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In silico evaluation of black seed oil from *Nigella sativa* as a therapeutic agent for rheumatoid arthritis: Molecular docking and structural analysis

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Rheumatoid arthritis (RA) is a chronic autoimmune disorder that causes joint pain and inflammation. Despite the availability of various treatments, their side effects often limit their long-term effectiveness, leading to increased interest in natural compounds with fewer adverse effects. *Nigella sativa* (black cumin), known for its diverse pharmacological properties, including anti-inflammatory effects, has gained attention as a potential treatment for RA. However, the specific pharmacological actions of black cumin seed oil in treating arthritis are not well-established. In this context, the current study aimed to evaluate the therapeutic potential of black seed oil by various computational studies like ADMET prediction, network pharmacology, molecular docking, and molecular dynamics simulations. Based on the network pharmacology results, the identified top 2 targets (IL6 and STAT3) are further screened for molecular docking studies with PDB IDs 1alu and 6NJS, respectively. The docking results revealed that eicosadienoic acid exhibited stronger binding activity than the standard methotrexate against the protein ID 1ALU and 6NJS, with docking scores of -7.82 and -8.66 kcal/mol, respectively. Molecular dynamics simulations showed that the eicosadienoic acid IL6 complex remained stable between 40 and 85 nanoseconds. These findings revealed that eicosadienoic acid is a promising therapeutic agent for targeting RA.

Keywords: ADMET studies, Black seed oil, Biological processes (BP), Cellular components (CC), Molecular functions (MF), Rheumatoid arthritis

Rheumatoid arthritis (RA) is a multifactorial autoimmune disease with an unknown etiology and primarily affects the articular joints. The pathophysiological mechanism is moderately elucidated due to the complexity of the disease[1,2]. The global burden of RA was first reported in 2014. The total number of disability-adjusted life years (DALYs) for RA in 2020 was 3.06 million, with an age-standardized rate of 36.4 per 100,000. Most of these DALYs (76.4%) were due to years lived with disability (YLDs). Smoking was responsible for 7.1% of RA-related DALYs. By 2050, it is expected that 31.7 million people will be living with RA worldwide. Rheumatoid Arthritis Global Statistics – 2020, as seen in (Table 1)[3].

However, despite advances in treatment, concerns regarding safety and efficacy necessitate further research[4]. Arthritis is broadly classified into non-inflammatory and inflammatory types, with inflammatory arthritis commonly associated with viral or bacterial infections. The drug classes that are used for the treatment of RA are synthetic disease-modifying antirheumatic drugs, targeted synthetic disease-modifying drugs, and biological disease-modifying antirheumatic drugs[5].

Although there is a wide variety of treatments available for RA, it has severe side effects like Nausea, vomiting, bone marrow suppression, and liver toxicity, *etc*., which in turn highlights the importance of novel drug discovery to eliminate the side effects. Natural compounds are now widely used to find the pharmacological activities with minimal side effects and high efficacy. From the previous research reports data, black cumin is used in traditional medicine with wide pharmacological actions[6,7]. *Nigella sativa*, a medicinal plant belonging to the Ranunculaceae family mainly seen in the bordering countries of the Mediterranean Sea. It has diverse pharmacological properties, including anti-inflammatory properties, anti-tussive, anti-diuretic, anti-cancer, and antipyretic properties[8,9]. The primary volatile

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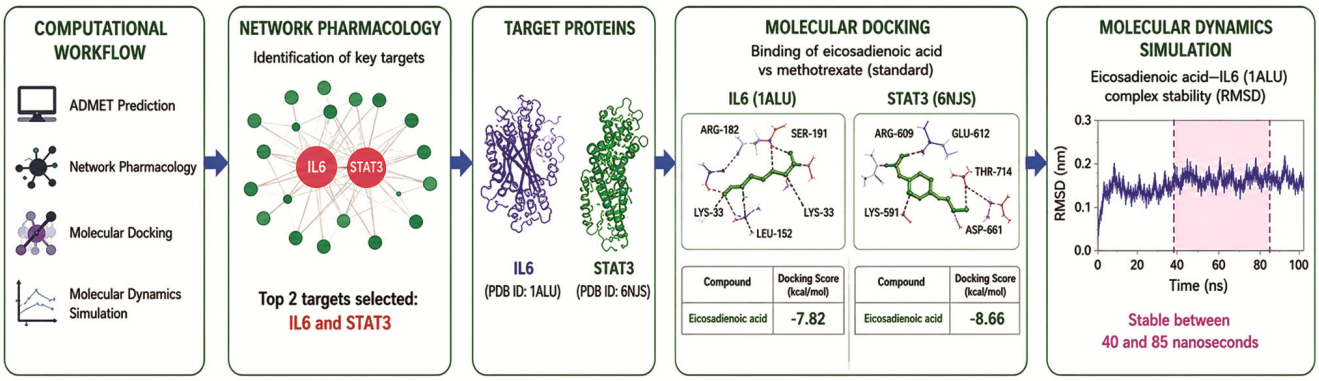

Graphical abstract

Table 1 — Rheumatoid Arthritis Global Statistics – 2020

Category	Value	Unit
People living with rheumatoid arthritis	17.6	million
Global prevalence rate	208.8	per 100,000 people
Death rate	0.47	per 100,000 people
Total deaths	38,300	people
Incidence increase	14.1	%
Death reduction	23.8	%
Female-to-male ratio	2.45	ratio

constituents of black seed are carvacrol, alpha-pinene, beta-pinene, thymol, thymoquinone, linoleic acid, eicosadienoic acid, and thymohydroquinone. It also consists of a wide range of phenolic acids and flavonoids such as gallic, ferulic, vanillic, p-coumaric, chlorogenic acid, catechin, quercetin, apigenin, rutin, flavonoids B, and flavone. Alkaloids in *Nigella sativa* comprise Nigellidine, Nigellimine, Nigellicine, Nigellone, Nigelline, and Magnoflorine. Saponins present in the plant consist of alpha-hederin, nigelloside, flaccinoside, kaempferol-3-rutinoside, and sapindoside[10-12]. Thymoquinone stands out as the most significant constituent of black seeds, along with essential oils like oleic acid, palmitic acid, nigellone, eicosadienoic acid, beta-sitosterol, limonene, gamma-terpinene, beta-thujene, and carvacrol[13]. Thymoquinone is the major constituent in black seed oil with numerous pharmacological activities; there is a notable lack of scientific literature addressing black cumin's diverse effects[14]. Both active phytochemicals and vital nutrients in *Nigella sativa* contribute significantly to human well-being, although it has limited reported pharmacological properties.

