limited side effects.

Spices have long been an important part of people's lives in many areas of the globe. Humans have been using herbs and spices for culinary and medicinal purposes for centuries. Plant-derived molecules are important for maintaining and promoting good health. Plant sources have diverse uses, such as colorants, flavoring agents, preservatives, food additives, and medication [9,10]. These effects are based on the molecular foundation of active phytochemical constituents from spices. Many spices are used in the kitchen and have therapeutic properties such as purgative, laxative, expectorant, carminative, diuretic, etc.; considering these therapeutic properties, these plants are throughout antiquity and continue to do so now. Spices like turmeric, cinnamon, cayenne pepper, ginger, and garlic have a broad range of bioactivities. Their additive or synergistic effects are likely to defend the human body against several threats [11]. Spices comprehensively impact human health when consumed as part of a balanced diet [9]. In this study, spices are chosen to explore the small molecule phytochemicals present in them and evaluate their anti-inflammatory properties using computational tools.

To date, many small molecules have been identified for TNF-α inhibition. Most of the molecules target intracellular key molecules of TNF-α pathways by reducing the expression of TNF-α and inhibiting TACE (TNF-α converting enzyme) [12]. However, direct inhibition of TNF-α remains a challenge even today [13]. As per the published reports, few small molecules that can act as an antagonist for TNF-α, such as suramin and SPD304, these molecules often show adverse side effects and are not used for anti-TNF-α therapies [12,14,15]. SPD304 includes the carcinogenic moiety of 3-alkyl indole, which is metabolized by cytochrome P450 enzymes via a dehydrogenation mechanism similar to the powerful pneumotoxin 3-methyl indole, resulting in a reactive electrophilic iminium substance that can react with protein and DNA targets [16]. Therefore, continuous research is in progress to identify effective inhibitors with low or no toxicity for TNF-α. In this study, some phytoconstituents are selected from a few well-known spices having anti-inflammatory properties, such as turmeric, ginger, cinnamon, cayenne pepper, and garlic. The bioactive compounds from these spices showing anti-inflammatory activities are extensively screened and evaluated using various virtual screening and molecular modeling tools and found two vital compounds, Bisdemethoxycurcumin and Zerumbone. Further systematic *in vitro* and *in vivo* validation studies may aid in developing herbal-derived therapeutic modalities for improving inflammatory disease management.

2. Materials and Methods

2.1. Protein preparation.

The TNF-α protein structure was selected from Protein Data Bank (https://www.rcsb.org/) with PDB ID: 2AZ5 and having a resolution of 2.10 Å. The protein dimer structure is bound to ligand SPD304 [17]. Using the structure analysis and verification

server (SAVES) (https://servicesn.mbi.ucla.edu/SAVES/), the protein characterization and validation, structural geometry, and stereochemistry were analyzed [18].

2.2. Prediction of the active sites.

The active sites of TNF-α are retrieved by the PDBsum server [19]. The TNF-α protein interaction with the ligand (SPD304) is predicted through a ligplot. Those interacted residues are considered active sites and used for the docking process.

2.3. Ligand preparation.

The bioactive compounds of Indian spices are selected as ligands through a literature survey [20-30]. The selected compound structures are downloaded from the PubChem database [31], and Lipinski's rule of 5 of all the downloaded compounds is analyzed by the molinspiration server (https://www.molinspiration.com/cgi-bin/properties) [32]. Further, the compounds are subjected to ADME property prediction through *swissADME* (http://www.swissadme.ch/index.php) [33], and toxicity was predicted through admetSAR and Protox-II servers [34,35].

2.4. Molecular docking.

The Autodock 1.5.6 tool is used for molecular docking studies [36,37], which enables us to know the interaction between protein and ligand molecule to generate a set of ligand binding poses and scoring functions. Also, help to predict the binding affinity of protein-ligand interaction. Simulation of docking was calculated by Gasteiger charge, and polar hydrogens were added. A grid map was placed to cover active protein sites. Lamarckian genetic algorithm is used for molecular docking simulation. Docking simulations were carried out 10 times and resulted in 10 docked conformations. Finally, the minimal energy conformation/s is considered the best binding conformation/s.

2.5. Molecular dynamic simulation studies.

Molecular Dynamic Simulation (MDS) is the study of the dynamics of any system. This is a computational technique used to describe the protein-ligand complex stability analysis, conformational changes, and internal motions. In the present study, MDS was performed to predict the stability of shortlisted compounds with good ADMET property prediction using the Desmond package of the academic version [38]. MDS (25nano seconds) of TNF-α complexes with shortlisted compounds derived from docking studies. The system was solvated in an orthorhombic box containing TIP3P water models and neutralized with Na+ counter ions to create an aqueous environment. For pressure coupling, the Martyna–Tobias–Klein method was utilized. Under specified periodic boundary conditions, the run was carried out at 300 K with constant volume and temperature (Isothermal-Isobaric ensemble (NPT) ensemble). To determine the relative stability of the ligand inside its binding pocket, root-mean-square deviation (RMSD) plots for the backbone atoms for both the protein and ligand-bound protein were constructed.

