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Molecular Docking of *Nigella Sativa* Phytocompound as Inhibitors of Transcription Factors NF-KB Implicated in Rheumatoid Arthritis

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Abstract

Objectives: *Nigella sativa, a plant considered around the world as the most treasured nutrient-rich herb for centuries together in different civilizations, is exceptionally known for its high levels of antioxidant properties. Free radical intensification due to oxidative stress plays a key role in the pathogenesis of rheumatoid arthritis by activating the NF-KB protein that regulates the expression of the genes involved in inflammation.* ***Methods:*** *This study involves in-silico approach to validate the antioxidant properties of the phytocompounds obtained from Nigella sativa in inhibiting the protein activity of NF-KB by contemplating the binding affinity of the phytochemicals with the target macromolecule virtually by Molecular Docking (PyRx).* ***Results:*** *Phytochemical compounds that surpassed the ADME test were screened based on the binding affinity against the protein. Ligands showing values less than -6 kcal/mol were further visualized, and the computation results manifested phytocompound Nigellone (Dithymoquinone) had the strongest binding affinity against the protein NF-KB, recommending the compound for future therapeutic interventions in the treatment of RA.*

Keywords: *Nigella sativa*, Nigellone, NF-KB, free radicals, oxidative stress, rheumatoid arthritis

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory autoimmune disease (AID) of the synovial membrane causing progressive and irreversible joint destruction left untreated, which leads to ankylosis [1]. RA can affect an individual at any age, predominantly occurring 2–3 times more in women. In 2020, an estimated 17.6 million people were affected globally, taking over 0.5–1% of the population, and it is forecasted to be taking over 31.7 million people worldwide by 2050 [2]. The Incident rates in the Indian populations are estimated to be 0.9% [3], in North Africa and the Middle East around 0.16% [3, 4], whereas in US reportedly 0.5–1% [4, 5]. RA is a progressive disease typically occurring in stages over a span of multiple years. The HLA-DRB1 genetic risk locus, the shared epitope (SE) alleles (conserved sequences of 5 amino acids), is the key genetic risk factor strongly associated with the development of RA [6, 7, 8]. In the initial stages of RA, malfunctioning mitochondria and increased DNA damage sustain the free radical generation in the synovium, causing oxidative stress (OS), which has shown a direct correlation associated with synovitis causing degeneration of the cartilage by denaturation of collagen and proteoglycan protein [1, 9].

Reactive oxygen species (ROS) in the immune system act as a signaling molecule and are observed to be at alleviated levels in patients with RA [10]. RA Pathogenesis is associated with decreased antioxidant levels, impaired antioxidant defenses, and increased oxidative stress, which leads to major consequences like lipid peroxidation. In spite of the cellular repair systems ability to correct most of

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these damages, DNA lesions caused due to free radicals over a prolonged period could be an important attribute towards understanding autoimmune diseases like RA [1]. One such transcription factor (TF) protein activated by free radicals (ROS) is nuclear factor-kappa B (NF-KB) P52 bound to DNA, which plays a major role in the pathogenesis of RA by regulating the expressions of genes involved in immune response, cell survival, and inflammation [11–13].

The main objective of this study is to inhibit the mechanism of free radicals and downregulate the functioning of NF-KB in the cellular destruction of RA by effectively utilizing the antioxidant properties of the bioactive compound obtained from *Nigella sativa* nigellone over NF-KB [14–16]. Current treatment plans include non-specific immune suppression, such as DMARDS, NSAIDS, corticosteroids, immunosuppressants leading to systemic toxicity, risk of infections, and malignancies [17]. Due to these serious side effects an important adjuvant and complementary therapy is taking an interesting twist in the treatment of RA concerning dietary supplements which involves extraction of polyphenols, a highly water-soluble chemical compound derived from a diverse class of medicinal plants. Polyphenols are shown to have definite clinical efficacy, properties, such as multi-mechanism in pharmacology, multi-component, multi target, no drug-resistance and less toxic side effects making them to be an interesting chemical component to be worked upon [18]. This study primarily focuses on phytochemistry and therapeutic properties of *Nigella sativa* (black seed), whose traditional use as a natural medicine dates back as early as 1st century A.D. [19]. Due to the high levels of various bioactive compounds this plant is considered for its therapeutic effects, antioxidant, anti-inflammatory, anti-allergic, anti-cancer, hypolipidemic and immunomodulating properties [20].

The current study is based on in-silico methodology–molecular docking to evaluate the efficacy of the bioactive compounds using computational tools by analyzing various properties of the compound that are already available in the databases. Therefore, in this research, we have thoroughly analyzed therapeutic properties of bioactive compounds obtained in *Nigella sativa* as a potential inhibitor of inflammatory responses by suppressing the upregulation of NF-KB protein in the treatment of RA.

MATERIALS AND METHODS

Preparation and Selection of the Target Protein

The 3D structure of the target protein NF-KBP52 homodimer-DNA complex (1A3Q) was downloaded in PDB format from RCSB Databank (https://www. rcsb. org/structure/1A3Q) [21]. Protein was prepared in Discovery Studio 2021 v21. 1. 0. 20298 (BIOVIA) software were the protein is analyzed and cleaned of hetatms, ligands, water molecules, polar hydrogen atoms were added and the protein binding sites were retained [22]. The 3D structure of the altered protein with A and B is then saved in PDB format to proceed ahead with the molecular docking using PYRX.

