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Research article

Exploring the mechanism of *Nigella sativa* against colorectal cancer by network pharmacology and molecular docking

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Abstract

Background: *Nigella sativa*, generally known as black seed or black cumin, has a rich historical background in traditional medicine for its diverse health benefits. **Objective:** The aim of the study explore the potential effects of *Nigella sativa* (NS) on colorectal cancer (CC) through the application of network pharmacology and molecular docking. **Methods:** Network pharmacology (NP) analysis was conducted to identify pertinent colorectal cancer targets and compounds sourced from relevant databases. Subsequently, protein-protein interaction (PPI) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were employed to delineate critical molecular pathways, while molecular docking simulations study were employed to validate the binding interactions at active sites. **Results:** Network analysis of compound-target interactions revealed a network comprising 319 nodes and 410 edges, indicating a complex interplay between the derivatives and their associated targets. The PPI analysis underscored the significant interactions within the network, particularly with targets known to be involved in colorectal cancer regulation. KEGG pathway analysis highlighted the importance of EGFR and PI3K pathways in the context of colorectal cancer. Notably, molecular docking studies identified Nigellicine as having the highest affinity for key colorectal cancer-related targets, including **AKT1** (-7.6 kcal/mol), **IL6** (-6.2 kcal/mol), **ALB** (-7.1 kcal/mol) and **HSP90AA1** (-8.2). **Conclusion:** The integration of network analysis and molecular docking studies provided collectively support the colorectal cancer characteristics of the compounds, emphasizing the need for further research to develop novel pharmacological interventions for colorectal cancer management.

Introduction

Cancer is characterized by excessive cell proliferation that can metastasis to other parts of the body and cause disease. The rising population, the aging population, and the effects of lifestyle changes all contribute to a rise in cancer incidence and mortality rates. Because cancer has such far-reaching effects, it degrades the quality of human resources [1]. As the second biggest cause of cancer-related death, colorectal cancer ranks third in terms of prevalence worldwide [2]. Hereditary disorders such as familial adenomatous polyposis (FAP) and hereditary nonpolyposis colorectal cancer (HNPCC) account for 10% of all cases of colorectal cancer [3]. The remaining 70% of cases arise at random.

Colorectal cancer treatment is complex and calls for input from other fields of expertise. There is a higher recurrence rate with local operations, surgical therapy is less successful,

especially for metastatic disease, and the adverse effects of chemotherapy and monoclonal antibodies are distinct [4]. This issue is mostly attributable to the fact that the medications being utilized are not selective, causing harm to both cancer cells and healthy cells. Numerous sets of genes are involved in most disorders. However, creating a new medication is a lengthy and expensive process [5]. Because of this issue, the focus of drug development has shifted from developing single-drug therapies to creating plant-based medicines that utilize many chemical compounds directed at different targets (multicomponent - network targets) [6]. Multicomponent medications have more than one active ingredient, and this can have a positive impact on the pharmacological effects. In silico computational methods have a synergistic effect on multicomponent approaches, allowing for faster execution and more encouraging outcomes. The concepts of network theory between chemicals and biological systems, as well as the outcomes of in vitro and in vivo testing for drug development, are explained by network pharmacology [7].

For correct findings and productive preclinical research, an early investigation providing an initial view is crucial. Compound content and mechanism of action are predicted using computational methods based on the compound's link to proteins in existing databases. The purpose of this research is to foretell *Nigella sativa* network pharmacology in colorectal cancer. Our findings can be used as a foundation for developing safe and effective new medicines or improvements in cancer treatment based on target genes.

Materials and Methods

Screening for active components of *Nigella sativa*

The constituents employed in this investigation were procured from the KNApSAcK Family database (http://www.KNApSAcKfamily.com/) and Dr. Duke's Phytochemical and Ethnobotanical Databases (https://phytochem.nal.usda.gov/phytochem/search). The selection of active compounds adhered to specific criteria, including a molecular weight (MW) within the range of 180 to 500 Dalton, oral bioavailability (OB) equal to or greater than 20%, drug-likeness (DL) equal to or greater than 0.1, and a blood–brain barrier (BBB) value equal to or greater than −0.3. The chemical structure validation of *Nigella sativa* components was conducted utilizing the PubChem database (https://pubchem.ncbi.nlm.nih.gov) and was generated using ChemDraw.

Screening for protein targets of *Nigella sativa*

The protein targets associated with *Nigella sativa* constituents were sourced from the Swiss Target Prediction database (http://www.swisstargetprediction.ch/). Target information relevant to colorectal cancer was extracted from the Gene Card Database (https://www.genecards.org/) and the Online Mendelian Inheritance in Man (OMIM) database (https://www.omim.org/). Putative targets of the *Nigella sativa* compounds were identified through a screening process that involved detecting common targets among the genes represented in a Venn plot (https://bioinformatics.psb.ugent.be/webtools/Venn/).

Protein-protein interaction (PPI) data

The exploration of protein-protein interactions (PPI) serves as a valuable approach to discern potential therapeutic targets in the management of disorders, given the pivotal role of PPI in orchestrating biological processes [8]. In this investigation, the shared targets of *Nigella sativa* compounds and colorectal cancer were delineated utilizing the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) V11.5 platform (https://string-db.org/) designed for multiple proteins in Homo sapiens [9]. A stringent minimal interaction score of 0.9 (indicative of high confidence) was applied in the establishment of the PPI network. Subsequent extraction of p-values was conducted to assess the statistical significance of the PPI network and its enrichment.

Gene and KEGG enrichment analysis

The investigation of signal pathways, biological processes, molecular functions, and cellular component terms associated with the intersecting targets between the drug and disease was conducted through Gene Ontology (GO) functional annotations and the Kyoto Encyclopedia of Genes and Genomes (KEGG), considering a significance threshold of $p < 0.05$. Enrichment analysis of these pathways was performed using Input 2.0 (http://cbcb.cdutcm.edu.cn/INPUT/Home/) and Shinygo (http://bioinformatics.sdstate.edu/go/) as analytical tools [10-11].

Compound-target network construction

The compounds derived from *Nigella sativa* were systematically linked to construct a compound-target network. Cytoscape 3.9.1 (Cytoscape Consortium, San Diego, CA, USA) [12], a software tool designed for visualizing interaction networks, was employed for the network construction. In this network, edges denote the interactions between the compounds and their corresponding protein targets, while nodes represent the individual compounds and their associated targets.

Molecular docking analysis

AutoDock Vina was employed to assess the interactions between *Nigella sativa* compounds and target proteins [13]. The three-dimensional (3D) structures of the target proteins, in Protein Data Bank (PDB) format, were retrieved from (https://www.rcsb.org) for subsequent docking analysis [14]. Additionally, the three-dimensional (3D) structures of *Nigella sativa* compounds were sourced from the PubChem database (https://pubchem.ncbi.nlm.nih.gov). AutoDock and PyMOL software were used to automatically determine

docking scores (binding affinities) and binding interaction between the compounds and target proteins [13].