3. Results and Discussion

3.1. Target protein preparation.

The 3D structure of the target protein (PDB id: 2az5) was fetched from PDB. PDB structure was allowed to bind with ligand SPD304. The SPD304 ligand can disassociate the trimer structure of TNF-α from the dimer structure, resulting in an inhibitory effect on TNF-α. The *SAVES* database was used to identify the quality of the structure, and the quality factor was found to be 98.70%. In the Ramachandran plot, 89.6% of residues in the most favored regions are depicted in Figure 1.

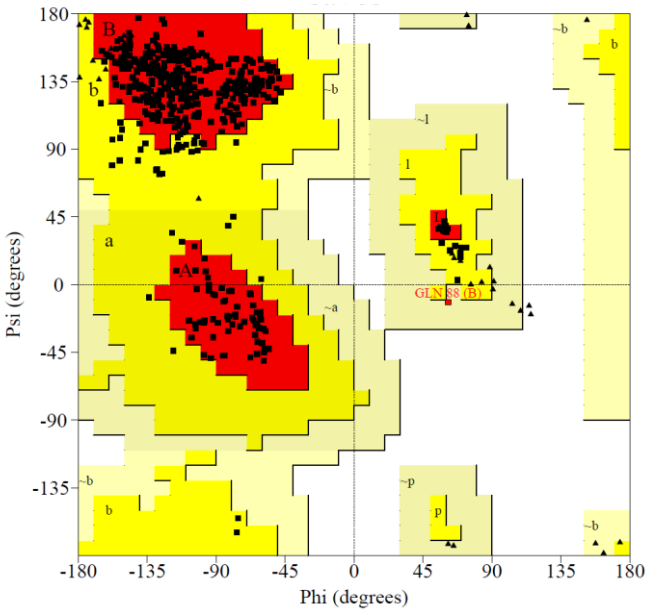


Figure 1. Ramachandran plot of 2az5 (TNF-α) by SAVES.

3.2. Prediction of the active sites.

The active sites of the target protein were predicted through a ligplot from the *PDBsum* of the RCSB (Research Collaboratory for Structural Bioinformatics) server. The active sites in the ligplot are shown as eyelid-like structures indicating the active sites of amino acid residues.

3.3. Selection of ligands.

Through extensive literature, we selected forty-five bioactive compounds from different species as ligand molecules. These molecules and 3D structures are taken from the *PubChem* database**.** The ligands were refined based on the 'Lipinski rule of 5' by using the *molinspiration* tool. The filtered compounds are within the range of octanol-water partitioning coefficient (Log P) -5 to 5, hydrogen bond Donor (nOHNH) ≤5, hydrogen bond acceptor (nON) ≤10, rotatable bonds (nrotb) ≤12, molecular weight (MW) ≤500 Dalton, and topological surface area (TPSA)≤1000. Table 1 represents the values of the Lipinski rule of 5.

Table 1. 'Lipinski rule of 5' property prediction of selected compounds.

Sl.No	Name of compound	LogP	TPSA	MW	nON	nOHNH	nrotb	Volume
1	Cinnamaldehyde	2.48	17.07	132.16	1	0	2	130.44
3	Cis-Cinnamaldehyde	2.48	17.07	132.16	1	0	2	130.44
4	Cinnamate	-0.81	40.13	147.15	2	0	2	135.72
5	Cinnamyl Acetate	2.74	26.3	176.22	2	0	4	172.79
6	DAS (diallyl sulfide)	2.13	0	114.21	0	0	4	119.83

Sl.No	Name of compound	LogP	TPSA	MW	nON	nOHNH	nrotb	Volume
7	DADS (diallyl disulfide)	2.63	0	146.28	0	0	5	137.96
8	Alliin	-3.39	80.39	177.22	4	3	5	154.76
9	Capsaicin	3.1	58.56	305.42	4	2	9	310.37
10	Dihydrocapsaicin	4.1	58.56	307.43	4	2	10	316.56
11	Nordihydrocapsaicin	3.6	58.56	293.41	4	2	9	299.75
12	Homocapsaicin	3.61	58.56	319.44	4	2	10	327.17
13	homodihydrocapsaicin	4.61	58.56	321.46	4	2	11	333.36
14	Curcumin	2.3	93.07	368.38	6	2	8	332.18
15	demethoxycurcumin	2.48	83.83	338.36	5	2	7	306.64
16	Bisdemethoxycurcumin	2.67	74.6	308.33	4	2	6	281.09
17	6-gingerol	3.22	66.76	294.39	4	2	10	295.61
18	6-paradol	4.6	46.53	278.39	3	1	10	287.57
19	6-shogaol	4.35	46.53	276.38	3	1	9	281.38
20	Zerumbone	4.2	17.07	218.34	1	0	0	236.18

3.4. Molecular docking.

Bioactive compounds from selected spices have undergone the process of molecular docking studies by using *Autodock 1.5.6.* The referral drug (SPD304) was docked to get the same confirmation available in the PDB structure but failed to get exact binding confirmation. Considering the confirmation, which is near to crystallographic structure binding confirmation, it shows binding energy -5.37. After performing all compound molecular docking studies, results showed that out of 19 compounds, 07 compounds show significant binding energy and hydrogen bond interaction compared to the referral drug (SPD304). 12 compounds were eliminated due to less binding energy than SPD304. Further, an *in silico* evaluation was conducted, and the results are depicted below in Table 2.

Table 2. Docking results of selected compounds.