Preparation and Selection of the Ligand

30 bioactive compounds from *Nigella sativa* plant were chosen from IMPPAT Database (https://cb. imsc. res. in/imppat/) [23]. The canonical smiles and 3D structure data file (SDF) format was downloaded from Pubchem (https://pubchem. ncbi. nlm. nih. gov/) [24]. Further analysis of the compounds for physicochemical, pharmacokinetics, drug likeness was done using web tool Swiss ADME (http://www. swissadme. ch/index. php#1) [25]. Out of 30 compounds, 18 most favorable compounds were shortlisted based on ADME screening to further determine the potential and binding affinity of the compounds via molecular docking against the target protein.

In Silico ADME Analysis

During the selection of the ligands, undesirable toxicity and pharmacokinetics of the compounds are to be strictly screened to avoid any adverse effects in the later stages of drug research and development processes. One such tool used to analyze the most desirable compound is Swiss ADME, which analyzes various parameters, such as absorption, distribution, metabolism, excretion, toxicity, pharmacokinetics, and medicinal chemistry, to mention a few. Emphasis on 0 violation Lipinski parameters is taken into consideration while screening and validating the most favorable compounds to carry further for docking analysis. 30 compounds from *Nigella sativa* were chosen for the ADME analysis. Compounds with high GIA absorption, Lipinski 0 violation filters (MW≤500, MLogP≤4. 15, N or O≤10, NH or OH≤5) were strictly followed. 18 compounds passed the above parameters and were carried forward in the research.

Molecular Docking

The in-silico target-based screening on the purified protein 1A3Q was carried out in PyRx [26]. The protein was purified in BIOVIA TOOL and was saved in pdbqt format. The PDB file of the prepared protein was loaded, and by autodock, converted into pdbqt format in PyRx. The ligands were loaded in SDF format into PyRx via open babel tool, energy was minimized and then converted into autodock ligand pdbqt format. The ligands and proteins were then selected, moving onto vina vizard, and the grid dimensions were set. The grid dimensions for purified protein 1A3Q for chains A and B were set in vina vizard are as follows: X:16. 7623 Y:61. 4114 Z:0. 6667 dimensions (angstrom) x:82. 8715 Y:89. 3828 Z:93. 8778 and was subjected to docking. The binding affinity scores obtained for ligands against the target protein were then saved in CVS file format. The results were analyzed, and the binding affinity with the lowest score that coordinated with 0 rmsd were preferred for further confirmation and visualization. The pdbqt file of the ligand was saved in PDB format. The pdbqt files of the protein and the selected ligand were then submitted in the biovia tool for visualization to observe the interaction between the target protein against the chosen ligand.

RESULTS

Phytocompound Selection

The seeds of the *Nigella sativa* plant are known infamously as cures for any disease except death. The 30 topmost phytochemicals were chosen from the IMPPAT database and screened. Canonical smiles and 3D SDF downloads of the compounds were obtained from PubChem. 18 compounds passed the screening in ADME analysis. The binding affinity of the ligands ranging from –3. 7, i.e., decanoic acid (PubChem ID: 2969) to –7. 6, i.e., nigellone (PubChem ID: 398941). Nigellone (Figure 1) showed the best binding affinity towards the target protein 1A3Q. The ligands scoring a binding affinity of less than –6 were considered for further analysis of, which the compound reflecting the highest binding is carried forward for molecular docking. The binding affinities of the screened compounds.

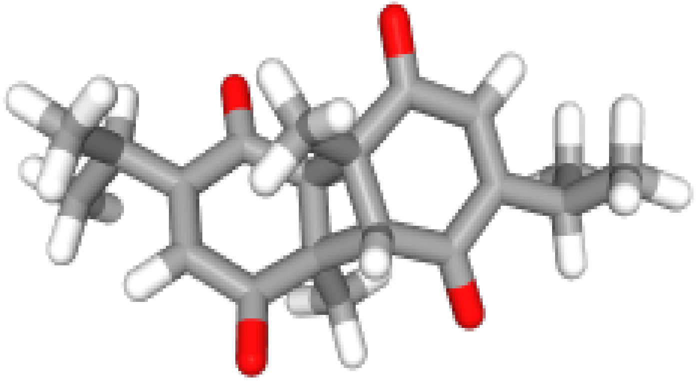

Figure 1. 3D structure of the phytocompound nigellone.

Selection and Purification of the Protein 1A3Q

The crystal structure of the human NF-kappaB p52 homodimer-DNA complex obtained through the X-ray diffraction method has a resolution of 2. 1Å with PDB ID-1A3Q and is downloaded in PDB format from the RCSB database (PDB DOI: https://doi. org/10. 2210/pdb1A3Q/pdb). The protein was then subjected to purification in the BIOVIA tool before the molecular docking process to get the best quality structure where the active sites, A & B chains, and ligand binding sites were restored whilst removing the rest of Figure 2(a, b).

