Research article

Integrating network pharmacology and molecular docking for the identification of key genes and therapeutic targets of *Nigella sativa* in multiple sclerosis treatment

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ABSTRACT

Introduction and Aim: Multiple sclerosis (MS) is a chronic neurodegenerative disease affecting around 2.8 million people worldwide. MS pathophysiology is not yet explained up to the mark, which is the cause of difficulty and complexity in treating the illness. Most present-day scenarios are engrossed in inhibiting central nervous system (CNS) inflammation. However, this is not enough, hence the present study aims at finding best neuroprotective treatment without adverse effects.

Materials and Methods: *In silico* attempt to validate the phytocompounds from *Nigella sativa* and showcase their use for targeting the neuroprotective mechanism involved in management of MS by finding the key potential genes which were derived from mRNA datasets of previous research. Various bioinformatics tools and software such as GEO, String, ShinyGO, PyRx were used to carry out the current study. The leading steps involve retrieval of targets from mRNA datasets, molecular docking of phytocompounds with the targets and pharmacological analysis.

Results: These phytocompounds from seeds of *N. sativa* showed promising results as therapeutic agents against target genes RPL27, RPS14 and FAU for management of MS during current *in silico* study, but any treatment prior its clinical practice should validate with large robust data, which lies as the future prospective here.

Conclusion: In summary notable progress in management of MS with better understanding of pathology has been made and many disease modifying therapies (DMT) are made available but the question of safety and efficacy is still challenging due to adverse effects associated with these therapies. Hence properties of *N. sativa* must be explored as a therapeutic agent that can reduce the neuronal degeneration.

Keywords: mRNA datasets; multiple sclerosis; *Nigella sativa;* significantly expressed genes; molecular docking.

INTRODUCTION

Multiple sclerosis (MS) is characterized as an autoimmune, inflammatory, chronic neurodegenerative condition of central nervous system (CNS) which eventually leads to demyelination of axonal transection imparting neurological nontraumatic disability (1,2). It is one of the utmost non-traumatic disease imparting disabilities among young adults and anticipated to have affected about 2.8 million individuals in the entire world (3). It ordinarily affects the young adults between 20 to 40 years of age, the one with emerging symptoms above the age of 50 are categorized under the late-onset multiple sclerosis (LOMS) (4). The principal event directing to characteristic pathophysiology is the penetration by peripheral immune cells which are primed in contrast to the myelin sheath. The major property in MS is the growth of demyelinating lesions and focal inflammatory which appears in white matters of the brain, vertebral column and optic nerve, however deep grey matter and intracortical lesions are also in existence. Peripheral immune cells, macrophages and primary B and T lymphocytes intrude into central nervous system parenchyma which leads to development of subependymal demyelination, perivascular demyelination and neuroaxonal degeneration. Weekend recovery ultimately results to axonal transection which leads to everlasting clinical debility and neurodegeneration (5). It is usually enumerated that the origin of MS is not known, which is misleading. Sunshine (UVB) vitamin D, smoking and Epstein-Barr virus (EBV) are the collective genetic features executing crucial roles in the pathway of the MS development. Past studies also suggest environmental exposure as the secondary factor in MS progression (3). Even though the accurate etiology of MS is not well-known, the access of the macrophages and lymphocytes to the CNS in accumulation of the already existing genetic and environmental aspects can cause the onset of the disease. Inflammatory cytokines such as tumour necrosis factor (TNF-α) and interferon (IFN-γ) are recognized to liberate from the activated T helper cell (Th1) leading to expression of the lymphocytes and antigen-presenting cell (APCs) surface receptors. Consequently, the existence of genetically susceptible MS antigens like myelin oligodendrocyte glycoprotein (MOG) and myelin basic protein (MBP) attached to stimulated immune cells of the CNS, heading to the constant inflammatory process of declining and devastation into CNS (6).

The definite diagnosis tests are not yet available, and due to its variable indications, the disease is not

predictable in its primary stages. Eventually it is diagnosed on the roots of clinical tests and measures like magnetic resonance imaging (MRI) scans of the brain, (apparatuses of McDonald Criteria 2017) also able to distinguish myelin damage in spine and brain. Meanwhile testing of cerebrospinal fluid (CSF) of the spinal canal specifies the occurrence of proteins or the anomalous level of white blood cells and might direct any fault associated to MS. Moreover, blood test can also help detect the viral agent that may provoke neurological symptoms same as that observed in MS individuals (7). miRNA outline in MS has been investigated to demonstrate modification in the immune system and the CNS, leading to altered levels in expression of genes of several cell forms contributing to the disease condition.