Sl.No	Compounds	Rank	Run	Binding energy (Kcal/mol)	No. of H. bonds	Amino acids present in H. bond
1	Cinnamaldehyde	1	4	-4.33	2	Leu120, Ser60
2	Cis-Cinnamaldehyde	1	5	-4.04	0	-
3	Cinnamate	1	4	-4.66	1	Ser60
4	Cinnamyl Acetate	1	10	-4.78	1	Tyr151
5	DAS (diallyl sulfide)	1	5	-3.03	0	-
6	DADS (diallyl disulfide)	1	2	-3.58	1	Tyr151
7	Alliin	1	1	-3.28	2	A: Lys98, B: Lys98
8	Capsaicin	1	8	-6.0	2	Tyr151, Gly121
9	Dihydrocapsaicin	1	1	-5.26	0	-
10	Nordihydrocapsaicin	1	2	-4.76	0	-
11	Homocapsaicin	1	5	-5.85	1	Tyr151
12	Homodihydrocapsaicin	3	1	-5.15	1	Ser60
13	Curcumin	3	6	-6.07	3	Tyr151, A: Gly121, B: Gly121
14	Demethoxycurcumin	3	8	-6.67	4	Leu120, A: Tyr151, B: Tyr151
15	Bisdemethoxycurcumin	2	4	-7.22	3	A: Tyr151, B: Tyr151,Gly121
16	6-gingerol	4	9	-2.89	0	-
17	6-paradol	1	8	-5.58	1	Tyr151
18	6-shogaol	1	5	-5.26	1	Tyr151
19	Zerumbone	1	2	-7.11	1	Tyr151
20	Referral drug (Spd304)	5	8	-5.37	1	GLY121

3.5. ADME analysis.

ADME properties were analyzed by *swissADME* sever for compounds. This gives value to solubility, lipophilicity, and medicinal chemistry [33]. The toxicity prediction of the compound was made by *admetSAR* and *Protox-II* [35,39].

3.5.1. Absorption of compounds.

One of the most important qualities that influence absorption is solubility. The solubility of the molecule in aqueous and non-aqueous media is significant during the drug development process and until oral administration [33]. Lipophilicity refers to a compound's effective solubility in non-aqueous media, and it's linked to a variety of pharmacological qualities like adsorption, distribution, metabolism, and toxicity [40]. There are five different forecasting models available, are iLOGP (implicit log $P_{o/w}$), WLOGP (Wildman and Crippen log $P_{o/w}$), XLOGP3(enhanced atomic/hybrid log $P_{o/w}$ 3), MLOGP (quantitative-structure log $P_{o/w}$), and SILICOS-IT all these methods were used to check the lipophilicity of the compounds. Consensus log P was the mean lipophilicity prediction value by these methods [41]. The consensus of log P values is shown in Table 3.

Several drugs should be highly water-soluble to carry a sufficient quantity of the active ingredient. Approximate solubility according to log S scale is <-10 poorly soluble, <-6 moderately soluble, <- 4 soluble, < -2 very soluble, and <0 highly soluble [33]. Based on these ranges, the solubility class was predicted in Table 3.

Table 3. Absorption parameter prediction of compounds.

Sl No.	Compound	Consensus Log P	Consensus log S	Solubility class
1	Capsaicin	3.43	-3.53	Soluble
2	Homocapsaicin	3.85	-3.88	Soluble
3	Curcumin	3.03	-3.94	Soluble
4	Demethoxycurcumin	3.00	-3.92	Soluble
5	Bisdemethoxycurcumin	2.83	-3.80	Soluble
6	6-paradol	3.96	-3.72	Soluble
7	Zerumbone	3.57	-3.68	Soluble
8	Referral drug (Spd304)	5.75	-8.49	Poorly soluble

3.5.2. Distribution of compounds.

After absorption of the drug in the human system because of its solubility, it comes across various membrane barriers like gastrointestinal epithelial cells, hepatocyte membrane, glomerulus, blood capillary wall, restrictive organ barriers (BBB-Blood-Brain Barrier), at last, targeted cell [40]. If the log Kp value is more negative, then the compound shows less skin permeant [33,42]; as a result, it specifies all the compounds are less skin permeant shown in Table 4. The gastrointestinal (GI) or human intestinal absorption (HIA) data are used to define adsorption and distribution. All the compounds are well absorbed and not brain penetrant by predicted values in Table 4. Compounds Curcumin and Demethoxycurcumin are large; therefore, it does not permit passing through the brain, which is not a blood-brain-barrier (BBB) permeant. Other remaining compounds are non-blood-brain permeants; when these are metabolized decrease the chance of inducing harmful toxins in the bloodstream and brain. The referral drug (SPD304) is Low in GI absorption and is not BBB permeant.

Table 4. Distribution parameter prediction of compounds.