Results

Screening for active components of *Nigella sativa*

Ten components inherent in *Nigella sativa* were anticipated through a comprehensive analysis utilizing the KNApSAcK Family and Dr. Duke's Phytochemical and Ethnobotanical databases. Specifically, five components were identified within the seed oil of *Nigella sativa* L., as detailed in Table 1. The KNApSAcK database, referenced in [15], provides a wealth of information encompassing the interrelation of components, biological activities, and associated species. Additionally, Dr. Duke's Phytochemical and Ethnobotanical database, accessible online at no cost, offers valuable ethno medicinal data of high significance. *Nigella sativa* seeds have historically played a pivotal role in treating various diseases, including cancer, within traditional medical systems prevalent in South and Southeast Asia, Arabia, Africa, and the Mediterranean [16]. Employing pharmacokinetic criteria and literature reviews, we identified nine potentially active chemicals from *Nigella sativa*, detailed in Table 2. These compounds exhibit molecular weights (MW) ranging from 180 to 500, oral bioavailability (OB) between 20 and 30, and blood–brain barrier (BBB) binding affinity constants between 0.3 and 0. The chemical structures of these selected compounds were delineated using ChemDraw, as illustrated in Figure 1, showcasing a diversity of structures encompassing glucosides, long-chain lipids, and simple chemical molecules.

The study reveals the capacity of *Nigella sativa* components to interact with a diverse array of proteins, including those implicated in cancer, as determined through protein target prediction utilizing the Swiss Target Prediction database. The database identified 410 potential chemical targets associated with *Nigella sativa*. To identify potential targets pertinent to colorectal cancer, the Gene Card Database was employed. The combined analysis of Swiss Target Prediction and GeneCards Database yielded a comprehensive list of *Nigella sativa* compound protein targets, as detailed in Table 3.

The selection of 410 targets predicted by the Swiss system was visually represented using Cytoscape 3.9.1, resulting in a compound-target network displayed in Figure 2A-2B. The Venn diagram illustrates shared targets and genes, involving 9 chemicals and 410 interacting target proteins. Notably, multiple components within this network were observed to target the same proteins. The network comprises 319 nodes and 410 edges, suggesting a potential synergistic effect of *Nigella sativa*'s active biochemicals on various targets. This aligns with the plant's documented therapeutic efficacy against a spectrum of diseases and ailments. Furthermore, topological characteristics, including betweenness centrality, closeness centrality, and degree, were assessed to elucidate the importance of nodes within the network, as summarized in Table 4. These findings underscore the multifaceted potential of *Nigella sativa* compounds in targeting specific proteins, thereby presenting a promising avenue for therapeutic interventions against diverse diseases, including colorectal cancer.

PPI network

Figure 3A-B illustrates the protein-protein interaction (PPI) network depicting potential colorectal cancer targets associated with *Nigella sativa*. The PPI enrichment analysis yielded a p-value of 0.0272, while the average node degree was determined to be 8.6. Notably, specific targets such as AKT1 exhibited a node degree of 120, IL6 demonstrated a node degree of 112, ALB exhibited a node degree of 110, and HSP90AA1 showed a node degree of 88. These findings suggest that these identified targets, particularly AKT1, IL6, ALB, and HSP90AA1, may play pivotal roles in mediating the effects of *Nigella sativa* on colorectal cancer.

GO and KEGG enrichment analysis

Genes with potential relevance to colorectal cancer treatment were identified through a Gene Ontology (GO) enrichment study of *Nigella sativa*. These genes were associated with various cellular components, molecular functions, and biological processes. Noteworthy biological processes included positive regulation of gene expression, response to endogenous stimulus, cellular localization, intracellular signal transduction, nitric oxide biosynthetic process, protein phosphorylation, epidermal growth factor response, apoptotic process, and acute inflammatory response, as indicated in Table 5. Molecular functions associated with these genes encompassed nitric-oxide synthase regulator activity, enzyme binding, binding of identical proteins, binding of phosphatases, ions, proteins, protein phosphatases, and small molecules, detailed in Table 6a. Additionally, Table 6b highlighted the predominant cellular component term as the Endoplasmic reticulum lumen in the GO analysis.

Furthermore, the Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis Table 6c and Figure 4 KEGG Pathways revealed several pathways linked to the potential colorectal cancer impact of *Nigella sativa*. These pathways included Pathways in cancer, EGFR tyrosine kinase inhibitor resistance, the IL-17 signaling pathway, prostate cancer, the ErbB signaling pathway, the TNF signaling pathway, the Estrogen signaling pathway, gastric cancer, Th17 cell differentiation, the MAPK signaling pathway, apoptosis, and the Rap1 signaling pathway (Figure 5A-E). The implicated genes within these pathways encompassed the epidermal growth factor receptor, nitrogen metabolism, cancerous pathway, PI3K, and vascular endothelial growth factor.

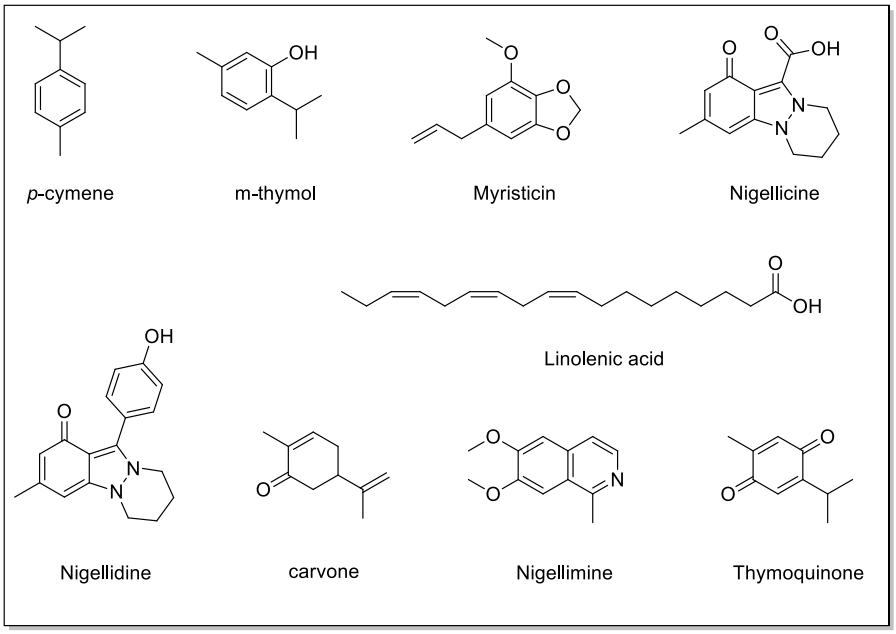


Figure 1. Structures of Phytoconstituents of *Nigella sativa* L.