Structural Analysis of the Protein

The 2D and 3D structural analysis and interaction of the protein 1A3Q was studied in PDBSum (https://www. ebi. ac. uk/thornton-srv/databases/pdbsum/) [27]. The protein chains A and B have a total of 285 a. a residue. The 2D structure of 1A3Q consists of 5 sheets, 4 beta hairpins, 4 beta bulges, 21 strands, 3 helices, 1 helix-helix interaction, 29 beta turns and 2 gammas turn, as shown in Figure 3.

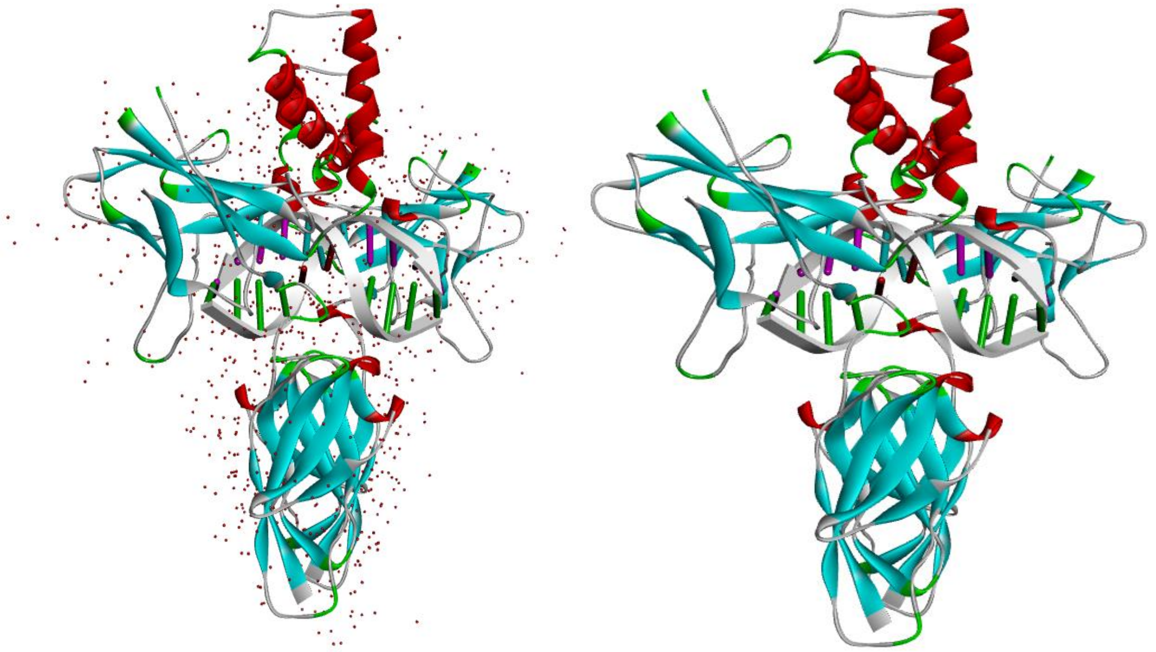

Figure 2. 3D Structure of protein 1A3Q. (a) 3D structure from RCSB PDB, (b) Purified 3D structure from BIOVIA.

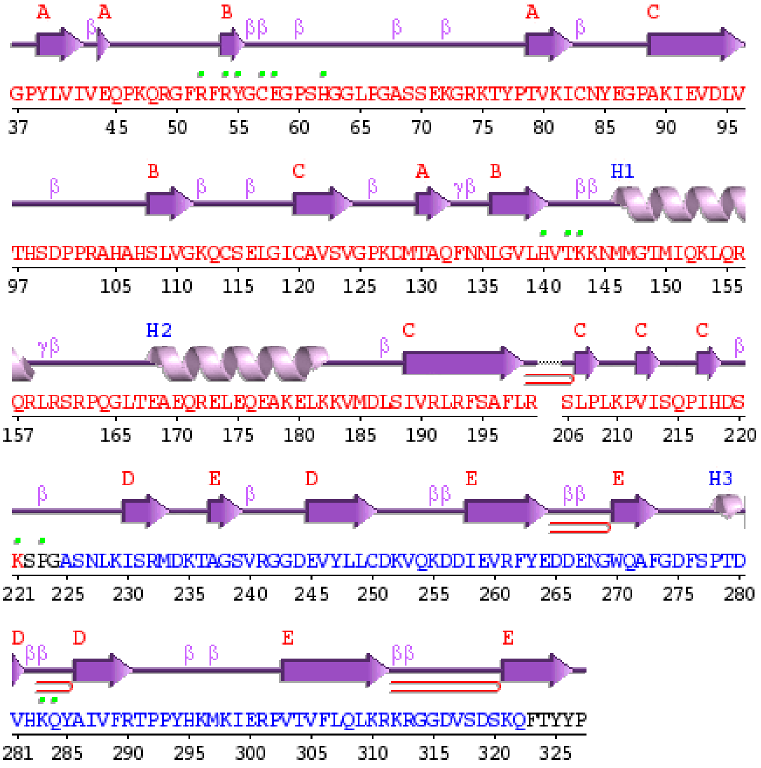

Figure 3. Secondary structure of protein 1A3Q.