Interleukin 6 (IL-6) is an important factor in the development of RA and is a key target for treatment[15,16]. Tocilizumab and sarilumab are interleukin-6 (IL-6) receptor inhibitors that have demonstrated efficacy and are generally well tolerated, either as monotherapy or in combination with other treatments, in patients with rheumatoid arthritis who have shown an inadequate response to conventional disease-modifying antirheumatic drugs (DMARDs) or tumor necrosis factor (TNF) inhibitors[17]. STAT3 is important in RA because it helps cause inflammation. It gets activated by certain proteins, like IL-6 and Oncostatin M, which start the inflammatory process. This leads to the release of other proteins that cause pain, swelling, and damage in the joints of people with RA[18].

Utilizing CADD, also known as the *in silico* method, plays a major role in drug discovery to improve the effectiveness of novel drug discovery and development. ADME is a crucial pharmacokinetic parameter in drug discovery[19]. The investigation of ADMET properties helps to understand the bioavailability, absorption, and permeability of the natural constituents[20]. Network pharmacology is a modern approach to discovering the possible mechanisms and pathways of natural compounds[21,22]. It also gives a correlation between the compound, the pathway, and the target. This method primarily focuses on exploring the multi-target pathways of natural compounds. Molecular

docking plays a crucial role in computer-aided drug discovery by predicting ligand–receptor binding affinities and elucidating the nature of molecular interactions, thereby supporting the identification of potential therapeutic candidates. This method has become a robust tool for investigating drug-active sites, thus significantly contributing to natural product research[23,24]. Molecular dynamics simulation (MD) is a computational simulation approach employed to understand the stability and behaviors of atoms and molecules. Additionally, it helps to determine the relationships between structure and function in biomolecules and to predict their responses in varying environments[25].

In the present study, the main aim was to systematically explore the major bioactive constituents of black seed oil against rheumatoid arthritis (RA) through an integrated computational approach. Network pharmacology was used to find the possible therapeutic targets and key signaling pathways, which were then analyzed by molecular docking and molecular dynamic simulation to assess the binding interactions and dynamic stability of the selected compounds with the best-ranked targets, IL6 (PDB ID: 1ALU) and STAT3 (PDB ID: 6NJS). Furthermore, physicochemical and *in silico* ADME analyses were carried out to evaluate the drug-likeness and the pharmacokinetic properties of the identified compounds. The novelty of this study is that it combined network pharmacology, molecular docking, molecular dynamics simulation, and ADME prediction to comprehensively analyze the potential interactions between the components of black seed oil and important targets related to RA.

Materials and Methods

In silico analysis

Screening of ADMET properties

The phytoconstituents of black seed oil were selected from the Indian Medicinal Plants, Phytochemistry and Therapeutics database (IMPPAT), and their smiles codes were obtained from PubChem. ADME properties were identified using the Swiss ADME online server, while ProTox was utilized to predict the toxicity profile by inserting the SMILES codes of the black seed oil constituents[26,27]. A set of knowledge-based criteria is used in the ADME assessment to determine optimal values for solubility, molecular weight, permeability, partition coefficient, number of metabolites, number of rotatable bonds, and percentage of human oral absorption. Log S (Drug solubility) affects the pharmacological action of the drug and provides an idea of how well the drug can dissolve in biological fluids. The optimal solubility of constituents guarantees the efficient distribution and absorption of the drug throughout the body. Molecular characteristics such as TPSA (Total Polar Surface Area), Log P, counts of hydrogen bond donors and acceptors, molecular weight, rotatable bond count, and bioactivity assessments[28].

Network pharmacology

Screening of hub genes

The selected plant phytoconstituents were collected from the IMPATT database using *Nigella sativa* as a keyword, and the oil constituents are summarized in (Table 4). Smile codes of the constituents were collected from PubChem for target prediction. Target-related genes of 11 constituents were collected using the Swiss Target Prediction database, and disease-related genes were collected from DISGENET. Venny 2.1 was used to identify the common genes between disease and target, referred to as hub genes[29,30].

Construction of PPI

Hub genes were imported into the STRING database using the "multiple protein" option to obtain the protein-protein interaction (PPI) diagram. The PPI network was visualized using Cytoscape 3.10.3. Cytoscape 3.10.3 is an open-source software platform used for visualizing and analyzing complex biological networks. It is commonly used in systems biology and bioinformatics and also allows researchers to import network data, perform analyses, and create graphical network maps. The top 10 ranked gene targets were predicted using the CytoHubba plugin. The pathway and target files from the KEGG enrichment analysis and the compound-target file from the Swiss Target Prediction database were imported into Cytoscape 3.10.3. Both networks were merged and analyzed to create the compound-pathway-target network, which provides insights into the correlations and interrelationships between the compounds and their mechanisms against rheumatoid arthritis[31-33].

GO Enrichment Analysis

Hub genes were imported into the DAVID database, and the functional annotation tool was selected. Information about biological processes,

cellular components, and molecular functions was obtained by selecting Gene Ontology (GO) terms. Visualization was done using the SR plot that provides a clear representation of the gene functions and the pathways involved[34].