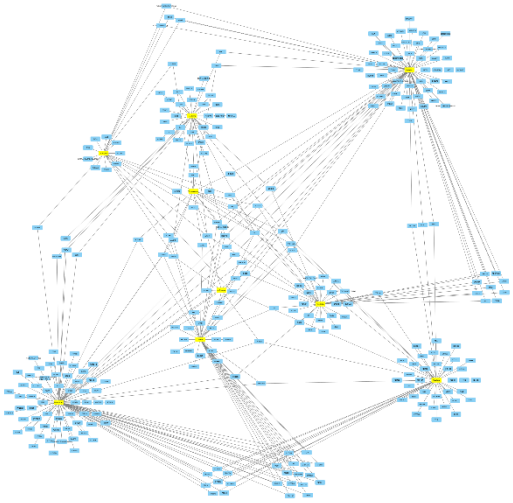


Figure 2A. Compound- Target Network.

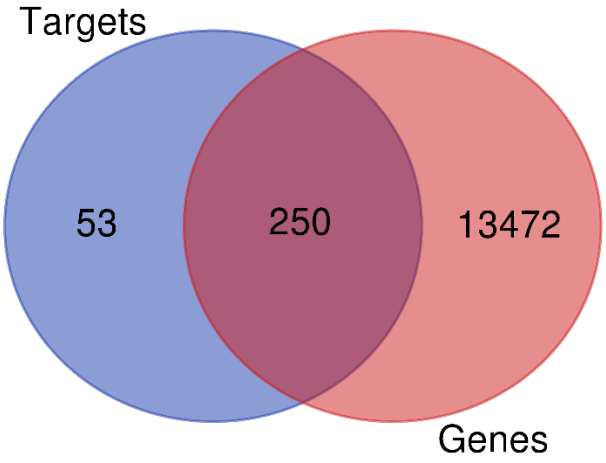


Figure 2B. Venn diagram shows the intersection between *Nigella Sativa* targets and colorectal cancer related genes.

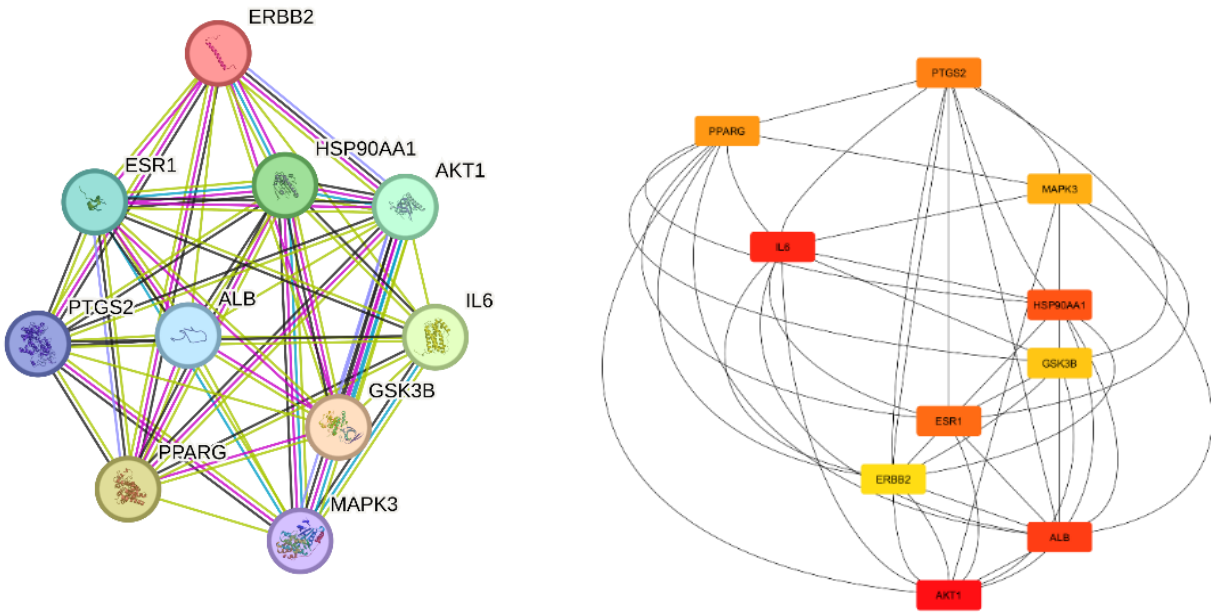


Figure 3. A) PPI network of potential colorectal cancer targets of *Nigella Sativa*. The PPI enrichment p-value was 0.0272 and the average node degree was 8.6. The node degrees of AKT1 was 120, IL6 was 112, ALB was 110, and HSP90AA1 88 was suggesting that these targets could play important roles in the effects of *Nigella Sativa* on colorectal cancer. B) Top 10 Hub Genes Network.