Protein-Protein Interface

Protein-protein interface statistics were retrieved from PDBsum. Chains A and B have an interface residue of 14 each. Chain A has an interface area (A^2) of 691, whereas chain B has an interface area (A^2) of 678. Chain A and B were interacting with a total no of 6 salt bridges, 8 Hydrogen bonds, and 91 non-bonded contacts, and there was no evidence of disulphide bonds between the 2 chains (Figure 4).

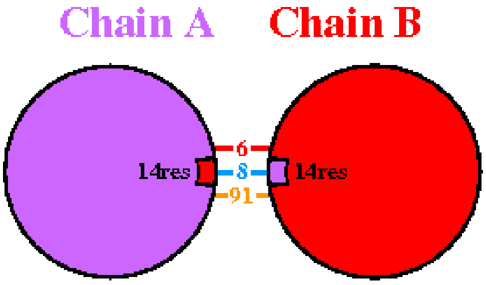

Figure 4. Protein-protein interface of protein 1A3Q from PDBsum.

Ramachandran Plot

Ramachandran Plot in each protein structure is a display of ϕ, ᴪ angle pairs of a polypeptide chain. The Ramachandran plot was analyzed from PDBSum and PROCHECK as shown in Figure 5. PROCHECK plot statistics observed a total number of 570 residues, of which 482 residues were non-glycine and non-proline. 411 residues (A, B, L) colored red in the plot were classified as more favored regions, 67 residues (a, b, l, p) colored mustard yellow in the additional allowed regions, 4 residues (~a, ~b, ~l, ~p) colored yellow in the additional allowed regions, 0 disallowed regions, and 4 end residues (excluding glycine and proline), of which 46 glycine and 38 proline residues are observed. In in-silico studies, stronger peptide confirmation is observed when the residues fall under the most favorable region.

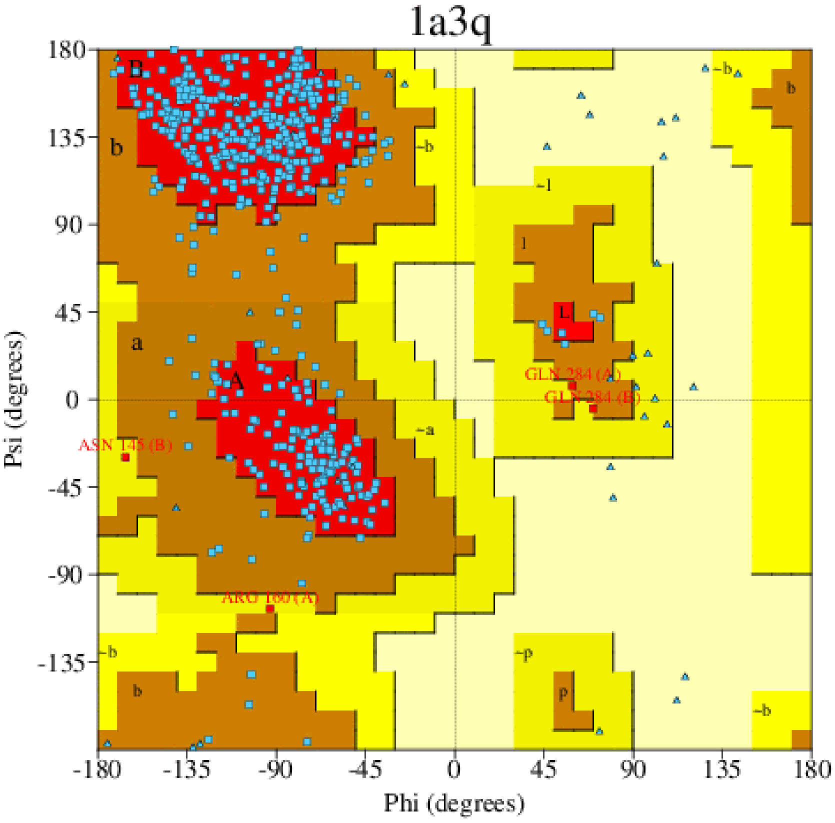

Figure 5. Ramachandran plot of protein 1A3Q.

Protein Statistics

EMBOSS:pepstats (https://www. ebi. ac. uk/jdispatcher/seqstats/emboss_pepstats) tool analyzes various sequence statistics on the protein properties and this data obtained is crucial for analysis for further protein purification and in R&D [28]. The following details were obtained for protein 1A3Q. molecular weight = 932.07, residues = 11, average residue weight = 84.733, charge = 0.0 isoelectric point = 5.4755. Protein dynamics like number of amino acid residues, their property and % mole were retrieved from EMBOSS pepstats (Table 1).

Table 1. 1A3Q Protein statistics data obtained from EMBOSS:pepstats.