Several studies showing the dysregulation of miRNA expression during analysis of whole blood, CNS and peripheral blood mononuclear cells have been reported. Hence miRNA analysis and the consequential variations in the rate of occurrence of mRNA/protein can help to comprehend the etiology of the MS and insight into the innovative method in treatment and diagnosis of the condition as well (8). MS pathophysiology is not yet explained up to the mark, which is the cause of difficulty and complexity in treating the illness. Most present-day scenarios are engrossed in inhibiting CNS inflammation. In 1993 interferon beta was definite as the first drug for treating MS. Later several drugs (Teriflunomide, Natalizumab, ofatumumab) were introduced. However, these drugs were not entirely effective and had antagonistic effects after prolonged usage. Hence, the study to find a harmless and effective treatment is still in process (9).

However, over the preceding decades using alternative medicine especially herbal medicine has increased notably (10). Numerous investigations conferred therapeutic use of medicinal plants and herbs in disorders like neurodegenerative, diabetes, cancers (11). One among various medicinal plants is *Nigella sativa (N. sativa)* (Ranunculaceae family), an evolving phenomenal herb with an ironic ancient and pious background since several studies discovered its varied scale of pharmacological possibility (12). There are numerous indications that depict the anti-inflammatory activity of *N. sativa* seed oil (13). Thereby in this research, we have investigated the therapeutic properties of *N. sativa* phytocompound in management of MS with the aid of molecular docking and *in-silico* pharmacology.

MATERIALS AND METHODS

Retrieval of mRNA dataset

The transcriptome data of control and MS patient samples was obtained from GEO database (https://www.ncbi.nlm.nih.gov/geo/) setting mRNA, human and multiple sclerosis as the keywords. The following criteria were set 1) The dataset should include test and control from human samples. 2) The datasets should be an analysis of mRNA expression. Total 48 results appeared of which 3 datasets were selected. The GSE ID along with the explanation of the selected datasets for the current research is exhibited (Table1). Then the Significantly Expressed Genes and Top Differentially Expressed Genes (DEGs) were categorised using the GEO2R package (https://www.ncbi.nlm.nih.gov/geo/geo2r/). Later, the Volcano plot and the top Significantly expressed genes table was downloaded from GEO2R.

Table 1: Insight into the selected mRNA datasets

GSE ID	Experimental Type	Platform	Source of sample	No. of Samples	No. of Control	No. of Test (MS patients)	Description
GSE21942	Expression by array	GPL570 [HG-U133_Plus_2] Affymetrix Genome U133 Plus 2.0 Array	PMBC	27	15	12	Expression data from peripheral blood mononuclear cells in multiple sclerosis patients and controls
GSE17048	Expression profiling by array	GPL6947 Illumina Human HT-12 V3.0 expression beadchip	Whole blood	144	45	99	Multiple Sclerosis Blood Cell mRNA Transcriptome
GSE193260	Expression profiling by high throughput sequencing	GPL24676 Illumina NovaSeq 6000 (Homo sapiens)	PBL	8	3	5	Brain antigens stimulate proliferation of T lymphocytes with a pathogenic phenotype in multiple sclerosis patients

PMBC: Peripheral Mononuclear blood Cell; **PBL:** Peripheral blood Leukocytes

Identification of overlapping gene

To discover the overlapping significantly expressed genes among the 3 mRNA expression datasets, Bioinformatics and Evolutionary genomics tool was used and Venn diagram illustrating the common genes was structured (https://bioinformatics.psb.ugent.be/webtools/Venn/). The gene symbols table of

the significantly expressed genes were downloaded from GEO2R and were given as input.

PPi network construction and identification of key genes

PPi network of the 37 overlapping genes with other 15 top expressed non-overlapping genes from all 3 datasets were considered as query taking *Homo sapiens* as an organism and association among them was authenticated using STRING database (https://string-db.org/). The genes encoding proteins depict the genomic association between proteins. Hence, this genomic association was evaluated and studied using the STRING database. The key genes in the network were nominated based on the highest degree of interaction among them.

Functional enrichment of network

For portraying the Gene Ontology (GO), ShinyGO 0.77 (http://bioinformatics.sdstate.edu/go/) which is a graphical tool for gene enrichment analysis was used. Enrichment analysis associates the gene list to functional categories and molecular pathway, the enrichment column table was also retrieved from string.

Identification of target proteins

The 3 key genes from the string network showing high degree of interaction were used as targets and were searched in NCBI-protein (https://www.ncbi.nlm.nih.gov/protein/) for the specific encoded protein. RPL27 encodes for ribosomal protein L27, RPS14 codes for 40S ribosomal protein S14, FAU coding for ubiquitin like and ribosomal protein S30 fusion.