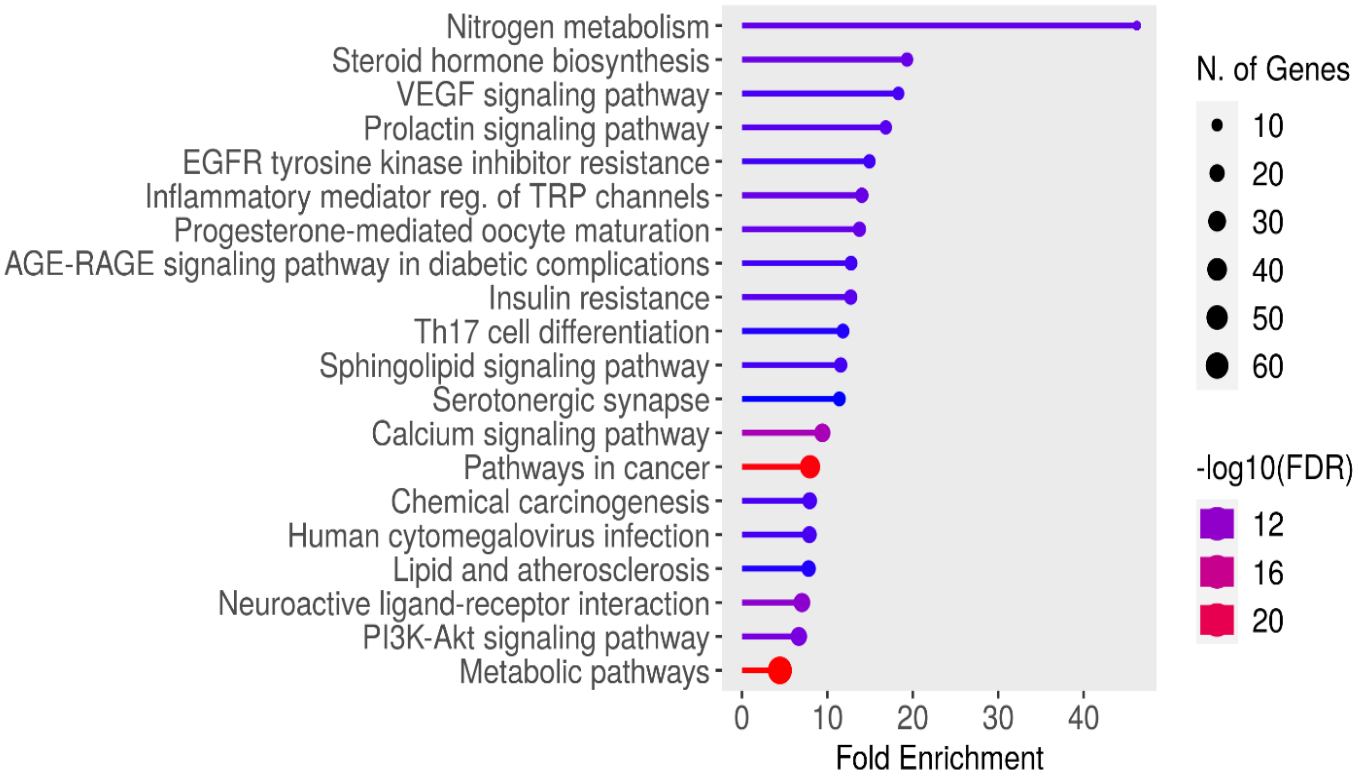


Figure 4. KEGG Plot.

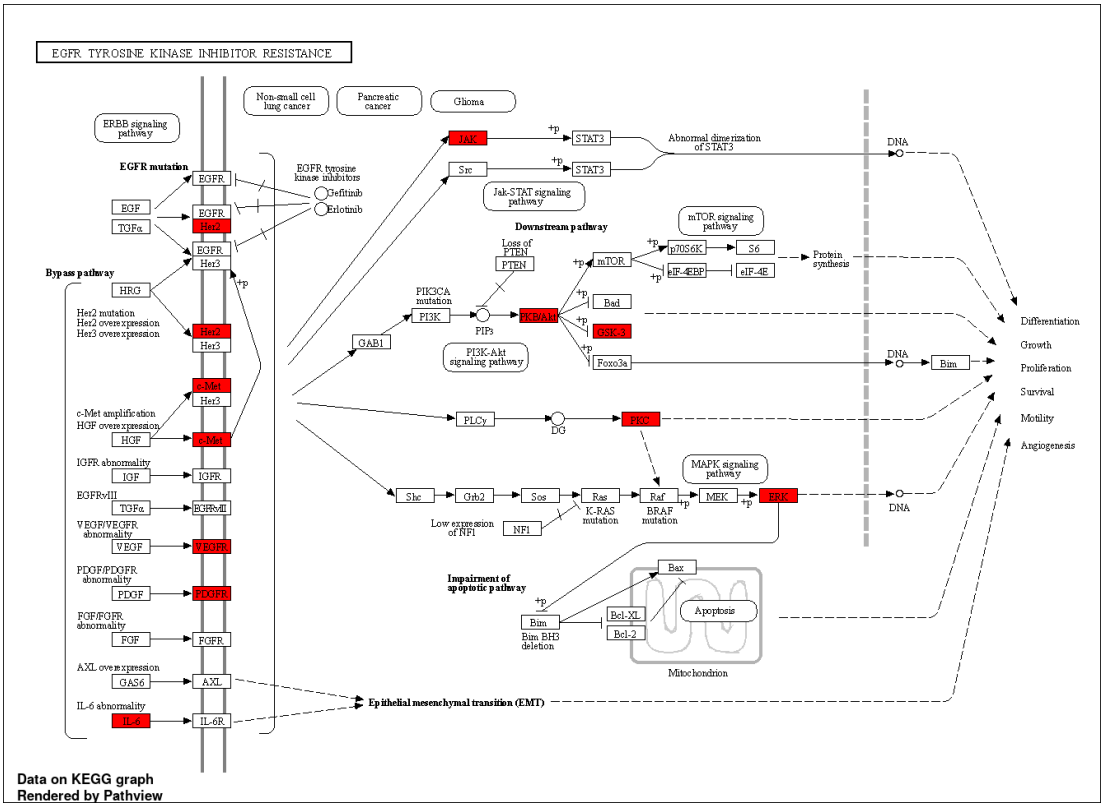


Figure 5A. EGFR Pathway.

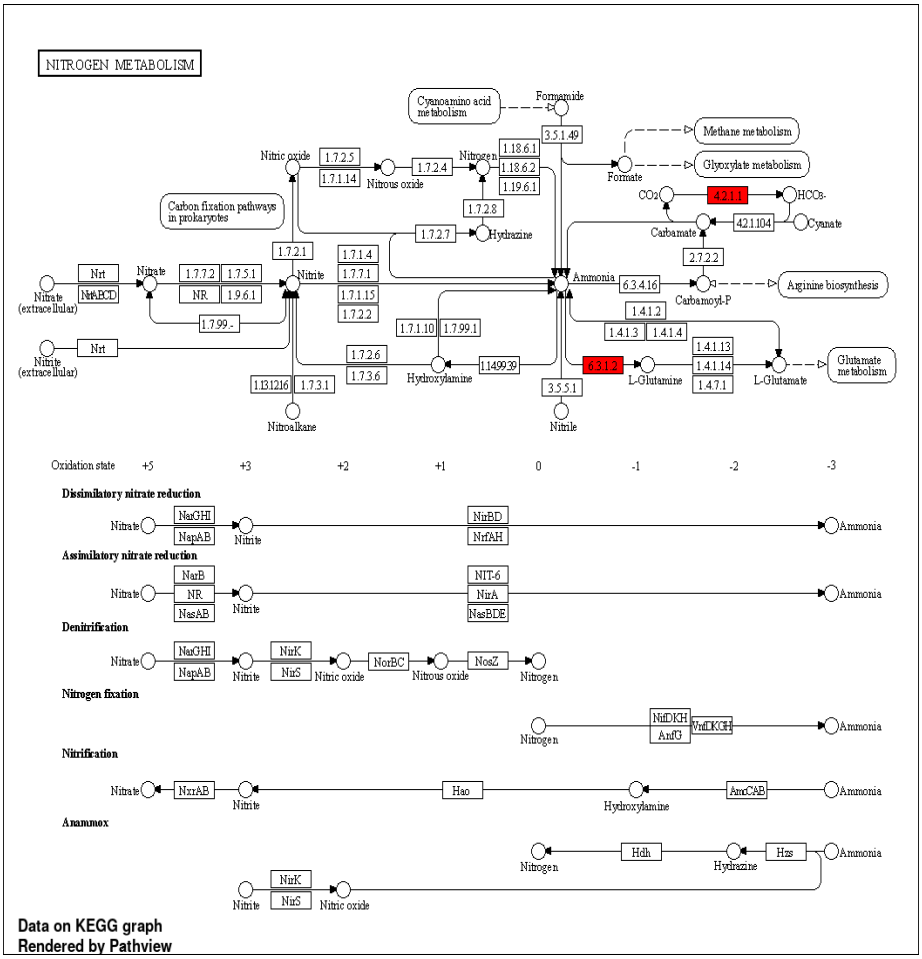


Figure 5B. Nitrogen Metabolism.