Sl. No.	Compound	GI absorption	BBB permeant	log Kp (cm/s)
1	Capsaicin	High	Yes	-5.62
2	Homocapsaicin	High	Yes	-5.32
3	Curcumin	High	No	-6.28
4	Demethoxycurcumin	High	No	-6.01
5	Bisdemethoxycurcumin	High	Yes	-5.87
6	6-paradol	High	Yes	-5.08
7	Zerumbone	High	Yes	-4.83
8	Referral drug (Spd304)	Low	No	-4.50

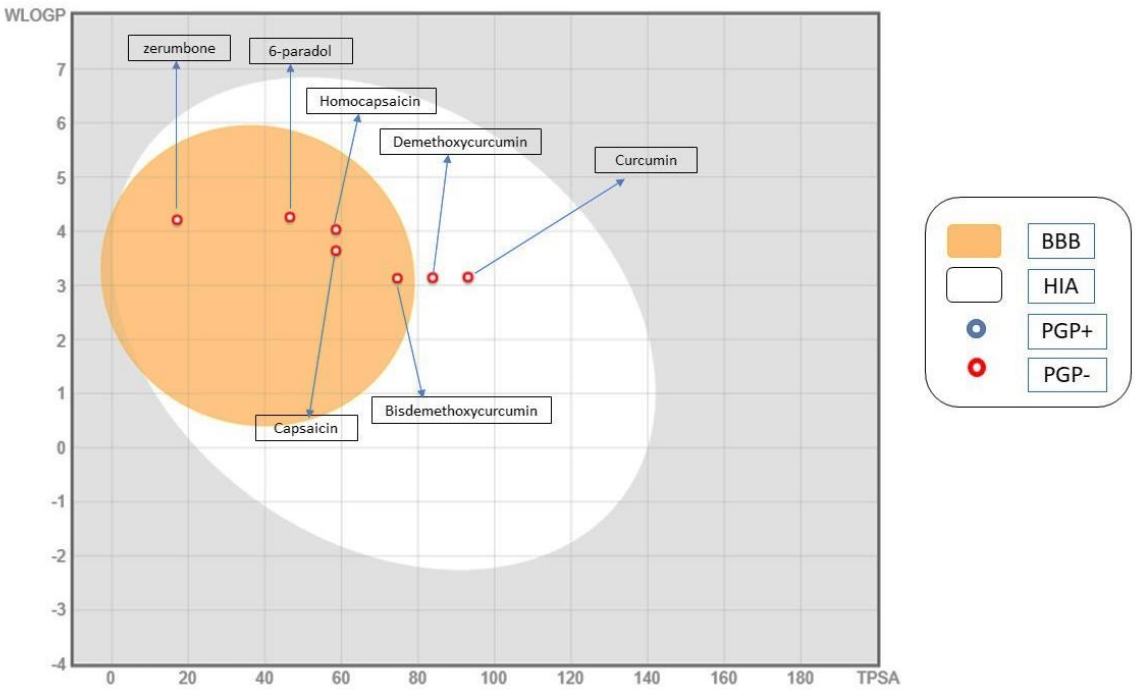


Figure 2. Boiled-egg graph shows the most potent drugs among the investigated ligands against TNF-α.

Bisdemethoxycurcumin, Capsaicin, Homocapsaicin, 6-paradol, and Zerumbone are present in the yellow region of the boiled egg, shown in Figure 2. It indicates that all these compounds are within the acceptable ranges of Human intestinal absorption and Blood Brain Barrier permeant. Curcumin and Demethoxycurcumin are only present in the white region. This shows these two compounds are in the range of Human intestinal absorption but not Blood Brain Barrier permeant.

3.5.3. Metabolism of compounds.

The compounds are metabolized after being delivered to the organism's system, and the metabolites are safely excreted. Drug bioavailability and drug-drug interactions are both heavily influenced by metabolism. It is helpful to know if a certain chemical is a substrate or non-substrate of the permeability glycoprotein (P-gp). The P-gp protein involves the ATP-binding cassette transporters that are major to accessing effective effluence over the biological membrane. It is important to observe the interaction of molecules with cytochrome P450 (CYP). These enzymes are dominant in drug elimination through a metabolic transformation [43]. CYP and P-gp can operate small molecules synergistically to enhance the protection of tissues and organisms [44]. Inhibition of isoenzymes is certainly one of the main causes of pharmacokinetics-related drug-drug interactions; adverse side effects will also occur, as well as the lower solubility and accumulation of the drug or its metabolites cause unwanted side effects [45]. To understand all the parameters of drug deposition, drug efficacy, and toxicity, All the bioactive compounds are determined for either being a P-gp substrate or inhibitor and CYPs. All the compounds are found to be non-substrate of P-gp. All the compounds are substrates of CYP2C19 substrate. All the compounds are CYP1A2 non-substrate except curcumin and zerumbone. CYP2C9 substrates or non-inhibitors are Capsaicin, Homocapsaicin, 6-paradol, and other, all are inhibitors. All compounds are CYP2D6 substrates except Capsaicin, Homocapsaicin, and 6-paradol. All compounds are CYP3A4 inhibitors except 6-

paradol and zerumbone. The referral drug shows substrate for P-gp and CYP2D6 and CYP3A4 inhibitors.

Table 5. Metabolism parameter prediction of compounds.