Molecular docking

Three-dimensional X-ray crystallographic structures of two proteins involved in rheumatoid arthritis, identified by PDB IDs 1ALU and 6NJS, were retrieved from the RCSB Protein Data Bank[35,36]. The canonical SMILES representations of the phytoconstituents (ligands) were obtained from the PubChem compound database, and their 3D structures were generated using ChemDraw and saved in .mol format. Docking studies were performed using MOE software to visualize the interactions between the ligands and the protein molecules. Prior to docking, active site analysis and energy minimization were conducted to optimize the protein. Finally, the docking complex was analyzed to determine the interactions and docking scores, providing a detailed overview of the ligand's effectiveness in binding to the respective protein receptors[37].

Molecular dynamics

Molecular dynamics simulations were conducted using Desmond V 5.9 from Schrödinger LLC on an Ubuntu platform to evaluate the stability of the protein complex for a 100ns time frame. For this, we configured a workstation with a 16GB Nvidia GTX3060 GPU and installed the CUDA platform to integrate the GPU with Desmond. The most stable conformer was saved as a PDB file and subjected to energy minimization with the OPLS force field. The protein complex was centered in an orthorhombic cubic box filled with TIP3P water molecules, maintaining a 10 Å buffer between the protein and the box edges. To neutralize the system, counter ions (Na^+ and Cl^-) were added, and the box volume was adjusted accordingly. Energy minimization was carried out using the OPLS-2005 force field, with the simulation employing NPT and NVT ensembles to maintain a temperature of 10K while constraining the heavy atoms of the solute[36]. The simulation ran with a 20 ps relaxation time, at 1 atm pressure and around 300 K temperature, with a reference simulation duration of 100 ns. The simulation trajectory was analyzed using Desmond's built-in interface[38-40].

Results and Discussion

***In silico* analysis**

Physiochemical properties analysis and ADMET studies

The ADMET (absorption, distribution, metabolism, excretion, and toxicity) properties are crucial for determining a compound's bioavailability, permeability, and solubility in drug discovery and development. In our study, we used SWISSADME to predict the ADME characteristics of the phytoconstituents. For a pharmaceutical compound to be considered viable, it must demonstrate strong biological activity and low toxicity.

According to Lipinski's rule of five, compounds with no more than one violation are considered potentially active. Thymoquinone, linoleic acid, oleic acid, palmitic acid, eicosadienoic acid, nigellone, and nigelline show high absorption in the gastrointestinal tract, while beta-thujene, limonene, gamma-terpinene, and beta-sitosterol exhibit low absorption. Additionally, thymoquinone, linoleic acid, palmitic acid, beta-thujene, limonene, gamma-terpinene, nigellone, and nigelline have a strong ability to cross the blood-brain barrier.

The molar refractivity of thymoquinone (47.52), linoleic acid (89.46), oleic acid (89.94), palmitic acid (80.80), eicosadienoic acid (45.22), nigellone (47.12), and nigelline (133.23) meets the criteria for high solubility. The LogP values for thymoquinone, linoleic acid, oleic acid, palmitic acid, beta-thujene, limonene, gamma-terpinene, beta-sitosterol, eicosadienoic acid, nigellone, and nigelline are 1.99, 4.14, 4.27, 3.85, 2.62, 2.72, 2.73, 4.79, 4.41, 2.46, and 2.28, respectively. These results suggest that the active constituents of black seed oil have LogP values between 1 and 3, indicating a good balance between hydrophilicity and lipophilicity. However, linoleic acid, oleic acid, palmitic acid, beta-sitosterol, and eicosadienoic acid have LogP values between 3 and 4, which indicates greater lipophilicity. The physicochemical properties of black seed oil constituents are summarized in (Tables 2 & 3).

Physicochemical and ADME parameters of the main constituents of black seed oil obtained from *in silico* were largely consistent with the experimental and literature-reported pharmacokinetic parameters. The molecular weight, TPSA, hydrogen-bonding properties, solubility class, and LogP of thymoquinone were well predicted by the published values, which confirm that thymoquinone is moderately lipophilic and is expected to have good

Table 2 — Physiochemical properties of active constituents in black seed oil

Molecule	Molecular weight	R. bonds	H-bond acceptor	H-bond donor	TPSA	Bioavailability Score	Lipinski violations
Thymoquinone	164.2	1	2	0	34.14	0.55	0
Linoleic acid	280.45	14	2	1	37.3	0.85	1
Oleic acid	282.46	15	2	1	37.3	0.85	1
Palmitic acid	256.42	14	2	1	37.3	0.85	1
Beta thujene	136.23	1	0	0	0	0.55	1
Limonene	136.23	1	0	0	0	0.55	0
Gama terpinene	136.23	1	0	0	0	0.55	0
Beta sitosterol	414.71	6	1	1	20.23	0.55	1
Eicosadienoic Acid	308.5	16	2	1	37.3	0.85	1
Nigellone	328.4	2	4	0	68.28	0.55	0
Nigelline	195.22	4	3	1	47.56	0.55	0

Table 3 — Solubility and Permeability parameters of active constituents in black seed oil

Compound name	MR	GI absorption	BBB permeability	LOG P	Solubility
Thymoquinone	47.52	High	Yes	1.99	-2.18
Linoleic acid	89.46	High	Yes	4.14	-5.05
Oleic acid	89.94	High	No	4.27	-5.41
Palmitic acid	80.80	High	Yes	3.85	-5.02
Beta thujene	45.22	Low	Yes	2.62	-2.77
Limonene	47.12	Low	Yes	2.72	-3.5
Gama terpinene	47.12	Low	Yes	2.73	-3.45
Beta sitosterol	133.23	Low	No	4.79	-7.9
Eicosadienoic Acid	99.08	High	No	4.41	-5.76
Nigellone	91.24	High	Yes	2.46	-3.05
Nigelline	53.52	High	Yes	2.28	-2.73

membrane permeability. The long-chain fatty acids (linoleic, oleic, and palmitic acids) showed high lipophilicity and low aqueous solubility, which are very similar to their well-documented physicochemical properties reported in the literature. Likewise, β-sitosterol was highly lipophilic and poorly soluble in water, and this is consistent with its experimentally reported low oral bioavailability. The monoterpenes and the alkaloid-like constituents (limonene, β-thujene, γ-terpinene, nigellone, and nigelline) were also shown to share ADME properties in line with their reported membrane permeability and pharmacokinetic properties. In general, there was good agreement between the predicted ADME parameters and the experimental and literature-reported pharmacokinetic data, thus demonstrating the reliability of the *in silico* prediction.