Property	Residues	Number	Mole%
Tiny	(A+C+G+S+T)	11	100
Small	(A+B+C+D+G+N+P+S+T+V)	11	100
Aliphatic	(A+I+L+V)	1	9.091
Aromatic	(F+H+W+Y)	0	0
Non-polar	(A+C+F+G+I+L+M+P+V+W+Y)	9	81.818
Polar	(D+E+H+K+N+Q+R+S+T+Z)	2	18.182
Charged	(B+D+E+H+K+R+Z)	0	0
Basic	(H+K+R)	0	0
Acidic	(B+D+E+Z)	0	0

Pharmacological Studies

The selected 18 ligands of the *Nigella sativa* were subjected to Swiss ADME analysis wherein the phytocompounds were observed under physicochemical parameters, Drug likeness properties pertaining to 0 violation Lipinski rule as shown in Tables 2, 3 & 4. The pharmacokinetics properties of the compounds showed high GI absorption indicating good efficacy, BBB (Blood brain barrier) is shown to be high in the compounds as well, which could be subjected to chemical synthesis to get a desirable composition during drug development considering other beneficial properties in the compounds and less side effects when compared to conventional treatment, Pg-p substrate except for nigellidine is shown to be favorable too.

Table 2. Data analyzed for the physicochemical properties from Swiss ADME.

Ligand	Formula	MW	H A (<10)	H D (<5)	TPSA
Myrtenol	$C_{10}H_{16}O$	152.23	1	1	20.23
Nigellidine	$C_{18}H_{18}N_2O_2$	294.35	2	1	47.16
Dillapiol	$C_{12}H_{14}O_4$	222.24	4	0	36.92
Thymoquinone	$C_{10}H_{12}O_2$	164.2	2	0	34.14
Carvacrol	$C_{10}H_{14}O$	150.22	1	1	20.23
Geranylacetone	$C_{13}H_{22}O$	194.31	1	0	17.07
Thymol methyl ether	$C_{11}H_{16}O$	164.24	1	0	9.23
Nigellicine	$C_{13}H_{14}N_2O_3$	246.26	3	1	64.23
2-Tridecanone	$C_{13}H_{26}O$	198.34	1	0	17.07
2-(4-Methylphenyl) propan-2-ol	$C_{10}H_{14}O$	150.22	1	1	20.23
Lauric acid	$C_{12}H_{24}O_2$	200.32	2	1	37.3
Decanoic acid	$C_{10}H_{20}O_2$	172.26	2	1	37.3
Myristicin	$C_{11}H_{12}O_3$	192.21	3	1	27.69
Nigellone	$C_{20}H_{24}O_4$	328.4	4	0	68.28
Coumarin	$C_9H_6O_2$	146.14	2	0	30.21
Nonanal	$C_9H_{18}O$	142.24	1	0	17.07
Eugenol	$C_{10}H_{12}O_2$	164.2	2	1	29.46
4-Isopropylbenzaldehyde	$C_{10}H_{12}O$	148.2	1	0	17.07

Note: MW: molecular weight (<500 daltons), H A: hydrogen acceptor, H D: hydrogen donor, TPSA: topological polar surface area.

Table 3. Pharmacokinetics data obtained from Swiss ADME.

Ligand	**GI**	**BBB**	**P-gp Substrate**
Myrtenol	High	Yes	No
Nigellidine	High	Yes	Yes
Dillapiol	High	Yes	No
Thymoquinone	High	Yes	No
Carvacrol	High	Yes	No
Geranylacetone	High	Yes	No
Thymol methyl ether	High	Yes	No
Nigellicine	High	Yes	No
2-Tridecanone	High	Yes	No
2- (4-Methylphenyl) propan-2-ol	High	Yes	No
Lauric acid	High	Yes	No
Decanoic acid	High	Yes	No
Myristicin	High	Yes	No
Nigellone	High	Yes	No
Coumarin	High	Yes	No
Nonanal	High	Yes	No
Eugenol	High	Yes	No
4-Isopropylbenzaldehyde	High	Yes	No

Note: GI: Gastrointestinal absorption, BBB: blood brain barrier, P-gp: permeable-glycoprotein substrate.

Table 4. Drug likeness of the phytochemicals obtained from Swiss ADME.

Ligand	**Lipinski # Violations**	**Bioavailability Score**
Myrtenol	0	0.55
Nigellidine	0	0.55
Dillapiol	0	0.55
Thymoquinone	0	0.55
Carvacrol	0	0.55
Geranylacetone	0	0.55
Thymol methyl ether	0	0.55
Nigellicine	0	0.85
2-Tridecanone	0	0.55
2- (4-Methylphenyl) propan-2-ol	0	0.55
Lauric acid	0	0.85
Decanoic acid	0	0.85
Myristicin	0	0.55
Nigellone	0	0.55
Coumarin	0	0.55
Nonanal	0	0.55
Eugenol	0	0.55
4-Isopropylbenzaldehyde	0	0.55