Sl No.	Compound	P-gp substrate	CYP1A2 inhibitor	CYP2C19 inhibitor	CYP2C9 inhibitor	CYP2D6 inhibitor	CYP3A4 inhibitor
1	Capsaicin	No	Yes	No	No	Yes	Yes
2	Homocapsaicin	No	Yes	No	No	Yes	Yes
3	Curcumin	No	No	No	Yes	No	Yes
4	demethoxycurcumin	No	Yes	No	Yes	No	Yes
5	Bisdemethoxycurcumin	No	Yes	No	Yes	No	Yes
6	6-paradol	No	Yes	No	No	Yes	No
7	Zerumbone	No	No	No	Yes	No	No
8	Referral drug (Spd304)	Yes	No	No	No	Yes	Yes

3.5.4. Toxicity prediction of compounds.

Toxicity prediction of compounds was made through the *admetSAR (*version 2.0) online tool [34,39], which can be accessed by the link (http://lmmd.ecust.edu.cn/admetsar2/) and *Protox-II* [35,46]. Toxicity values are predicted from admetSAR in Table 6. In this table, "+" means toxic, and "-" means non-toxic. The numbers in brackets indicate the probability values [47]. Based on the results table, all compounds are non-carcinogenic. These compounds have less probability of being carcinogenic. All compounds are non-eye corrosion. All compounds are Eye irritants except capsaicin and Homocaspsaicin. Compounds capsaicin and Homocaspsaicin are Ames mutagenesis. Compounds Curcumin, Demethoxycurcumin, and Bisdemethoxycurcumin have hepatotoxicity. All the compounds come under class III of acute oral toxicity, which means that, based on the US EPA classification, their LD_{50} values are less than 5000mg/kg but greater than 500 mg/kg.

Table 6. Toxicity parameters of compounds are predicted through admetSAR.

Sl No.	Compounds	Carcinogenicity	Eye corrosion	Eye irritation	Ames mutagenesis	Hepatotoxicity	Toxicity class	Acute oral toxicity
1	Capsaicin	-(0.8429)	-(0.9914)	-(0.492)	+(0.82)	-(0.725)	III	0.6752
2	Homocapsaicin	-(0.7571)	-(0.9885)	-(0.816)	+(0.75)	-(0.575)	III	0.6676
3	Curcumin	-(0.8061)	-(0.9778)	+(0.7044)	-(0.96)	+(0.725)	III	0.6349
4	Demethoxycurcumin	-(0.8061)	-(0.9743)	+(0.5507)	-(0.92)	+(0.8)	III	0.625
5	Bisdemethoxycurcumin	-(0.8051)	-(0.9772)	+(0.84)	-(0.78)	+(0.825)	III	0.6484
6	6-paradol	-(0.6571)	-(0.8733)	+(0.9525)	-(0.57)	-(0.85)	III	0.8126
7	Zerumbone	-(0.7)	-(0.8938)	+(0.9455)	-(0.8)	-(0.825)	III	0.602
8	Spd304	-(0.9000)	-(0.9891)	-(0.9446)	+ (0.5800)	+(0.7000)	III	0.7069

The tabulated results in Table 6 show that all the compound's toxicology profiles are important to know the route of administration and preferred dosage form. Although different probability values indicate that these are preliminary studies, they should be validated experimentally.

Protox-II: the *in silico* toxicity was predicted using *Protox-II*, and the results are shown in Tables 7, 8 & 9. Table 7 illustrates the values of LD50 and toxicity class. LD50 means a median lethal dose of a compound at which the test substrate drops off upon disclosure. The range of toxicity class is from 1 to 6. Here, 1 is toxic if consumed, and 6 is non-toxic [48]. All the compounds were predicted to be orally non-toxic except Capsaicin and Homocapsaicin.

Table 7. LD50 and Toxicity class were predicted.

Sl.No.	Compound	Predicted LD50 (mg/kg)	Toxicity class
1	Capsaicin	47	2
2	Homocapsaicin	47	2
3	Curcumin	2000	4
4	Demethoxycurcumin	2000	4
5	bisdemethoxycurcumin	2560	5
6	6-paradol	2580	5
7	Zerumbone	4590	5
8	Referral drug (Spd304)	1190	4

The toxicity endpoint was also predicted by the *protox-II* server. The toxicity endpoints are Hepatotoxicity, Carcinogenicity, Immunotoxicity, Mutagenicity, and Cytotoxicity. All the compounds are Non-Hepatotoxic except Homocaspaicin. Hepatotoxicity was predicted to know whether the compound can cause liver dysfunction or not [35,48]. Capsaicin is showing carcinogenic. All the compounds are immunotoxic except bisdemethoxycurcumin and Zerumbone compound 6-paradol showing mild immunotoxicity. Immunotoxic compounds will change the function of the immune system by inhibiting B cell growth [35,48]. Mutagenetic compounds will mutate the genetic material (DNA) of an organism. The result shows capsaicin, homocapsaicin, and 6-paradol have mild mutagenicity, and all the compounds show no cytotoxicity, which means these compounds are non-toxic to cells. Referral drug is hepatotoxic and immunotoxic based on predictions.

Table 8. Toxicity endpoints of compounds were predicted.