Pharmacological studies

Retrieval of ligand from medicinal plant

IMPPAT, Indian Medicinal Plants, Phytochemistry and Therapeutics (https://cb.imsc.res.in/imppat/) was employed to retrieve primary ligands from the medicinal plant *Nigella sativa.* Only the compounds from seed were taken into consideration, a total of 167 ligands excluding duplicates and large molecules were screened against the target molecule. Later, PubChem database (https://pubchem.ncbi.nlm.nih.gov/) was utilized to gather the canonical smiles, PubChem CID, and the 2D structure (SDF format) for further exploration.

Retrieval of protein, its purification and protein structure validation

The significant molecular targets for the current research were RPL27, RPS14 and FAU. The 3D structure was retrieved from Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) (https://www.rcsb.org/). The 3D structure of human RPL27 (PDB ID-6Y2L), RPS14 (PDB ID-5OA3) and FAU (PDB ID-2L7R) without any mutation were downloaded as .pdb format. The experimental method and resolution were recorded. Before docking the proteins were purified by DS BIOVIA discovery studio, where retaining the protein group, active group and chain of interest all other heteroatoms, water molecules and ligand groups were removed and polar H atoms were incorporated to stabilize the purified protein. After this the purified proteins were saved as .pdb format. The purified proteins were validated considering Ramachandran plot generated using PDB sumgenerate (https://bio.tools/pdbsum_generate). The torsion angle (Phi and Psi) and the protein flavoured region of the Ramachandran plot was considered to validate the stability of targeted protein molecules. As the hydrophilicity and hydrophobicity of the identified druggable protein is also necessary, the hydropathy plot was considered which was evaluated using EMBOSS pepinfo (https://www.ebi.ac.uk/Tools/seqstats/emboss_pep info/).

Primary screening of ligands

Total of 167 ligands were screened for BBB (Blood Brain Barrier) permeability and Lipinski acceptances using SwissADME tool (http://www.swissadme.ch/). Out of all, 117 ligands fulfilled the criteria of BBB 'Yes' and Lipinski '0 or 1'. These 117 ligands were further used for molecular docking.

Molecular docking

Focusing on the current research molecular docking is the crucial step for evaluating the affinity of ligands with the targeted proteins. The software named PyRx was used to determine the docking. It is a virtual screening tool for computer aided drug designing (CADD).

The purified protein as a macromolecule and the SDF ligands files were uploaded separately into the software. With the openBabel tool all the ligands were subjected to minimize energy level and were converted to. pbqt format. Later using Vina Wizard blind docking was performed covering the entire protein. Each protein was docked against all the ligands and binding affinity results were downloaded as .csv file format for all the 3-target proteins. Later binding affinity corresponding to 0 RMSD (Root Mean Square Deviation) depicting least binding score amongst all the available conformation was considered as the best docking pose.

Visualization

The first ligand showing best binding score was considered for visualisation. The ligand along with its interacting protein was displayed in PyRx and the model one of the ligands was downloaded as .pdb file format. Then visualization was done using DS Biovia. The 3D structure of target protein and the ligand file were distinctly opened and ligand was copied to the protein, later ligand interaction was monitored following the defined ligand from the view interaction section. 3D docked structure was downloaded after shading the ligand and the protein group distinctly.

Pharmacological analysis

For appraising the pharmacological worth and drug likeness of the of the active compounds from *N. sativa* scrutinizing its ADMET properties becomes a prominent criterion. It estimates the medicinal chemistry, absorption, distribution, physiochemical properties and toxicity. Here, ADMET analysis was performed using ADMETlab2.0 (https://admetmesh.scbdd.com/). The top 7 ligands were considered for pharmacological analysis. The canonical smiles were subjected to the webserver and ADMET results were downloaded as .csv file.

RESULTS

Identification of significant genes

The gene expression profiles of GSE21942, GSE17048, GSE193260 are shown (Table 2). The criteria were set as log2FC > 1 for upregulated genes and log2FC < 1 for downregulated genes.

The volcano plots of the datasets concerned in current research are shown in Fig. 1. The significantly differentially expressed genes are highlighted in blue (downregulated) and red (upregulated) colour at a default cut-off of p-value -0.05 and black dots represent the genes with no significant differences.

Table 2: Showing number of significantly expressed genes

Dataset GSE ID	Top Differentially Expressed Genes	No. of Significantly Expressed Genes	No. of Upregulated Genes	No. of Downregulated Genes
GSE21942	54674	10035	420	9614
GSE17048	10158	168	0	168
GSE193260	20276	9034	2225	6807