Structural analysis of protein 1A3Q

The NF-Kappa B p52 a homodimer and eukaryotic transcription factor plays a significant role in regulating many mammalian genes encoding pro inflammatory cytokines, major histocompatibility complex (MHC) proteins, cell adhesion molecules, growth factors, interferones including viruses, such as herpes, human immunodeficiency virus (HIV) [29]. 1A3Q human NF-Kappa-B P52 bound to DNA is selected as a macromolecule for this study from RCSB PDB. The 1A3Q protein has 285 sequences and consists of C [auth A], D [auth B]. Analysis of the protein using prankweb server retrieved 8 active sites. 12 amino acids residues were obtained in pocket 1 B_140 B_142 B_143 B_187 B_188 B_219 B_221 B_222 B_223 B_55 B_57 B_58, 11 in pocket 2B_140 B_142 B_143 B_187 B_188 B_219 B_221 B_222 B_223 B_55 B_57 B_58, 11 in pocket 3 B_140 B_142 B_143 B_187 B_188 B_219 B_221 B_222 B_223 B_55 B_57 B_58, 9 from pocket 4 B_140 B_142 B_143 B_187 B_188 B_219 B_221 B_222 B_223 B_55 B_57 B_58, 11 from pocket 5 B_140 B_142 B_143 B_187 B_188 B_219 B_221 B_222 B_223 B_55 B_57 B_58, 12 from pocket 6 B_140 B_142 B_143 B_187 B_188 B_219 B_221 B_222 B_223 B_55 B_57 B_58, 10 from pocket 7 B_140 B_142 B_143 B_187 B_188 B_219 B_221 B_222 B_223 B_55 B_57 B_58, 7 from pocket 8 B_140 B_142 B_143 B_187 B_188 B_219 B_221 B_222 B_223 B_55 B_57 B_58 [30]. No mutagenesis was reported on protein 1A3Q explored with computed atlas of surface topography of proteins (CASTp) (http://sts. bioe. uic. edu/castp/index. html? j_65e41d83b2dbf) [31].

Molecular Docking Analysis

Prepared protein 1A3Q and the ligands were docked in PyRx. Docking results were obtained from PyRx and the results were visualized in BIOVIA (Table 5). Receptor protein and the ligand were defined and the 2D visualization confirmed the interaction of the selected ligand with amino acids in the protein by conventional hydrogen bond and Pi-alkyl bond. The binding affinity of the 18 ligands with respect to A and B chains of the proteins are as shown in Table 6. Binding score less than -6 were considered for further analysis and the ligands nigellone (398941), nigellidine (138628302), nigellicine (11402337) as referred in Table 1 showed favorable binding affinity of which the highest binding affinity observed in nigellone was carried forward for further visualization in BIOVIA.

Protein Rretrieved from RCSB PDB

Binding Affinity of Nigella Sativa Phytocompounds with 1A3Q

18 compounds were screened and the binding affinity of less than –6 was favored. 3 compounds that displayed binding affinity towards the target protein are as shown in Table7. Nigellone with binding affinity of -7.6 was docked and visualized against the target protein in BIOVIA. Confirmation of interactions in 2D of the docked complex can be observed in Figure 6.

Table 5. 1A3Q protein details obtained from RCSB PDB.

Molecule	**Chain**	**Sequence Length**	**Organism**	**Details**
NF-KAPPA B PROTEIN	C [auth A], D [auth B]	285	*Homo sapiens*	Transcription/DNA Mutation (s) –NO

2D Visualization and Molecular Interactions of 1A3Q Protein with Ligand Nigellone

Amino acids ARG B-193, ARG B-103, ARG B-103, and ARG B-160 (Table 8) is shown to have strong conventional hydrogen bond interaction and amino acid PHE B-197 is interacting with pi-alkyl bond with the ligand nigellone (398941) obtaining a binding affinity of –7.6 with respect to B chains of 1A3Q protein as shown in Figure 6.

DISCUSSION

RA is a chronic inflammatory progressive autoimmune disease that gradually affects the synovial joints and leads to subsequent erosion and deformity of bone and joints. The true etiology of this disease is complex and multifactorial and certain conditions like environmental factors and genetics play a crucial role in outset and predisposition of this disease. RA manifests in either of 2 states active or inactive, while in active state tissues are inflamed and the relapse symptoms flares up, in an inactive state the inflammation subsides [3, 32, 33]. RA linked to environmental factors has gained attention in recent years from epidemiological and animal studies for their apparent role in provoking autoimmunity in an already genetically predisposed individual [34, 35]. These factors have shown to increase the oxidative stress in an individual creating a negative balance in the pool of oxidative molecules favoring predominance of the prooxidants leading to elevated concentrations of ROS, RNS, lipid peroxidation damaging the cellular components which are directly involved in the pathogenesis of RA [10, 36–37].

Table 6. A and B chain binding affinity data of protein 1A3Q with Phytocompounds of *Nigella sativa* from PyRx.

Ligand	**Binding Affinity**
Myrtenol	–5.9
Nigellidine	–6.9
Dillapiol	–5.2
Thymoquinone	–5.4
Carvacrol	–5.4
Geranylacetone	–4.8
Thymol methyl ether	–5.1
Nigellicine	–6.4
2-Tridecanone	–4
2-(4-Methylphenyl) propan-2-ol	–5.4
Lauric acid	–4.3
Decanoic acid	–3.7
Myristicin	–4.8
Nigellone	–7.6
Coumarin	–5.7
Nonanal	–4.2
Eugenol	–4.8
4-Isopropylbenzaldehyde	–5

Table 7. Binding affinity of top 3 phytocompounds with protein 1A3Q.