Toxicity Profile

In the drug discovery process, a significant challenge is drug toxicity. To address this, the selected constituents were screened for toxicity prediction using the free, open-source tool ProTox II. Preliminary toxicity studies evaluating various endpoints, including hepatotoxicity, neurotoxicity, nephrotoxicity, respiratory toxicity, cardiotoxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity, were conducted. The compounds thymoquinone, nigellone, palmitic acid, oleic acid, linoleic acid, and ecosadienoic acid were found to be inactive in all toxicity assessments. The *in silico* predictions of toxicity are reasonably consistent with the experimental data that are available. Thymoquinone exhibits low acute oral toxicity and no apparent organ toxicity at pharmacologically relevant doses in rodents, but it has been reported to have concentration-dependent hepatocyte cytotoxicity and genotoxicity at higher concentrations *in vitro*. Nigellone data are limited, but nigellone-containing preparations of *Nigella sativa* are relatively nontoxic in experimental models. Palmitic acid, oleic acid, linoleic acid, and eicosadienoic acid are endogenous or dietary fatty acids that have been shown to be safe and inactive in ProTox-II: there is no evidence of direct hepatotoxicity, nephrotoxicity, mutagenicity, or carcinogenicity.

Network pharmacology

Screening of hubgenes

A total of 343 genes were collected from the DISGENET database, and 299 genes were gathered

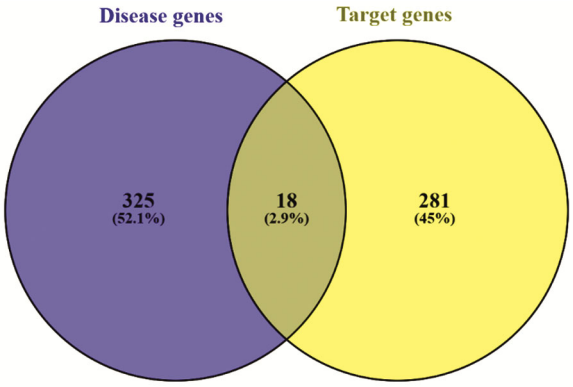

Fig. 1 — Venn diagram of the common genes of the target and the disease

Table 4 — Top 10 targets of black seed oil with degree

Rank	Name	Score (Degree)
1	IL6	15
2	STAT3	12
3	PTGS2	11
4	MMP2	7
5	MAPK1	7
6	PTPRC	6
7	ALOX5	6
8	ESR2	5
9	ABCB1	5
10	PTGS1	4

from the Swiss Target Prediction database. Eighteen hub genes were imported into STRING, and the Protein-Protein Interaction (PPI) network was visualized using Cytoscape. Hubgenes were visualized by Venny 2.1, which is given in (Fig. 1).

Construction of PPI and compound target pathway network

The PPI interactions network was constructed by imputing 18 hub genes into the STRING database. A network consists of nodes and edges. Nodes symbolize either proteins or genes and the edges represent the associations between them. PPI network helps in predicting the interactions and their connections with the receptors. The Cytohubba plugin of Cytoscape is used to find out the top 10 ranked genes. The top 10 target genes of Black Seed Oil, ranked by their significance, are IL6, STAT3, PTGS2, MMP2, MAPK1, PTPRC, ALOX5, ESR2, ABCB1, and PTGS1, with degree scores of 15, 12, 11, 7, 7, 6, 6, 5, 5, and 4. The top target genes were given in (Table 4). The top 10 genes of phytoconstituents of *Nigella sativa* involved in various processes such as inflammation, immune response, and cell signaling are given in (Fig. 2).

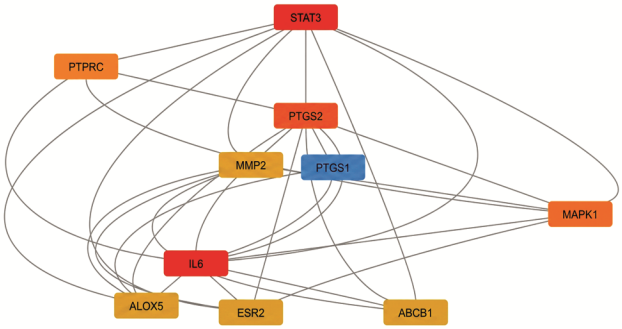

Fig. 2 — Top 10 target genes of black seed oil against rheumatoid arthritis

GO enrichment analysis

GO and KEGG enrichment analyses were conducted to investigate the biological processes, cellular components, and molecular functions related to the identified genes. Gene Ontology was used to predict the pathways involved in the biological processes. The top 10 enriched GO terms for each category were displayed in a bar graph for biological processes (BP), cellular components (CC), and molecular functions (MF) using the SR Plot (Fig. 3). The biological process (BP) analysis predicted that the potential targets were primarily involved in the prostaglandin biosynthetic process. In terms of cellular components, the targets were mainly found on the external side of the apical plasma membrane. For molecular functions (MF), the targets were enriched in prostaglandin-endoperoxide synthase activity.

Molecular docking

Eleven active constituents were selected and screened for ADMET properties. Further, by using network pharmacology, the top 10 targets were identified. Docking was performed using MOE 2022 against two rheumatoid arthritis targets, IL-6 (PDB ID: 1ALU) and STAT3 (PDB ID: 6NJS), which were selected for molecular docking analysis as they were identified as the top two hub genes in the protein–protein interaction (PPI) network using STRING and ranked by CytoHubba in Cytoscape. This selection was supported by their crucial roles in RA pathogenesis, particularly in the regulation of inflammatory signaling. Methotrexate was selected as the reference drug because it is the first-line antirheumatoid drug. The docking protocol was validated using the co-crystal ligand, and the superimposed and redocked ligand poses are given in (Fig. 5).