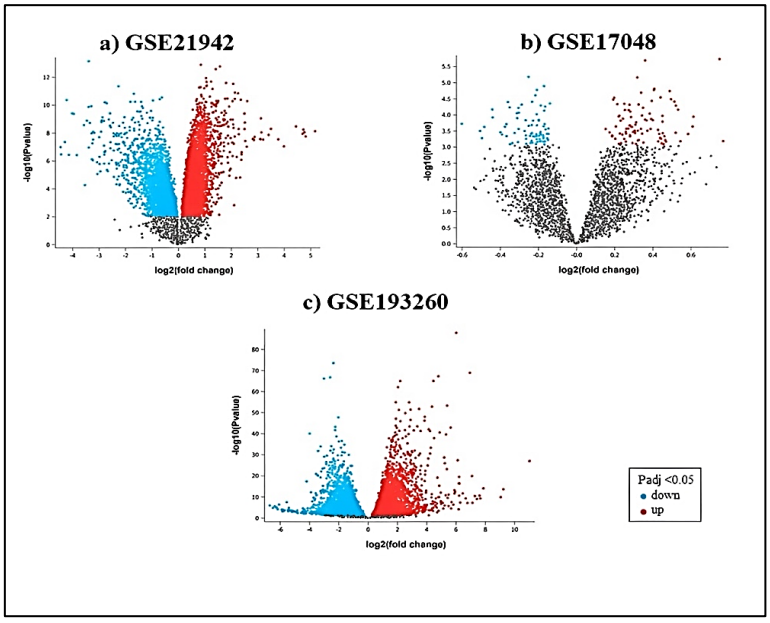

Fig. 1: Volcano plot of the top significantly expressed gene

Identification of overlapping genes

The Venn diagram showing a total 37 common genes among all significantly expressed genes irrespective of up and down regulation in all 3 mRNA datasets in concern is depicted in Fig. 2.

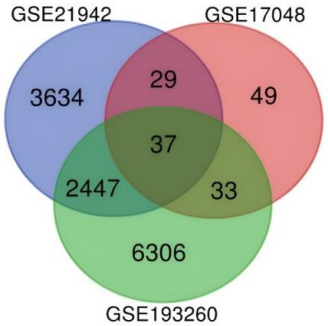

Fig. 2: Venn diagram showing overlapping genes among 3 mRNA datasets

PPi network construction & Identification of key genes

The PPi network association of the query genes at confidence level of 0.700 is shown in Fig. 3. The PPi cluster of the 3 datasets include 63 nodes with an average node degree of 0.635. Here the genes displaying the highest degree of association are RPL27, RPS14, and FAU. These were considered as targets for further evaluation.

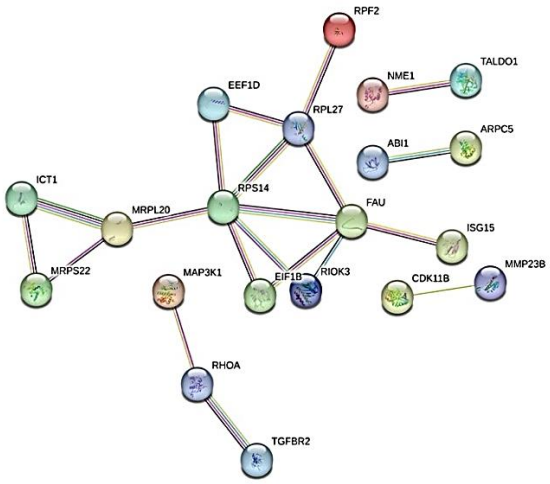

Fig. 3: PPi network of the overlapping genes

Functional enrichment of network

The GO networks derived from the ShinyGO 0.77 tool are represented in Fig. 4 showing various interactions. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway represents a major signalling pathway and molecular interaction of human diseases including several other processes. Here, the KEGG pathway for the genes associated with MS patients from the retrieved datasets is shown in Fig. 5.