Phytocompound Name	**PubChem ID**	**Binding Affinity in kcal/mol**
Nigellone	398941	–7.6
Nigellidine	138628302	–6.8
Nigellicine	11402337	–6.4

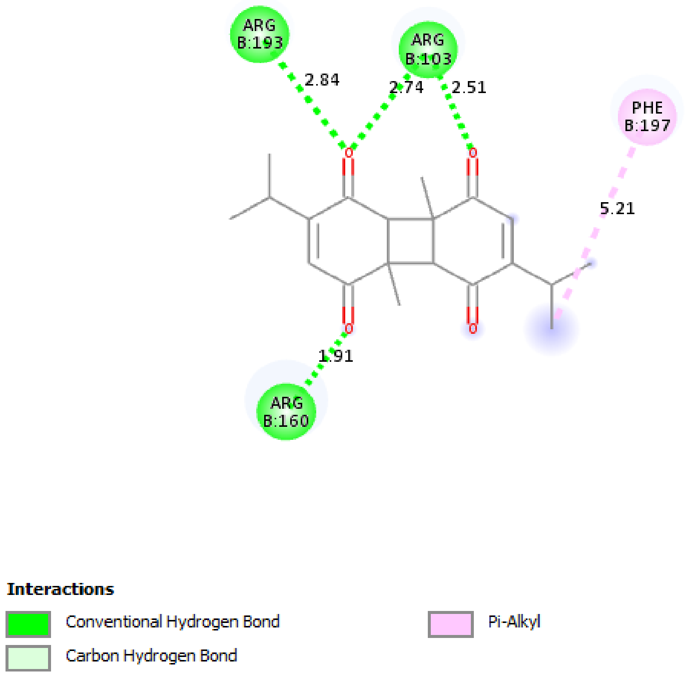

Figure 6. 2D visualization of nigellone interacting with protein 1A3Q.

Table 8. Protein complex 1A3Q and nigellone docking interactions.

Name	Distance	Category	Types
ARG B-193	2.84	Hydrogen bond	Conventional hydrogen bond.
ARG B-103	2.74	Hydrogen bond	Conventional hydrogen bond.
ARG B-103	2.51	Hydrogen bond	Conventional hydrogen bond.
ARG B-160	1.91	Hydrogen bond	Conventional hydrogen bond.
PHE B-197	5.21	Hydrophobic	Pi-alkyl.

Cytoplasmic eukaryotic transcription factor NF-KB was shown to be directly influenced by oxidative stress and takes a key role in regulating the genes involved in immune and inflammatory processes [38–41]. NF-KB signals can either be triggered or suppressed by free radicals and are variably context dependent and induce pro and antioxidant role in inducing oxidative stress. The upregulation of NF-KB target genes is led by 2 signaling pathways described as canonical (classic pathway) and non-canonical (alternative pathway). In RA NF-KB p52 is involved in inducing inflammation through non-canonical pathway that primarily activate the TNF receptors triggered by lymphotoxin β receptor, CD40 and B cell activation factor receptor 3 usually in concurrence with RelA by cytokine receptors (IL-1R, TNFR) [42–44]. NF-KB plays a key role in inducing inflammatory pathways in RA related to oxidative stress induced by free radicals, working on inhibiting free radicals through powerful antioxidants by reducing oxidative stress which inturn would downregulate the activity of NF-KB and hence reducing the inflammation is the primary principle involved in targeting NF-KB as a protein of choice in this study. The current treatment plan that has proven to suppress inflammation on targeting free radicals and has shown subsequent reduction in pannus proliferation is NOS inhibitor L-NAME GW274150 [45, 46]. Among the other treatment plans, first-line management: NSAIDS and corticosteroids, opioid analgesics, second-line management: disease-modifying antirheumatic drugs (DMARD) taking part as immunosuppressants are directed towards reducing the inflammation however the adverse side effects like high liver toxicity, bone marrow deterioration, cirrhosis, hypertensions, leukopenia, GI upset, interstitial lung disease are some of the drawbacks that are accompanied along with the treatment [47].