Sl. No	Compound	Hepatotoxicity	Carcinogenicity	Immunotoxicity	Mutagenicity	Cytotoxicity
1	Capsaicin	Inactive	**Active**	**Active**	Active	Inactive
2	Homocapsaicin	Inactive	**Active**	**Active**	Active	Inactive
3	Curcumin	Inactive	Inactive	**Active**	Inactive	Inactive
4	demethoxycurcumin	Inactive	Inactive	**Active**	Inactive	Inactive
5	bisdemethoxycurcumin	Inactive	Inactive	Inactive	Inactive	Inactive
6	6-paradol	Inactive	Inactive	Active	Active	Inactive
7	zerumbone	Inactive	Inactive	Inactive	Inactive	Inactive
8	Referral drug (Spd304)	Active	Inactive	Active	Inactive	Inactive

Here, we predicted the toxicity through two different servers to get more accurate results. *In silico* toxicity, prediction is on-demand because it's a cheaper and faster way. By adopting this method, we can reduce animal sacrifice for toxicity experiments. *In silico* evaluation reveals toxicity theoretically. However, *in vitro, in vivo* experimental evidence is necessary to validate toxicity, which is beyond the computational methods.

After analyzing ADMET property prediction, Bisdemethoxycurcumin and Zerumbone were shortlisted for molecular dynamics simulation studies. The docking pose of these compounds is shown in 3D and 2D images in Figure 3.

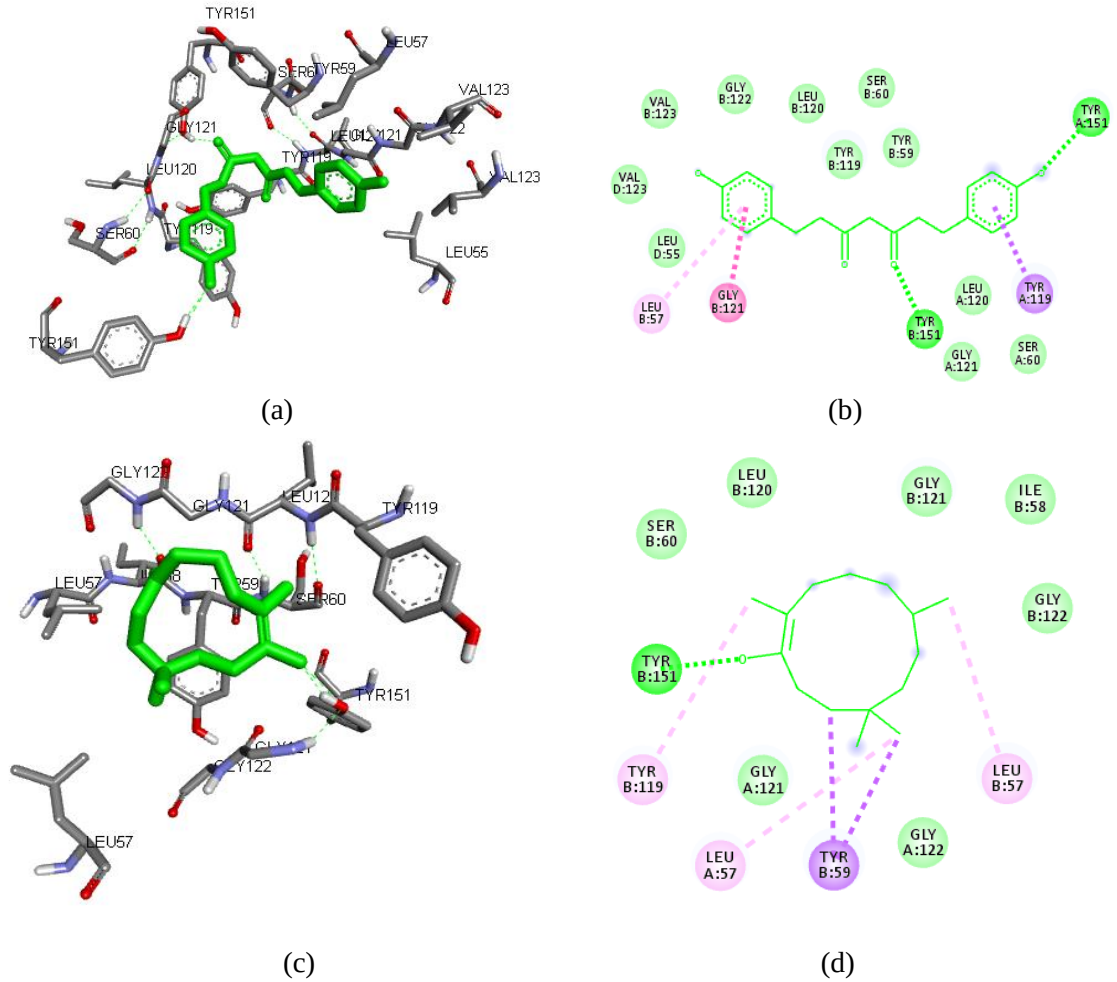
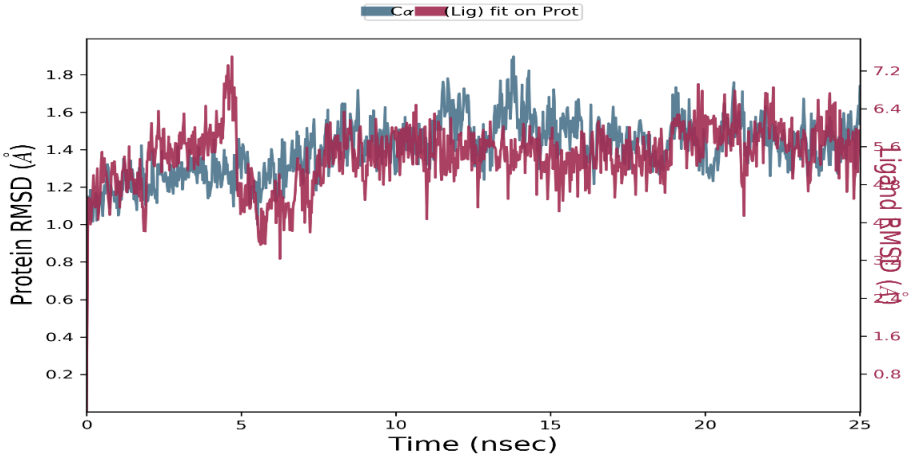