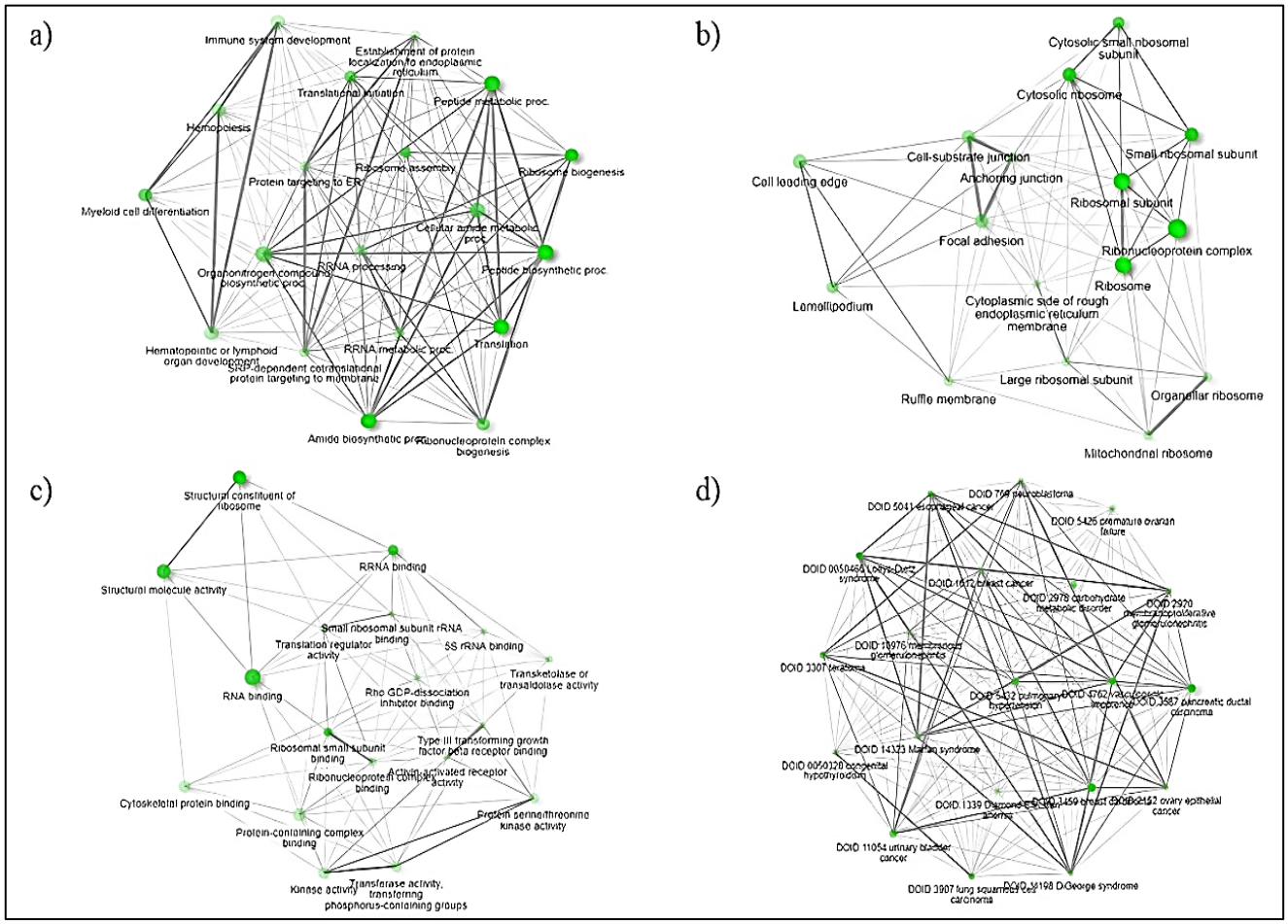

Fig. 4: GO and disease associated pathway
a) GO biological process **b)** GO cellular component **c)** GO molecular processes **d)** disease alliance

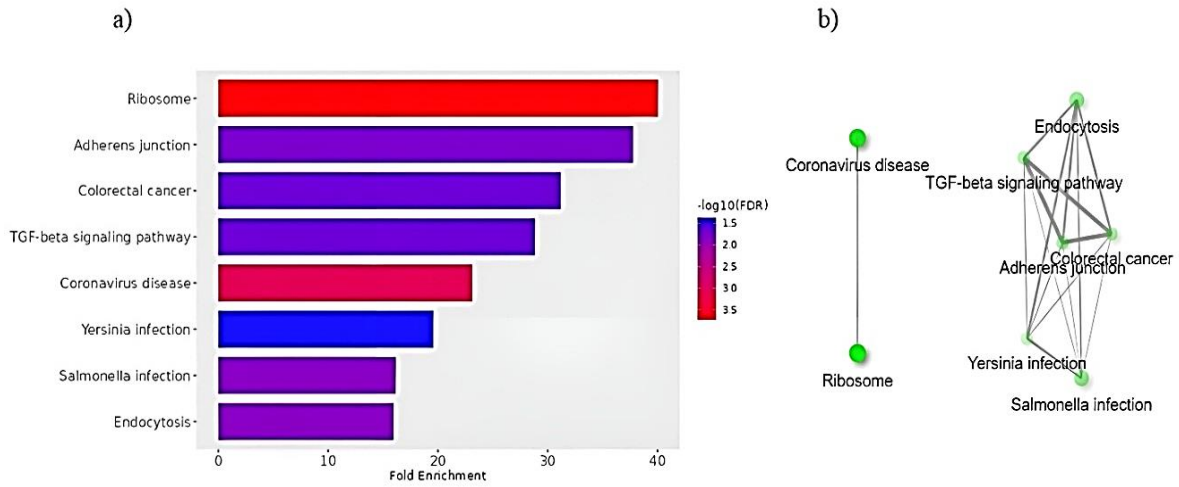

Fig. 5: KEGG pathway analysis
a: Process associated with disease in a bar plot format **b:** KEGG pathway