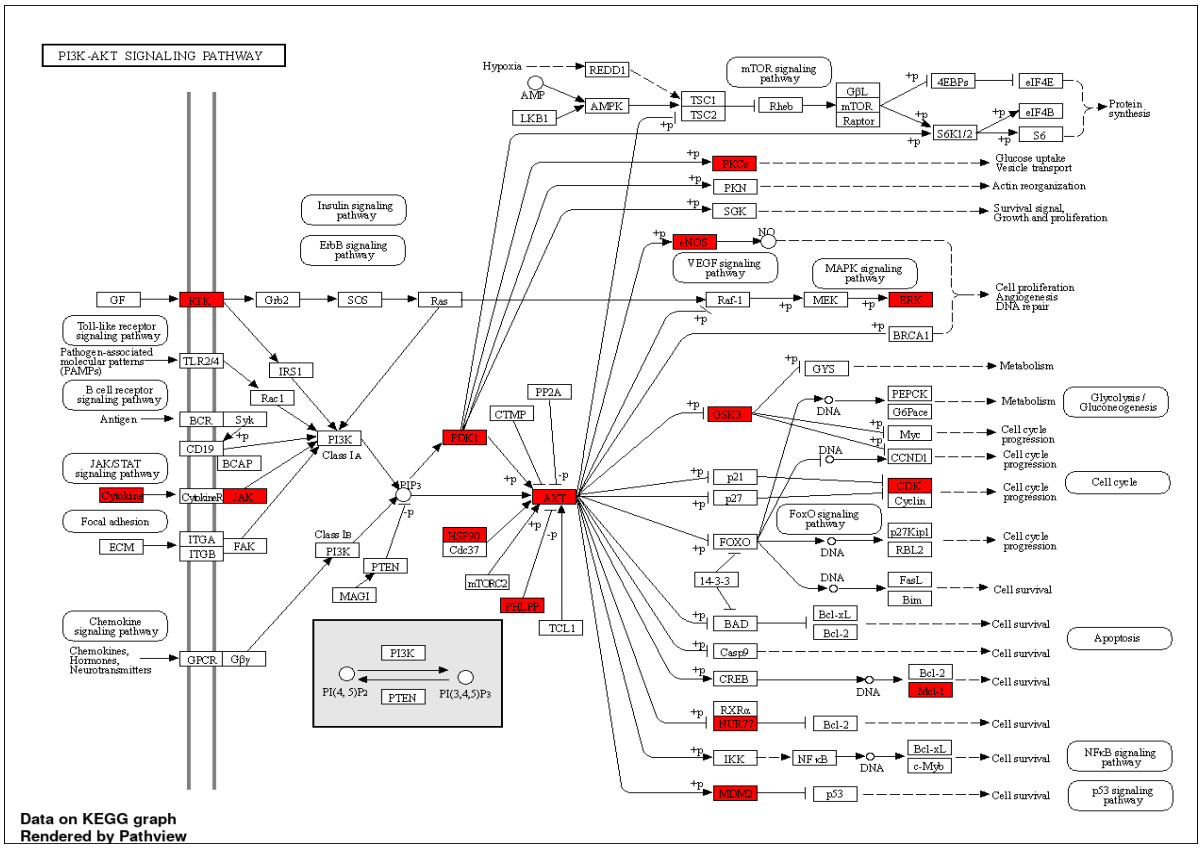


Figure 5C. PI3K Pathway.

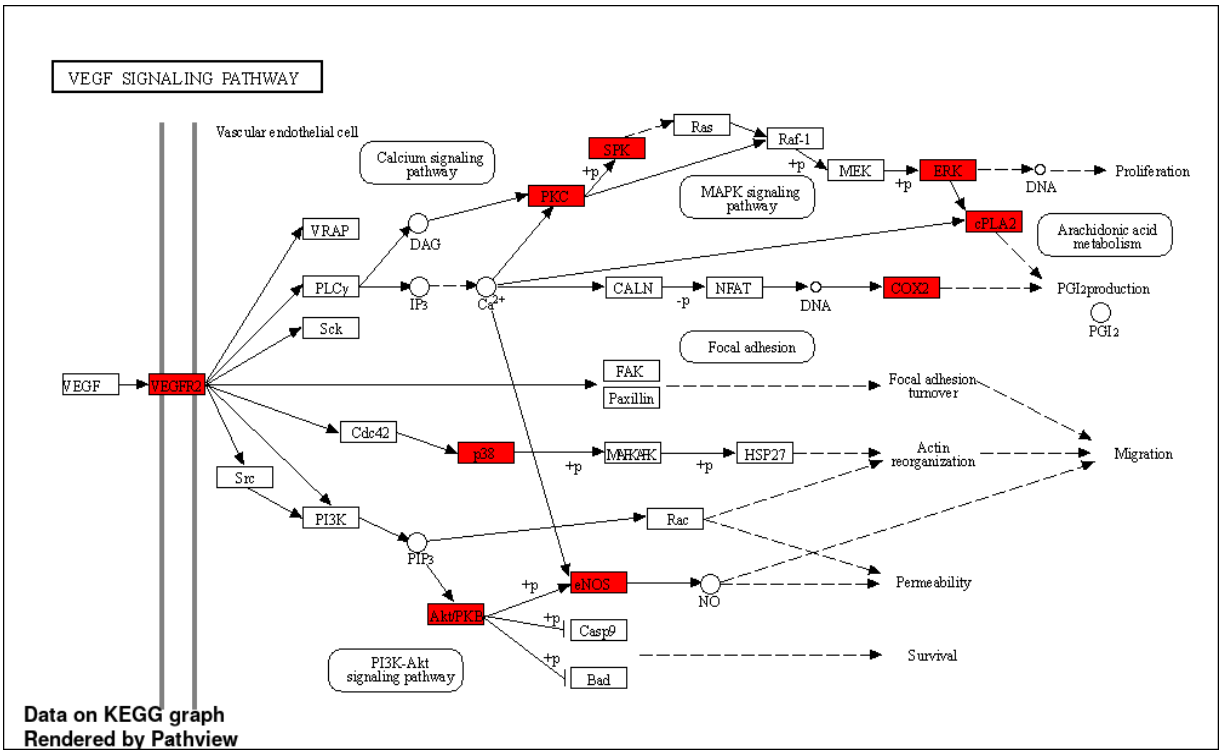


Figure 5D. VEGF.