Figure 3. (**a**&**b**) represents bisdemethoxycurcumin, (**c**&**d**) represent zerumbone protein-ligand interaction plot from discovery studio (3D) and (2D). 3D structure - green indicates ligand, grey indicates protein residues, and green dotted lines are interactions between ligand and protein. 2D structure – green indicates ligand molecule, ball-like structures are interacting residues, and green dotted lines are hydrogen bonds.

3.6. Molecular dynamic simulation.

MDS was performed by Desmond package [38]. Protein-ligand complex structure stability was evaluated by observing the root mean square deviation (RMSD) against a period of 25 ns. The RMSD plot of the protein-ligand complex shown in Figures a and c. blue indicates protein RMSD, and the maroon color indicates ligand RMSD and reveals the binding of bisdemethoxycurcumin and zerumbone stabilizes the protein structure with an average RMSD of 1.4 Å and 1.5 Å respectively (Figure 4).



(a)

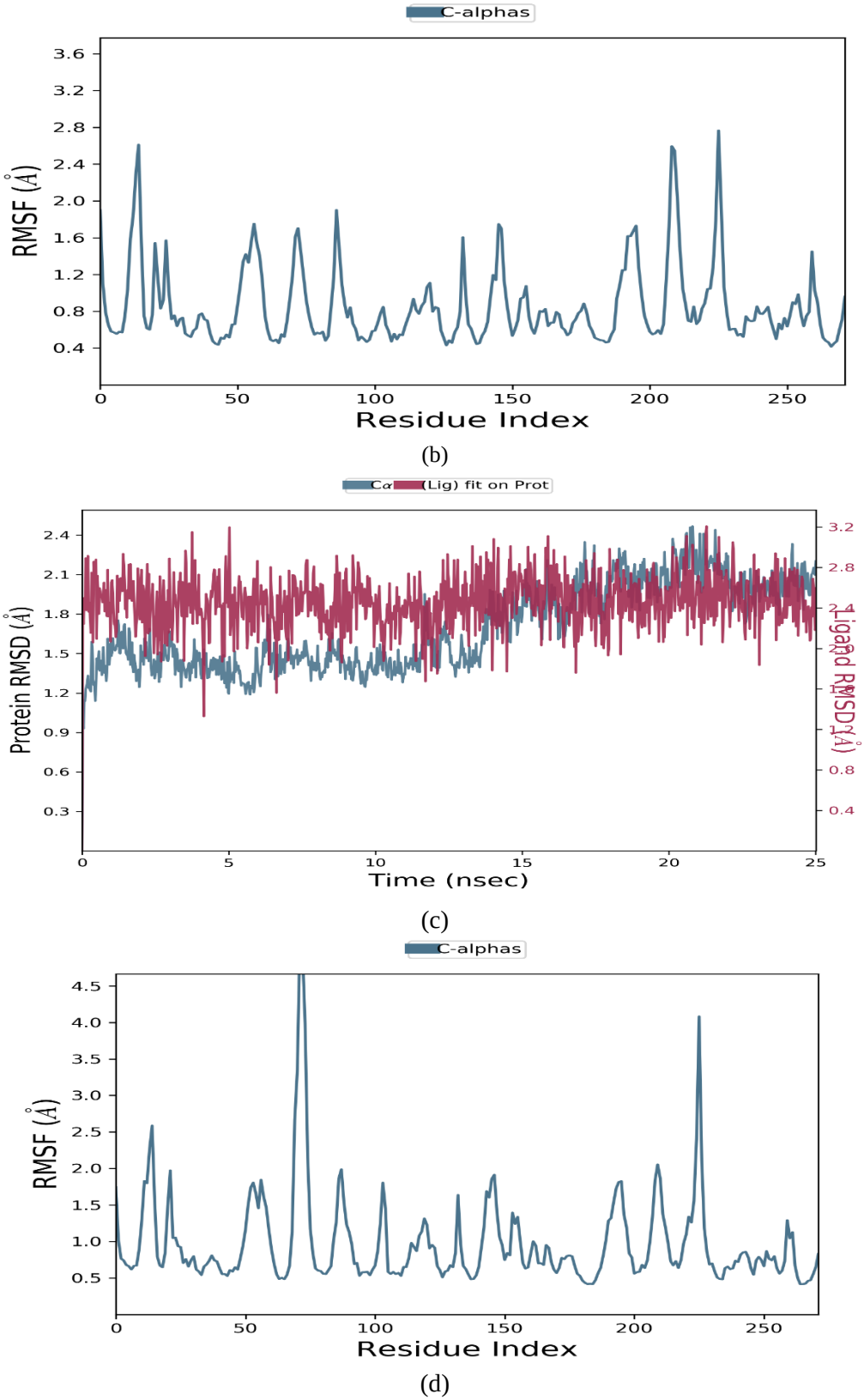


Figure 4. Molecular dynamic simulation of protein-ligand complex (a-b) representing RMSD of protein-ligand and RMSF of protein (PDB ID:2az5) with ligand bisdemethoxycurcumin and zerumbone (c-d) representing the same.