The interactions between the ligands and proteins were evaluated to determine their binding affinities.

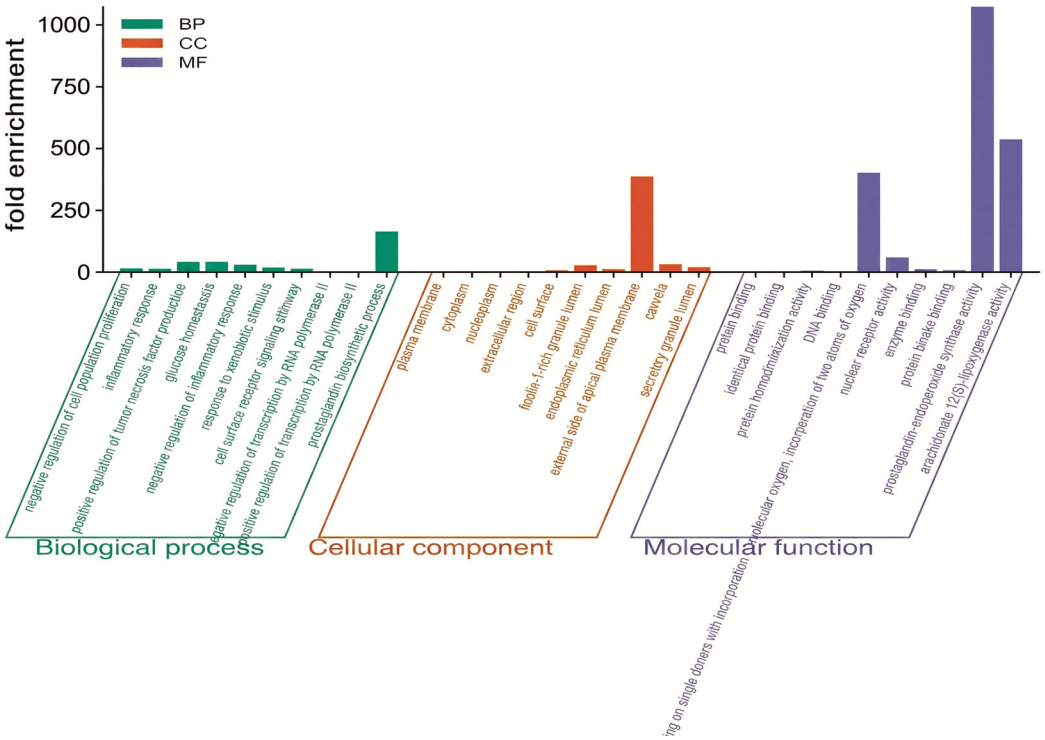

Fig. 3 — Biological process, cellular components, and molecular function of phytoconstituents

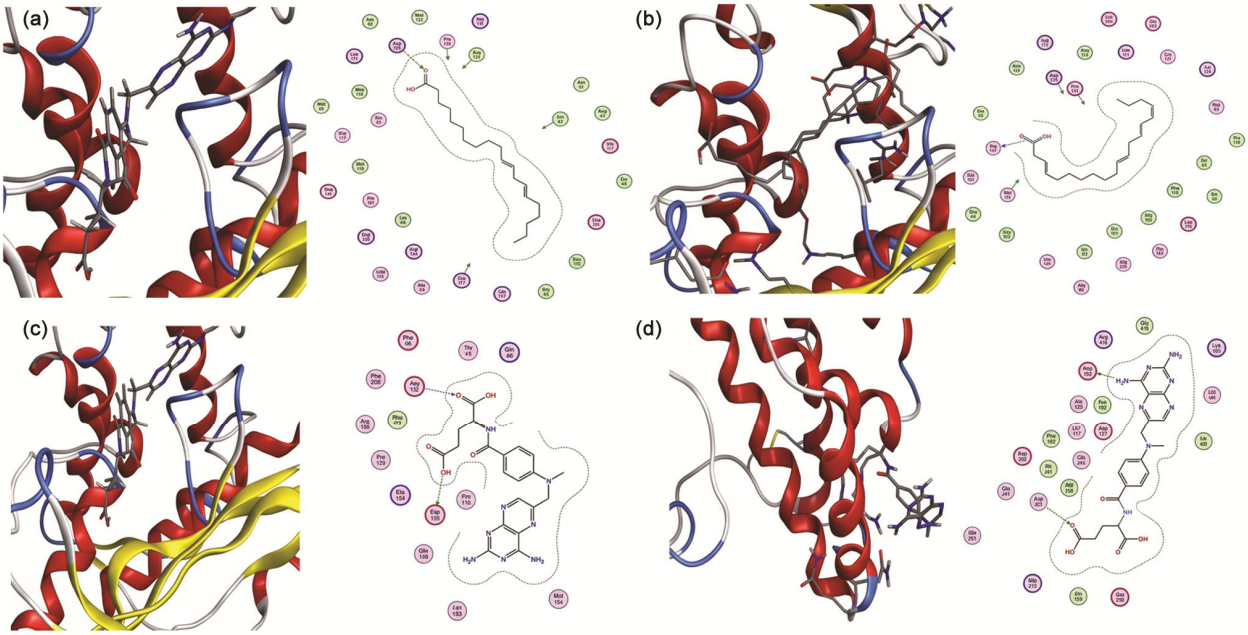

Fig. 4 — Superimposition of the co-crystallized and redocked ligand poses within the binding pocket of 1ALU and 6NJS

All eleven compounds exhibited relatively strong interactions with the target proteins. The docking scores for the compounds against protein 1ALU were as follows: thymoquinone (-5.62), linoleic acid (-7.14), oleic acid (-6.53), palmitic acid (-6.09), beta-thujene (-5.26), (-)-limonene (-5.39), γ-terpinene (-5.25), beta-sitosterol (-5.78), eicosadienoic acid (-7.82), nigellone (-5.167), and nigelline (-6.041 kcal/mol). Against the second protein target (6NJS), the docking scores were: thymoquinone