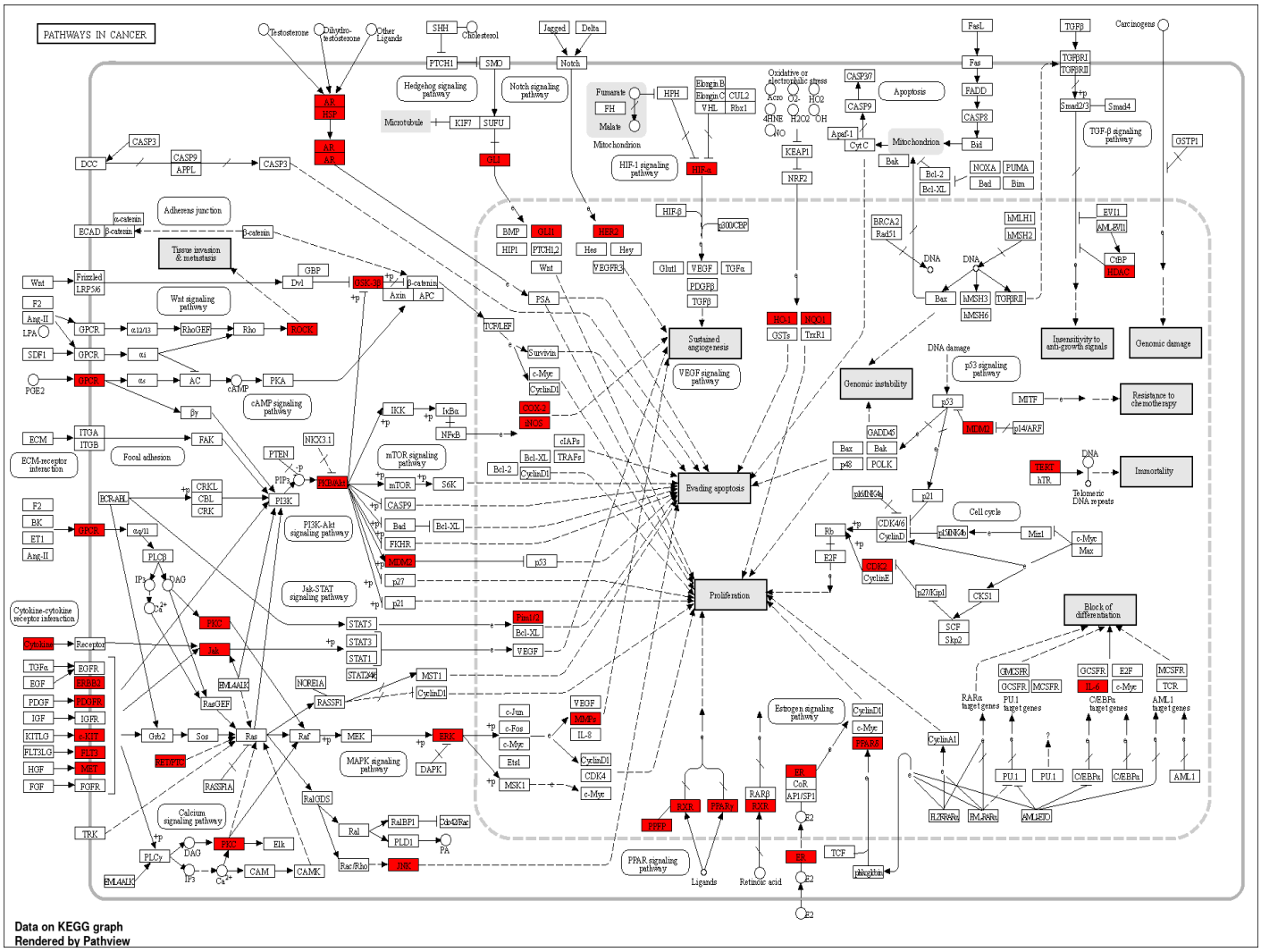


Figure 5E. Pathways in Cancer.

Table 1. Prediction of components contents of *Nigella Sativa* with KNApSAcK Family and Dr. Duke's Phytochemical and Ethnobotanical Databases.

Compound Name	CAS_ID	SMILES	Plant Part
m-Thymol	89-83-8	c1cc(c(cc1C)O)C(C)C	Seed Oil
Oleic acid	112-80-1	CCCCCCCC/C=C\CCCCCCCC(=O)O	Seed
Myristicin	607-91-0	c12c(c(cc(c1)CC=C)OC)OCO2	Seed Oil
p-Cymene	99-87-6	c1cc(ccc1C)C(C)C	Seed
beta-Amyrin	559-70-6	CC1(CCC2(CCC3(C(=CCC4C3(CCC5C4(CCC(C5(C)C)O)C)C)C2C1)C)C)C	Seed Oil
Linolenic acid	463-40-1	OC(CCCCCCC/C=CC/C=CC/C=CCC)=O	Seed
Thymoquinone	490-91-5	C1=C(C(=O)C=C(C1=O)C)C(C)C	Seed Oil
Kaempferol 3-glucosyl-(1->2)-galactosyl-(1->2)-glucoside	197250-98-9	C1=CC(=CC=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)OC4C(C(C(C(O4)CO)O)O)OC5C(C(C(C(O5)CO)O)O)O)OC6C(C(C(C(O6)CO)O)O)O)O	Seed Oil
Quercetin 3-glucosyl-(1->2)-galactosyl-(1->2)-glucoside	197250-97-8	C1=CC(=C(C=C1C2=C(C(=O)C3=C(C=C(C=C3O2)O)O)OC4C(C(C(C(O4)CO)O)O)OC5C(C(C(C(O5)CO)O)O)OC6C(C(C(C(O6)CO)O)O)O)O)O	Seed
Quercetin 3-(6””-feruloylglucosyl)-(1->2)	197294-29-4	COC1=C(C=CC(=C1)C=CC(=O)OCC2C(C(C(C(O2)OC3C(C(C(OC3OC4C(C(C(OC4OC5=C(OC6=CC(=CC(=C6C5=O)O)O)O)C7=CC(=C(C=C7)O)O)CO)O)	Seed