Table 3: Functional enrichment of network

	GO-term	Description	Count in network	Strength	False discovery rate
Biological Process (GO)	GO:0042255	Ribosomal assembly	3 of 61	1.7	0.0148
	GO:0006414	Translational elongation	4 of 119	1.54	0.0036
	GO:0070125	Mitochondrial translational elongation	3 of 90	1.54	0.0349
	GO:0070126	Mitochondrial translational termination	3 of 91	1.53	0.0450
	GO:0006614	SRP-dependent cotranslational protein targeting to membrane	3 of 96	1.51	0.0398
Molecular Function (GO)	GO:0019843	rRNA binding	3 of 62	1.7	0.0211
	GO:0008135	Translation factor activity, RNA binding	3 of 82	1.58	0.0392
	GO:0003735	Structural constituent of ribosome	5 of 159	1.51	0.0013
	GO:0045182	Translational regulator activity	4 of 131	1.5	0.0083
	GO:0005198	Structural molecule activity	6 of 635	0.99	0.0183
Cellular Component (GO)	GO:0015935	Small ribosomal subunit	3 of 68	1.66	0.0180
	GO:0005761	Mitochondrial ribosome	3 of 87	1.55	0.0293
	GO:0044391	Ribosomal subunit	6 of 178	1.54	2.64e-05
	GO:0022626	Cytosolic ribosome	3 of 97	1.5	0.0293
	GO:0015934	Large ribosomal subunit	3 of 113	1.44	0.0389
KEGG Pathway	hsa03010	Ribosome	4 of 130	1.5	0.0025

GO: gene ontology

The major pathway associated in the GO biological process involves ribosomal assembly, GO molecular function is rRNA binding, GO cellular component is small ribosomal subunit and KEGG pathway include ribosome (Table 3).

Pharmacological studies

Retrieval of protein and its purification and protein structure validation

The targeted proteins RPL27 and RPS14 were resolved by Electron Microscopy at a resolution of 3.00 Å and 4.20 Å respectively while FAU was resolved using solution NMR. The saved purified proteins in .pdb format were used for further investigation.

Molecular docking

The two-core information were drawn from the docking results 1) accurate configuration of receptor-ligand intricate 2) binding affinity score. The binding affinity of the top 5 ligands with each targeted protein are depicted, after removing the duplicates top 7 ligands showing affinity less than -5.0 were considered for further scrutiny. (Table 4). The ligand with Pub-Chem ID 91752502 showed high degree of binding affinity with all the targeted proteins in subject.

Table 4: Binding affinity of *N. sativa* phytocompounds with the target proteins

Ligand	Pub-Chem ID	Binding Affinity		
		2L7R	5OA3	6Y2L
Longiborneol acetate	91752502	-7.8	-9.8	-9.5
7-epi-alpha-Eudesmol	12304196	-6.8	-9.0	-8.9
Dithymoquinone	398941	-5.5	-6.7	-6.5
Longicyclene	564934	-5.4	-7.1	-5.5
Trans-4-Methoxythujane	71338689	-5.4	-7.1	-7.2
Copaene	12303902	-5.1	-7.0	-5.5
Nigellicine	11402337	-5.1	-5.8	-5.9

Visualization

The 3D docked structures are shown in Fig. 6. Visualization portrays the major bonds involved in receptor-protein binding. In case of protein ID 2L7R the ligand with PubChem CID 91752502, foremost showed hydrophobic and electrostatic interaction with Phe14 and Leu10. While 6Y2L predominantly, showed hydrogen bond, electrostatic bond and hydrophobic bonds with Phe101, Lys73, Val74, Arg36. 5OA3 showed only hydrophobic interaction with Phe34. Phe41 and Arg 55 as depicted in Fig.6.

Pharmacological analysis

The top 7 ligands with their absorption and distribution properties (Table 5), medicinal chemistry (Table 6) and toxicity (Table 7) are depicted. This evaluates the therapeutic potential of the phytocompounds extracted from the medicinal plant *N. sativa* and shows their efficiency as therapeutic targets for management of multiple sclerosis.