Pharmaceutical industries have always been attracted towards plant-based drugs for their convenience in standardization and purification of the compounds while being more efficacious with minimum side effects, by large harmless, easy metabolism and absorption in the body when compared to synthetic medicine [48]. For many centuries medicinal plants have been used in herbal medicines for their abundance in two major antioxidant phytochemicals carotenoids and polyphenols. *Nigella sativa* (*N. sativa*) (family *Ranunculaceae*) commonly known as black seed is well known for its wide spectrum of pharmacological potential as a anticancer and immunomodulatory, antimicrobial, analgesic and anti-inflammatory, gastroprotective, renal protective, hepatoprotective, anthelmintics, diuretic, antidiabetic, and antioxidant properties [49]. In this study we have chosen *Nigella sativa* as a potential drug supplement and as alternative therapy for allopathic medicine in RA to treat the free radical formation by high antioxidant properties of the phytocompounds present in *N. sativa.* In vitro studies indicate the bioactive compound thymoquinone at a dose 1–5 μM can effectively cause apoptosis and inhibit IL-6 and IL-8 induced by TNF-α by blocking the expression of myeloid leukemia (Mcl) [15]. Experiments done by Arjumand et al. have reported bioactive compound thymoquinone (TQ, 5-isopropyl-2-methyl-1, 4-benzoquinone) treatment conducted on 6–8 weeks old 40 male rats sprague-dawley with a dosage of 10 mg/kg body weight for 20 days showed reduced macroscopic arthritis score, CRP levels, synovitis, bone erosion and pannus formation, downregulation of NF-KBmRNA, decreased levels of TLR2, TLR4, IL-1 and TNF-α and showed no signs of hepatotoxicity or nephrotoxicity [15, 50]. Clinical trials on humans by Hadi et al. prescribed with black cumin seed oil capsules 2 capsules, 500 mg/day (1 g/day) for 8 weeks, on a study group of 50 patients16-placebo group, 23 intervention group with 39 complete completion showed significant decrease in intervention group in the DAS-28 compared to placebo group, reduction in MDA and NO to baseline value ($p < 0.\ 05$) comparison in the serum levels, increased serum IL-10 levels ($p < 0.\ 01$) in intervention group. There was no significant difference in serum levels of TNF-α, MDA, catalase, IL-10 and TAC among intervention and placebo group [15]. In silico molecular docking analysis on the bioactive compounds in *N. sativa* observed nigellone (PunChem ID-398941) to be the most bioactive compound having the highest binding affinity against the target protein 1A3Q followed by nigellidine and nigellicine shown in Table 1, whereas thymoquinone (PubChem ID-10281) showed a binding affinity of –5.4. The current study has proven nigellone to be the most preferred compound when targeted against the protein NF-KB at a molecular level. ADMETLab2. 0 toxicity on the liver DILI was shown to be high in the compound, which is also the main side effect observed in the current DMARDS methotrexate treatment. Insight into this concern can be taken into consideration during the drug design and development process to chemically synthesize the compound to a lesser toxic level and this should be able to outweigh the limitation when compared to the potentiality of the compound. Therefore, further in vitro and in vivo studies on nigellone may show better inhibition of NF-KB thus alleviating inflammation and free radical generation in the patients and could be considered as an antioxidant dietary supplement gradually increasing the dosage alongside conventional treatment with minimal side effects.

Previous studies conducted on *Nigella sativa* in vitro and in-vivo have shown thymoquinone to be the highly potent among the phytochemicals, however in-silico analysis of the phytocompounds targeted against NF-KB protein responsible in perpetuating inflammatory responses revealed nigellone (dithymoquinone) to have stronger binding affinity with the protein as discussed previously hypothesizing the emphasis on the compound for extensive research. Validation of the experimental results can further be concluded by dynamic simulation to capture the interactions, movement, functioning and behavior of protein 1A3Q at an atomic level with nigellone (dithymoquinone) in action over time and could be considered as a novel compound to carry forward in future studies in suppressing the inflammation in RA caused by free radicals.

CONCLUSIONS

Free radicals formed due to decrease in antioxidant levels in the body leading to oxidative stress plays a major role in the pathogenesis of RA by upstreaming the activity of NF-KB causing DNA damage. Raising the antioxidant levels in the body and by negating the proliferation of free radicals could be a propitious strategy in decreasing the inflammation in RA patients. *Nigella sativa's* highly potent antioxidant properties evidenced for centuries together and believed to be a miracle herb across civilizations marked and proven by scientific studies for its healing traits could turn out to be revolutionary as an antioxidant drug, which is the root cause for escalating pathological conditions. Previous experimental studies have shown desirable results with respect to thymoquinone as the most effective phytocompound. The current molecular docking interactions have unveiled nigellone (dithymoquinone) to be exhibiting the highest binding affinity with NF-KB, however data specific to nigellone is currently scarce to support this study. On the positive end due to the similarities between the structures of the phytocompounds, nigellone can be considered as a stronger alternative prospect expected to bring forth better results in the future research studies.

Abbreviations

GI	:	Gastro intestinal
BBB	:	Blood brain barrier
P-gp	:	Permeable-glycoprotein substrate
OS	:	Oxidative stress
DMARD	:	Disease modifying anti-rheumatic drug
NSAIDS	:	Non-steroidal anti-rheumatic drugs
P-gp	:	Permeable-glycoprotein substrate
RNS	:	Reactive nitrogen species
TNF	:	Tumor necrosis factor
CD40	:	Luster of differentiation 40
IL-1R	:	Interleukin 1 receptor
NOS	:	Nitric oxide synthase

L-NAME	:	Levo-nitroarginine methyl ester
TLR	:	Toll like a receptor
MDA	:	Malondialdehyde
TAC	:	Total antioxidant capacity
DILI	:	Drug induced liver injury

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Authors' Contribution

All the authors have equally contributed to the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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