-galactosyl-(1->2)-glucoside		O)CO)O)O)O)O)O)O	
Nigellicine	98063-20-8	CC1=CC(=O)C2=C(N3CCCCN3C2=C1)C(=O)O	Seed
Nigellidine	120993-86-4	CC1=CC(=O)C2=C(N3CCCCN3C2=C1)C4=CC=C(C=C4)O	Seed
Nigellimine	4594-02-9	CC1=NC=CC2=CC(=C(C=C12)OC)OC	Seed
Carvone	99-49-0	CC1=CCC(CC1=O)C(=C)C	Seed
Nigellidine 4-O-sulfite	1032622-86-8	CC1=CC2=C(C(=C1)OS(=O)(=O)O)C(=[N+]3N2CCCC3)C4=CC=C(C=C4)O	Seed

Table 2. Active components of *Nigella Sativa.*

Molecule Name	MW (g/mol)	B (%)	BBB	DL
m-Thymol	150.22	0.55	Yes	0.95
Oleic acid	282.46	0.85	No	1.0
Myristicin	192.21	0.55	Yes	0.65
p-Cymene	134.22	0.55	Yes	0.94
beta-Amyrin	426.72	0.55	No	0.98
Linolenic acid	278.43	0.85	Yes	1.0
Thymoquinone	164.20	0.55	Yes	0.93
Kaempferol 3-glucosyl-(1->2)-galactosyl-(1->2)-glucoside	772.66	0.17	No	1.0
Quercetin 3-glucosyl-(1->2)-galactosyl-(1->2)-glucoside	788.66	0.17	No	1.0
Quercetin 3-(6””-feruloylglucosyl)-(1->2)-galactosyl-(1->2)-glucoside	964.83	0.17	No	1.0
Nigellicine	246.26	0.85	Yes	0.77
Nigellidine	294.35	0.55	Yes	0.88
Nigellimine	203.24	0.55	Yes	0.89
Carvone	150.22	0.55	Yes	0.63
Nigellidine 4-O-sulfite	375.42	0.55	No	0.53

Table 3. Target proteins of *Nigella sativa* L. with Swiss Target Prediction.

Compound name	PubChem ID	Target genes linked to cancer
m-Thymol	6989	TRPA1, PTGS1, GABRA1, GABRG2, HTR2B, GABRB3, HTR2C, CA2, CHRM2, FLT3, JAK1, JAK2, PRKCA, AURKA, ESRRG, CDK2, CCNA1, CCNA2, ACHE
Myristicin	4276	CDK5R1, CDK5, CDK2, CCNA1, CCNA2, CDK9, CCNT1, DYRK1A, METAP2, PDE7A, ADORA2B, ADORA3, ADORA2A, DYRK1B, GRM4, JAK1, JAK2, CHRNA3, PDE5A, JAK3, NUDT1, AKT1, MAPK3K14, GRM5, TNKS2, NAMPT, ERBB2, CNR1, HSP90AA1, ALPL, HTR2A, HTR2C, LIMK1, CDK2, CDC7, GABRB3, GABRA3, GABRG2, GABRA1, GABRA5, GABRA2, CCNE1, CDK2, CDK5, CHEK1, KAT2B, KDR, HMOX1, PTPRC, STS, NOS1, NOS2, NOS3, TERT, CD38, TGM2, TYMP, CDK1, TSPO, MAPK8, GSK3B, MET, MAPK10
p-Cymene	7463	CYP2A6, ACHE, TAAR1, PPARA, PTGS1, TRPA1
Linolenic acid	5280934	PPARG, PPARA, PPARD, FABP4, FFAR1, FABP3, PTGS1, FABP5, TERT, FABP1, CNR1, PTPN1, ALOX5, PTPN2, PTGES, LTB4R, POLB, ESR2, PTPN6, RORC, TOP1, PTGER2, ALOX12, CDC25A, PTPRF, PTGS2, NOS2, PGR, SRD5A2, PDE4D, PTGER1, PLA2G1B, ESR1, CDC25B, NR3C1, PTPN11, ADORA3, NR1H3, CD81, PRKCH, FNTA, FNTB,

		RXRB, SERPINA6, SHBG, G6PD, ITGAL, ICAM1, ITGB2, PTGER4, CYP26A1, NPC1L1, CYP17A1, AKR1B10, IMPDH2, 1L6, HTR2B, MAPK14, MAPK1, PREP, AR, MDM2, FFAR4, ENPP2, MAPK3, RBP4, PRKAG1, PRKAB1, PRKAA2, ALOX15, TOP2A, RORA, MMP2
Thymoquinone	10281	PLK1, GLI2, GLI1, ALOX5, CYP19A1, PTPN2, CHRM2, CA2, ACHE, SHBG
Nigellicine	11402337	HTR2A, ADRA1D, HTR2B, ADRA1A, HTR2C, ADRA1B, TSPO, JAK3, DBF4, CDC7, MMP3, HPGD, DYRK1A, CDK5R1, CDK5, AR, EGFR, KDR, AURKA, AKT1, SIGMAR1, CSF1R, MMP13, MMP2, MIF, ALOX15, ALOX12, CNR1, MMP9, FGFR1, DYRK1B, GABRA1, CCNE2, CDK2, CCNE1, CDK1, CCNB1, CCNB2, PIM1, MAPKAPK2, GSK3B, CTSK, CDK2, CDK1, GABRA5, ROCK1, ERN1, AKR1B1, JAK2, CA12, AKR1B10, RBP4, PIK3CG, PIK3CA,TGFBR1, SIRT2, RORC, MAPK1, CASP3
Nigellimine	20725	ACHE, NQO1, CYP1A2, GRM4, CCNE2, CDK2, CCNE1, DYRK1A, DYRK1B, JAK1, JAK2, CTSK, GSK3B, CSNK1D, GRM5, ADORA2B, IDO1, PDE5A, CDK1, CCNB1, CCNB2
Carvone	7439	CYP19A1, SRD5A1, NR3C1, PGR, SERPINA6, SHBG, FABP1, NR1I3, SRD5A2, AR, ADH1A, SIGMAR1, ADH1C, NPC1L1, PTGES, CA2, CYP17A1, PARP1, ADORA3, MAPK3, PRKCH, PTPN11, AKR1B10, PTPN2, PTGS1, NR1H3, TOP1

Table 4. Topological analysis of important nodes with network analyzer results.

Name	Closeness Centrality	Betweenness Centrality	Degree	Average Shortest Path Length	Topological Coefficient
MAPK3	0.818182	0	7	1.22222222222222	1
ALB	1	0.007937	9	1	0.950617
IL6	1	0.007937	9	1	0.950617
AKT1	1	0.007937	9	1	0.950617
GSK3B	0.9	0	8	1.11111111111111	0.986111
PTGS2	1	0.007937	9	1	0.986111
ESR1	1	0.007937	9	1	0.950617
PPARG	1	0.007937	9	1	0.950617
HSP90AA1	1	0.007937	9	1	0.950617
ERBB2	0.9	0	8	1.11111111111111	0.986111

Table 5. Go Biological Process.