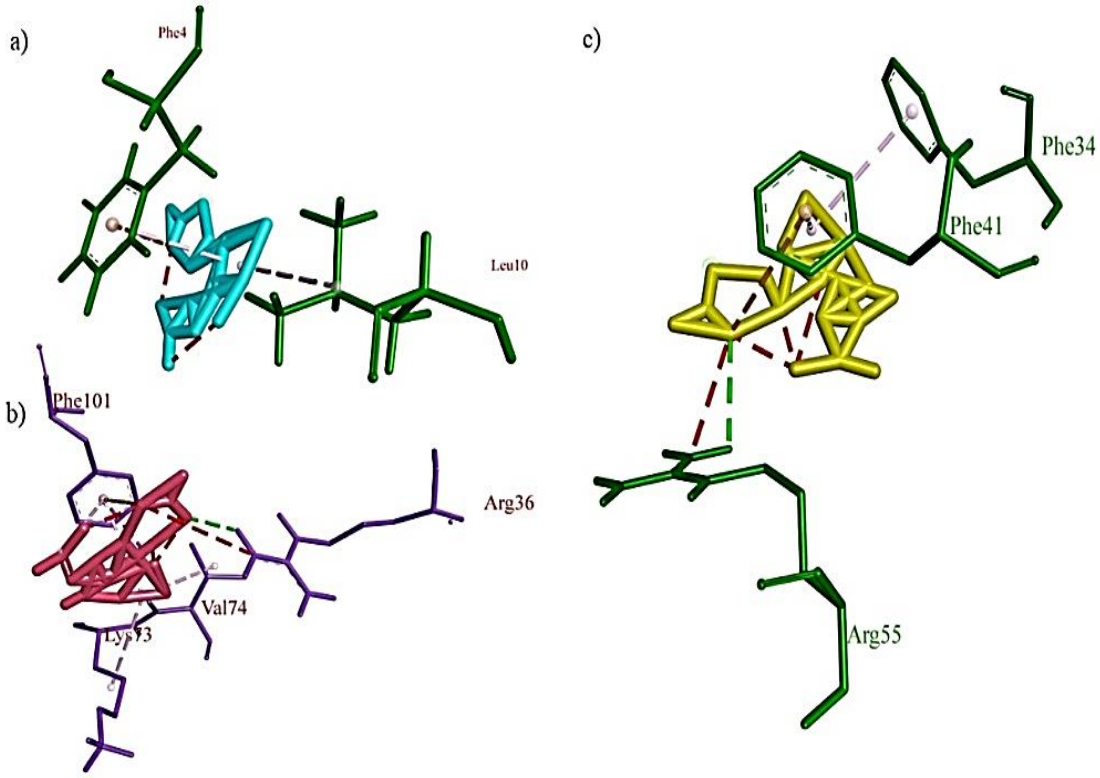

Fig. 6: 3D dock structures of all target proteins with the top ligand (PubChem CID-91752502)
a: Protein ID- 2L7R **b:** Protein ID- 6Y2L **c:** Protein ID- 5OA3

Table 5: Adsorption and distribution properties

Pub-Chem ID	Caco-2	Pgp-		HIA	BBB	PPB	VDss
		inh	sub				
91752502	-4.798	0.729	0	0.009	0.525	95.57%	1.134
12304196	-4.35	0.007	0	0.004	0.884	95.49%	1.504
398941	-5.25	0.089	0.99	0.028	0.011	67.37%	0.553
564934	-4.763	0.015	0	0.006	0.713	95.43%	1.846
71338689	-4.392	0.001	0.001	0.008	0.466	84.22%	1.452
12303902	-4.357	0.000	0	0.004	0.664	97.26%	3.393
11402337	-5.418	0.003	0.175	0.57	0.184	66.09%	1.12

Caco-2**:** Caco-2 Permeability; Pgp-inh/Pgp-sub: Inhibitor and Substrate of P-glycoprotein; HIA: Human intestinal adsorption; BBB: Blood-Brain barrier; PPB: Plasma Protein binding; VDss: Volume Distribution

Table 6: Medicinal properties

Pub-Chem ID	QED	SA score	PAINS	Lipinski	CL	T (1/2)
91752502	0.664	5.048	0	Accepted	5.892	0.087
12304196	0.667	4,007	0	Accepted	10.126	0.145
398941	0.641	3.964	0	Accepted	3.18	0.272
564934	0.557	5.857	0	Accepted	16.961	0.055
71338689	0.616	4.233	0	Accepted	12.827	0.339
12303902	0.559	5.199	0	Accepted	19.832	0.059
11402337	0.83	3.129	0	Accepted	6.115	0.226

QED: Measure of drug-likeness based on the concept of desirability; SA score: Synthetic accessibility score; PAINS**:** Pan Assay Interference compound; Lipinski Rule of 5: Molecular weight less than 500 daltons. nHD<5, nHD<10 and lipophilicity<4.15; CL: Clearance rate; T1/2: Half-life of the small molecules