The root means square fluctuation was also calculated for protein backbone residues upon the binding of both bisdemethoxycurcumin and zerumbone (Figure 4). The Root-Mean-Square Fluctuation (RMSF) obtained for protein upon binding with bisdemethoxycurcumin was less than that of zerumbone.

4. Discussion

TNF-α is a pleiotropic cytokine that plays a major role in the host immune system. It is mainly secreted by monocytes and macrophages. The overexpression of the TNF-α may lead to several inflammatory diseases, such as Psoriasis, Rheumatoid arthritis, septic shock, and inflammatory bowel diseases. *D*ue to this, it becomes an excellent therapeutic target [49-51]. Several TNF-α antibodies were developed, including adalimumab, infliximab, etanercept [52-54]. These are directly binding to TNF-α and prevent diseases by avoiding the binding of its receptors, TNFR1 and TNFR2 [55]. These antibodies are shown to be an effective treatment for many inflammatory diseases because of their high specificity. Global Industrial market analyzed, TNF-α antibodies alone accounts for 30 billion dollars in share [13]. However, these antibodies have adverse effects. Therefore, there is a continuous hunt for a new molecule which is having limited or no side effects for the management of inflammatory disease, and small molecule inhibitors are likely to be cheaper to produce as compared with mAbs or Ig-Fc-based fusion proteins [56]. In this connection, some small molecules have been identified through *in vivo* studies as the inhibitors for TNF-α. Still, among them, not even a single molecule has not been reducing the TNF-α induced inflammatory responses with no toxic effect and high efficiency.

The present study identified new TNF-α inhibitors by the *in silico* approach based on the TNF crystal structure complexed with SPD304 and spices compounds as ligands. The biologically active TNF-α is a trimer structure of homogeneous subunits, and SPD304 disassociates the subunits, resulting in inactive protein, which means that functional inhibition was affected by obstructing trimerization [17,57]. The glycine and tyrosine residues in the binding region of the TNF dimer in this protein-protein interaction are primarily hydrophobic. Small-molecule interactions have been described as hydrophobic and shape-driven [58,59] since the molecular structures must be big enough to connect with two subunits to prevent the third subunit from attaching to the TNF dimer these residues are considered active sites to perform the molecular docking studies. The TNF-α structure was fetched from PDB (PDB ID:2az5), and a docking study was performed, resulting in 19 compounds selected for further analysis.

Referral drug (SPD304) contains a toxic moiety of 3-alkylindole metabolized by cytochrome P450 enzymes via a dehydrogenation pathway same as potent pneumotoxin 3-methylindole, generating reactive electrophilic iminium material that could react with protein and DNA targets. ADMET prediction of a referral drug molecule was also performed to compare the results of spices. After docking studies based on referral drug binding interaction, 07 compounds were selected for *in silico* ADMET analysis. The results revealed that compounds Cinnamate, Alliin, Curcumin, and demethoxycurcumin are well absorbed and found non-blood brain permeant. All 07 compounds passed the ADME evaluation. Toxicity prediction by admetSAR and *Protox-II* suggested that the referral drug shows hepatotoxicity and immunotoxicity, but 6-paradol shows mild immunotoxicity and mutagenicity. bisdemethoxycurcumin and zerumbone are non-toxic in all toxicity parameters. Finally, bioactive compounds bisdemethoxycurcumin and zerumbone were selected as inhibitors of TNF-α with obeyed ADMET parameters.

The finalized compounds show docking interaction with the same residues in hydrophobic interaction residues of referral drug (SPD304), i.e., glycine and tyrosine. Due to these interactions, residues make the sub-units disassociation, and the compounds show less

binding energy than the referral drug, which shows stronger binding interaction with the target protein and ADMET properties compared with the referral drug in each parameter. As a result, bisdemethoxycurcumin and zerumbone can be better inhibitors than referral drugs. *In silico* validation of stable binding complexity of ligand and protein was analyzed by molecular dynamic simulation. The results suggest both ligands are showing good stability with targeted protein.

5. Conclusions

Many researchers and pharmaceutical companies use computer-aided drug discovery methods to introduce a new drug into the market with a minimum failure rate because the traditional and rational method of discovering a drug is very difficult, time-consuming, and expensive [60]. The present study uses the in silico approach to identify inhibitors for TNF-α. Here five major spice bioactive compounds are taken to validate whether these compounds can inhibit TNF-α or not. Our study identifies bisdemethoxycurcumin, and zerumbone compounds are showing inhibitory activity against TNF-α with obeyed ADMET parameters. The molecular dynamic simulation also shows the structural stability of the molecules. Further, more experimental validation by *In vitro* and *In vivo* experiments may explore the herbal-based treatment regime for managing inflammatory diseases.

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Conflicts of Interest

The author does not have any Conflicts of Interest.

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