Table 5 — Docking score and binding interactions of active constituents of Black seed oil

Compound Name	Docking score kcal/mol (PDB ID: 1ALU)	Interaction (PDB ID: 1ALU)	Docking score kcal/mol (PDB ID: 6NJS)	Interaction (PDB ID: 6NJS)
Thymoquinone	-5.62	LEU 64	-4.93	-
Linoleic acid	-7.14	-	-7.63	-
Oleic acid	-6.53	LYS 66	-7.58	-
Palmitic acid	-6.09	SER 176	-7.36	-
Beta thujene	-5.26	-	-4.54	-
Limonene	-5.39	-	-5.023	-
Gama terpinene	-5.252	-	-4.74	-
Beta sitosterol	-5.781	ARG 182	-6.81	-
Eicosadienoic Acid	-7.828	ASP 179	-8.66	GLY 254
Nigellone	-5.167	-	-5.90	-
Nigelline	-6.041	-	-5.02	LYS 574, ASP 570
Methotextrate	-6.81	GLU 106, ASP 160	-7.49	CYS251, ASP334

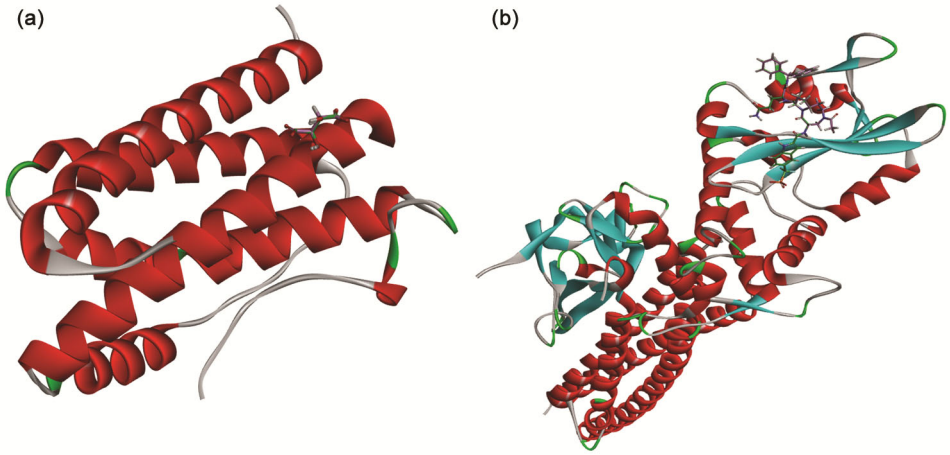

Fig. 5 — 3D and 2D interactions of eicosadienoic acid (a & b) and methotrexate (c & d) with 1ALU and 6NJS

(-4.93), linoleic acid (-7.63), oleic acid (-7.58), palmitic acid (-7.36), beta-thujene (-4.54), (-)-limonene (-4.87), γ-terpinene (-4.74), beta-sitosterol (-6.81), eicosadienoic acid (-8.66), nigellone (-5.90), nigelline (-5.0 kcal/mol). The 2D interaction diagram of the ligand-protein complex showed how the ligands interacted with their target proteins. Thymoquinone showed one interaction with residue LEU 64, while palmitic acid interacted with residues SER 176 and PRO 65, and beta-sitosterol and eicosadienoic acid interacted with residues ARG 182 and ARG 179 in 1ALU. Eicosadienoic acid also interacted with the key residue GLU 254. Nigellone interacted with residues LYS 574 and ASP 570 in 6NJS. Among the docked phytoconstituents, eicosadienoic acid exhibited docking scores of -7.82 and -8.66 kcal/mol against IL-6 and STAT3, respectively. In comparison, the reference drug methotrexate showed docking scores of -6.81 and -7.49 kcal/mol. The docking values and the interaction profiles of eicosadienoic acid and methotrexate with IL-6 and STAT3 are illustrated in (Table 5). Methotrexate, the standard drug, interacted with residues GLU 106 and ASP 160 in 1ALU and CYS 251, ASP 334 against 6NJS as shown in (Fig. 4). Furthermore, eicosadienoic acid was selected for a molecular dynamics study due to its favorable binding energy and interaction profile with the target proteins.

Molecular dynamics

The molecular dynamics simulation for the IL6-eicosaidenoic acid and STAT3-eicosaidenoic acid complex was used to determine the stability and interaction profile. The protein-ligand complex of the selected four compounds was subjected to dynamic simulation studies (100 ns), and the analysis of the complex was performed based on the root mean square deviation, root mean square fluctuation, and protein-ligand contacts. The average distance generated by the displacement of the selected atoms over a certain period relative to the reference time frame is measured using the RMSD. RMSD value of

the C-alpha and protein fit ligand is computed for all the time frames in the reference time (100 ns). Protein analysis during the molecular simulation studies of IL-6 eicosaidenoic acid revealed conformational changes whenever the ligand interacts with the residues. The stability of the complex eicosaideinoic acid is within the limit, having a maximum deviation of 3.2 Å, and the complex is stable throughout the 100ns simulation time with the target IL6. The Ligand RMSD value of eicosadienoic acid showed fluctuations for the first 40 nanoseconds but remained stable from 40 nanoseconds to 85 nanoseconds. Though some conformational changes occur, the complex remains in an equilibrium condition.