Table 7: Toxicity

Pub-Chem ID	hEGR Blockers	DILI	AMES Toxicity	Carcinogenicity	IGC50	LC50 FM
91752502	0.017	0.358	0.043	0.048	4.298	4.729
12304196	0.011	0.027	0.004	0.079	4.068	4.583
398941	0.001	0.933	0.878	0.482	4.061	5.315
564934	0.03	0.04	0.022	0.039	4.576	5.044
71338689	0.015	0.044	0.021	0.108	3.399	3.727
12303902	0.027	0.073	0.015	0.061	4.293	4.834
11402337	0.028	0.977	0.021	0.062	4.681	3.017

hERG: Human ether -a-go-go related gene; DILI: Drug-induced liver injury; AMES: Ames test for mutagenicity; IGC50: inhibitory concentration 50; LC50 FM: 96-hour fathead minnow: LC50

DISCUSSION

The threat of recurring relapses in the MS patients extensively declines by immunotherapies. Moreover, the neurodegeneration and persistent inflammation is not averted through these therapies leading to brain and spinal cord damage ensuing disability. Consequently, by uniting contemporary immunotherapies with regenerative, remyelinating and neuroprotective therapies must be practised (14). Treatment for MS has been innovative since its foremost disease modifying therapies (DMT) were made official back in 1993. Presently several DMT for MS with erratic mechanism of action, adverse effect profile, dosing, and various routes of administration are available with majorly targeting inflammation and fewer targeting neurodegenerative disease. Novel treatment aiming at neurodegeneration and remyelination is in progressive stage but additional dynamic data is always needed after its officialization (15). Formerly medicinal plants have achieved new elevations in managing neurodegenerative diseases such as MS, Alzheimer's, and Parkinson's. Reviewed facts showed the effectiveness of herbal medicine in handling MS and its symptom management by refining remyelination, demoting demyelination and conquering inflammation into the central nervous system (CNS). Anti-inflammatory properties of the *N. sativa* are the core reason for its therapeutic activity in MS. It usually arises by less production of inflammatory and pro-inflammatory cytokines and inhibiting infiltration of inflammatory cells in CNS. More studies are essential to reveal the accurate mechanisms of action underlying which phytochemical compounds demonstrate their neuroprotective and anti-inflammatory properties. Along with these benefits of *N. sativa*, it also helps in improving sleep quality, sedation, muscle stiffness relief and showcase anti-depressant effects. *In-vitro* studies showed a repressive outcome of thymoquinone and *N. sativa* seed oil as inflammatory mediators like IFN-c, IL-6, PGE2, IL-1b, TNF-α. In accumulation, black seed oil inhibited 5-LO and COX paths of arachidonate metabolism. Non-enzymatic peroxidation in the brain is also potently inhibited by thymoquinone. In MS animal model therapeutic effects of *N. sativa* have been reported. It is known to improve remyelination in CNS repressed TGF-β1 expression and inflammatory process reduction in the experimental autoimmune encephalomyelitis (EAE) models of MS disease (16). EAE is a well-recognised animal model for MS in which an attack by the immune system to myelin protein in the central nervous system leads to oligodendrocyte loss and inflammatory demyelination. EAE is also used in the drug discovery of the beneficial detection of new therapeutic agents for the MS individual (17). However, natural products are an advanced function of the drug development process. Due to its chemically varied nature, they can control numerous targets of the complex system at a time. Microarrays are suited better for simultaneous scrutiny of multiple genes simultaneously (18). Introduction of novel drugs is a complex, long, highly risky and costly process. Hence CADD method is broadly used in recent times which helps in speeding pharmaceutical industries' process. The advantage of cost and time is significant. Wet lab techniques in combination with CADD help in accelerating the process of novel drug discovery (19).

Here, in current research we have investigated molecular levels of pathogenesis involved in MS patients by considering their mRNA expression data. The current finding depicts the engrossment of RPL27, RPS14 and FAU genes misplay in ribosomal assembly and translation of protein pathways in MS patients, which might involve in coding protein leading to demyelination of axonal transaction. Targeting these proteins, phytocompounds showing therapeutic affinity towards such misplayed proteins can potentially be used for management of MS. Here such compounds (Table-4) are studied to perceive the binding affinity and therapeutic potential in treatment of MS pathogenesis by phytocompounds extracted from seed oil of *N. sativa.* These compounds showed promising results during the current *in-silico* study, but as mentioned earlier, prior to any treatment its clinical practice should be validated with large robust data, which lies as the future prospective here.

CONCLUSION

In summary notable progress in management of MS with better understanding of pathophysiology have been made and many DMTs are made available but the question of safety and efficacy is still challenging due to adverse effects associated with these therapies. Hence properties of herbal plants must be explored as a therapeutic agent that can reduce the neuronal degeneration. Anti-inflammatory properties of medicinal plants help reduce inflammatory mechanisms. Moreover, most studies based on herbal therapies outcome are experimented on animal models, which requires more clinical trials for validation. Here, in current in-silico study therapeutic potential of *N. sativa* against MS is investigated, still there is immense requirement for approval of this analysis by further clinical trials to recommend these currently investigated phytocompounds for MS patients.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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