RMSF in molecular dynamics simulations provides a clear idea about the extent of fluctuations within a complex as it undergoes conformational changes. The stability of the complexes may be influenced by the interactions between the various ligands. The fluctuations do not indicate instability; they are often intensified by the ligand-protein binding. From the results of IL-6 RMSF, it is revealed that our complex exhibits fewer fluctuations. In contrast, regions corresponding to secondary structure elements show greater rigidity compared to other parts of the protein, contributing to a manageable level of fluctuation. The RMSF values remain within an acceptable range, like RMSD, and stay below 4 Å, indicating a stable and manageable level of fluctuation throughout the simulation. Protein-ligand interaction studies play an important role in designing novel and effective medications. The interactions are of various types: hydrogen bonds, hydrophobic bonds, ionic interactions, and water bridges. The fraction on the Y-axis represents the proportion of time each residue remains in contact with the ligand during the simulation. A fraction of 1 means that a residue is in contact with the ligand for 100% of the simulation time. If the fraction exceeds 1, it indicates that the interaction is especially strong and sustained, with the residue interacting with the ligand for more than 100% of the simulation time, reflecting a high level of stability and persistence in the interaction. The ligand interactions within the protein-receptor complex involve several key residues, including ASN 48, LEU 87, ALA 58, LEU 62, LEU 64, MET 67, GLU 69, CYS 73, PHE 74, GLN 75, PHE 74, GLN 75, GLY 77, PHE 78, GLU 80, LEU 165, PHE 173, ARG 179, ALA 180, ARG 182, and GLN 183. Among these residues, GLN 75, PHE 18, ARG 179, and GLN 183 stand out as key residues, with interaction fractions greater than 0.2, indicating that these residues maintain significant and sustained contact with the ligand throughout a considerable portion of the simulation time.

The molecular dynamics simulation of the eicosadienoic acid-IL-6 complex revealed that the ligand properties remained stable with minor fluctuations. The radius of gyration (Rg) increased from 6.5 Å to 7.5 Å after 30 ns, indicating slight conformational changes. The molecular surface area (MOLSA) remained stable at 400 Å² for the first 25 ns, fluctuated to 340 Å², and then returned to 400 Å² from 60 to 100 ns. Solvent-accessible surface area (SASA) remained stable at 300 Å² between 65 and 100 nanoseconds, indicating minimal surface exposure changes. The polar surface area (PSA) remained mostly stable at 100 Å², with minor fluctuations at 25, 45, and 60 nanoseconds (45 Å², 60 Å², and 40 Å²). These results reveal that eicosadienoic acid forms a stable complex with IL6, with only minor conformational adjustments, indicating its potential to target IL6 as a therapeutic agent. The RMSD, RMSF, ligand contacts, and ligand properties are given in (Fig. 6).

In contrast, the protein RMSD of the STAT3 complex showed an initial rise from approximately 1.2 to 2.0 Å. Between 20 and 35 ns, the RMSD fluctuated between 2.5 and 3.0 Å. From 40 to 50 ns, a slight decrease in RMSD was observed, followed by an increase from 50 to 60 ns around 2.5–3.0 Å. From 70 to 90 ns, the RMSD ranged between 2.4 and 2.6 Å and then remained stable. The ligand RMSD remained below 2 Å for the initial 5 ns and then fluctuated further up to ~4.5 Å. Further, there was a sharp increase in RMSD, reaching around 10-11 Å at 25 ns, decreasing to around 7 at 34-37 ns, and then rising again to around 7. It further decreased from 50 to 85 ns at a distance of approximately 4.5 to 6 Å. From 85 ns, the RMSD increased to approximately 9 and 12 Å. According to the RMSF analysis performed on the STAT3-ligand complex, most of the residues had fluctuations of less than 1.5 Å, thus implying strong stability of the structure during the simulation process. However, high fluctuations were evident at some flexible loops, particularly those found between residues 28 and 40, with an RMSF of around 4.1 Å. There was also a second area of increased fluctuations between residues 113 and 125, with an RMSF of about 2.5–3.8 Å. Other than these areas, the rest of the residues had minimal fluctuations, meaning that no major changes in the protein occurred due to the ligand binding.

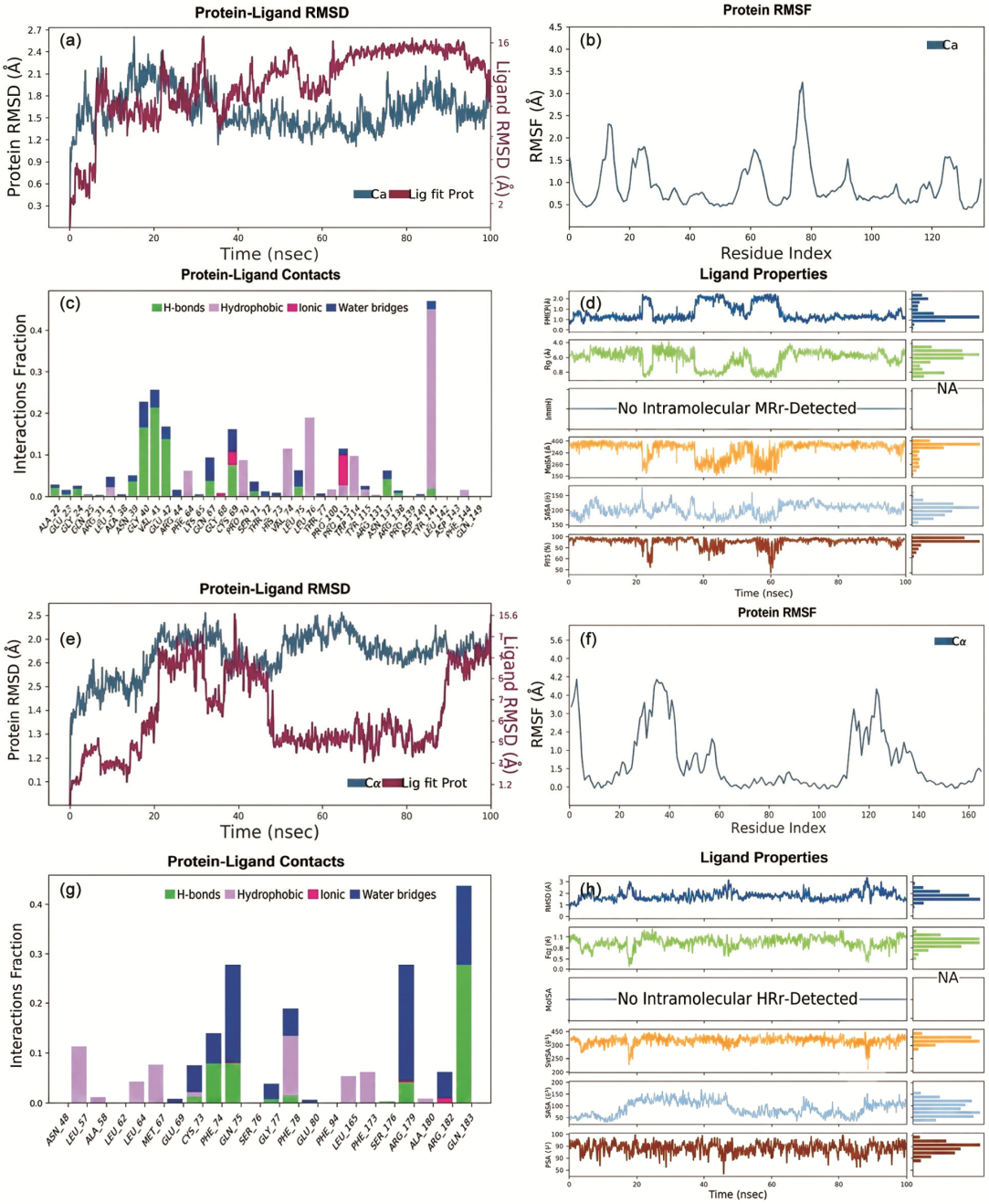

Fig. 6 — (a-d) IL-6-eicosadienoic acid complex showing (A) RMSD, (B) RMSF, (C) Protein-ligand contacts, and (D) ligand properties of eicosadienoic acid. (E-H) STAT3-eicosadienoic acid complex showing (E) RMSD, (F) RMSF, (G) Protein-ligand contacts, and (H) ligand properties

The interacting residues of STAT3 complex included ASN48, LEU57, ALA58, LEU62, LEU64, MET67, GLU69, CYS73, PHE74, GLN75, SER76, GLY77, PHE78, GLU80, PHE94, LEU165, PHE173, SER176, ARG179, ALA180, ARG182, and GLN183. Among these residues, GLN183 exhibited the highest interaction fraction (approximately 0.47), followed by GLN75 (0.28), ARG179 (0.28), PHE78 (0.19), GLU80 (0.17), and CYS73 (0.14). For the other "interacting" residues, the interaction fractions were < 0.10, suggesting a transient interaction during the simulation. Eicosadienoic acid stably resided in the STAT3 binding pocket throughout the 100 ns simulation, as indicated by the persistent interactions with GLN183, GLN75, ARG179, PHE78, GLU80, and CYS73.

The ligand property analysis also supported the stability of the eicosadienoic acid–STAT3 complex during the 100 ns simulation. The rGyr remained relatively constant, from about 6.0 Å up to 7.3 Å, with only a short drop to about 5.0 Å at about 18 ns, where the compactness of the ligand was not significantly different. Likewise, MolSA was almost unchanged at around 390–400 Å², except for two brief decreases at ~ 18 ns and ~ 89 ns, followed by rapid recoveries at these times. The SASA ranged between 60 and 240 Å², with moderate changes in the solvent exposure of the ligand during the simulation but no changes that persisted for the entire simulation.

In summary, the molecular dynamics simulations revealed that eicosadienoic acid complexes with IL-6 and STAT3 with different binding interactions and that both complexes are stable. The IL-6 complex reached a stable state after initial ligand adjustments and maintained a stable protein structure with consistent protein–ligand interactions. The STAT3 complex, however, exhibited greater mobility of the ligands throughout the simulation while the structure of the protein remained stable. The two complexes remained stable during the simulation because of continuous contact with residues that bind to the key. These results suggest that eicosadienoic acid forms a more stable complex with IL-6 than with STAT3 and a more flexible ligand with STAT3 than with IL-6.

The results of this study were obtained by using integrated computational techniques such as molecular docking, molecular dynamics simulation, and *in silico* ADME studies, which offer valuable insights into the likely interactions, stability, and pharmacokinetics of the key constituents of black seed oil. Both approaches are predictive and depend on what is already known about the structures, force-field parameters, and statistical models of the biological systems, and these may not be complete representations of the complexity of biological systems. Thus, the present results should be viewed as a hypothesis and should be further validated using *in vitro* and *in vivo* experiments with appropriate cell lines and animal models to validate the predicted biological activity and pharmacokinetic profile.

Conclusion

Rheumatoid arthritis is a crippling autoimmune disease that involves the immune system attacking healthy tissue. The results of the present *in silico* study revealed that one of the main constituents of black seed oil, eicosadienoic acid, had promising interactions with IL6 and STAT3, which are important mediators in the pathogenesis of RA. Physicochemical property analysis, molecular docking, molecular dynamics simulations, and predictions of ADME suggested good drug-like characteristics, stability of protein/ligand interaction, and acceptable pharmacokinetic properties. The docking results revealed that the protein structures 1ALU and 6NJS have higher docking scores for eicosadienoic acid, with predicted values of −7.82 and −8.66, respectively, compared to methotrexate's docking score. In addition, the molecular dynamics simulation showed that the overall structural stability of the IL6–eicosadienoic acid complex is retained during the 100 ns simulation period. The ligand RMSD varied during the simulation, but no protein-ligand interaction was lost throughout the run, indicating that the ligand stayed within the protein's binding site under the simulated conditions. In general, the results suggest that eicosadienoic acid could be a potential candidate for future studies on RA. The findings indicated in the present study are based solely on computational analyses, and the predicted biological activity and therapeutic potential need to be confirmed by suitable *in vitro* and *in vivo* studies.

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Conflict of interest

All authors declare no conflict of interest.

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