Description	Count in Gene Set	False Discovery Rate
Positive regulation of gene expression	9	1.18E-06
Response to endogenous stimulus	9	2.75E-06
Regulation of cellular localization	8	7.27E-06
Regulation of intracellular signal transduction	9	1.13E-05
Positive regulation of nitric oxide biosynthetic process	4	1.85E-05
Positive regulation of protein phosphorylation	7	2.84E-05
Response to epidermal growth factor	3	0.00031
Regulation of acute inflammatory response	3	0.00035

Table 6a. Go Molecular Function.

Description	Count in Gene Set	False Discovery Rate
Nitric-oxide synthase regulator activity	3	7.64E-05
Enzyme binding	8	0.0015
Identical protein binding	8	0.0015
Phosphatase binding	4	0.0026
Anion binding	7	0.0343
Protein binding	10	0.0372
Protein phosphatase binding	3	0.0372
Small molecule binding	7	0.0372

Table 6b. Go Cellular Components.

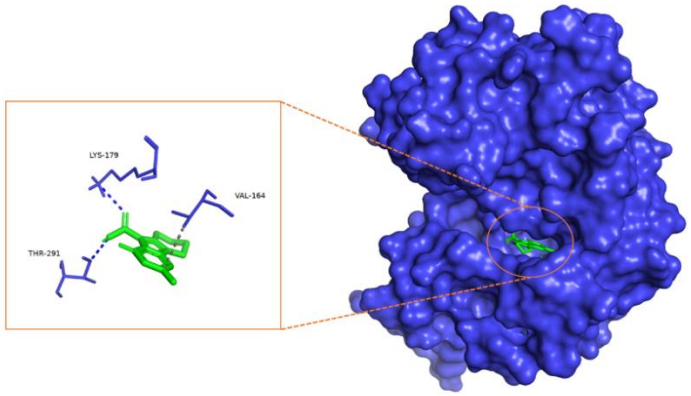
Description	Count in Gene Set	False Discovery Rate
Endoplasmic reticulum lumen	4	0.0258

Table 6c. KEGG Pathways.

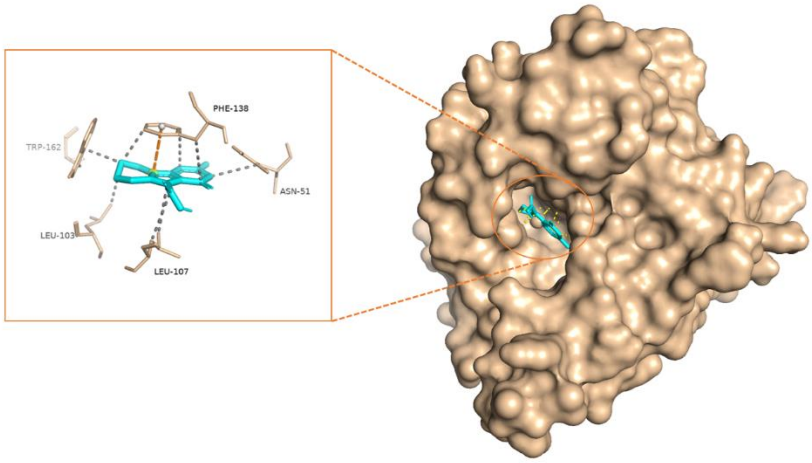
Description	Count in Gene Set	False Discovery Rate
Pathways in cancer	9	2.04E-11
EGFR tyrosine kinase inhibitor resistance	5	4.59E-08
IL-17 signaling pathway	5	6.83E-08
Prostate cancer	5	6.97E-08
ErbB signaling pathway	4	2.03E-06
TNF signaling pathway	4	5.04E-06
Estrogen signaling pathway	4	8.48E-06
Gastric cancer	4	1.10E-05
Th17 cell differentiation	3	0.00015
MAPK signaling pathway	3	0.0019
Apoptosis	2	0.0074
Rap1 signaling pathway	2	0.0147

Molecular docking analysis

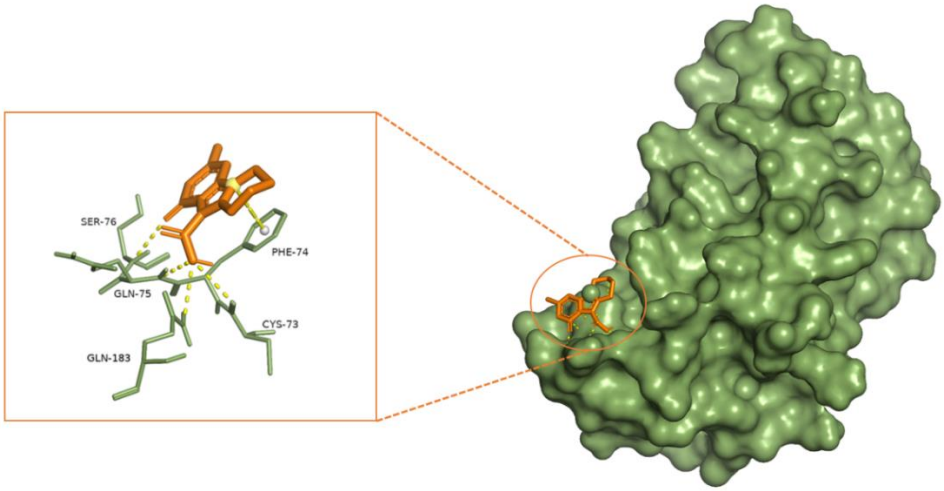
The study conducted molecular docking analyses to assess the binding affinities of active constituents from *Nigella sativa* with associated protein targets in the protein-protein interaction (PPI) network, including AKT1, IL6, ALB, and HSP90AA1. Molecular docking scores, indicative of the strength and stability of chemical-protein binding, were employed, where a more negative score indicated a stronger interaction. Table 7 presents the docking scores for the interactions between *Nigella sativa* compounds and their respective protein targets. Figures 6A-D illustrate diverse binding mechanisms between the compounds and proteins. Crystal structures from the Protein Data Bank (PDB) for AKT1 (PDB: 4EKL), IL6 (PDB: 4ZS7), ALB (PDB: 6M4R), and HSP90AA1 (PDB: 3O0I) were obtained. Auto Dock Vina was employed for docking ligands into receptors to anticipate potential binding interactions, with the grid centered on amino acid residues surrounding the active sites. Free binding energies, expressed in kcal/mol, were utilized to convey the results. Molecular docking experiments revealed estimated free energy of binding ranging from -5.7 to -7.6 kcal/mol for AKT1 (PDB: 4EKL), -4.3 to -6.2 kcal/mol for IL6 (PDB: 4ZS7), -5.4 to -7.1 kcal/mol for ALB (PDB: 6M4R), and -7 to -8.2 kcal/mol for HSP90AA1 (PDB: 3O0I). Nigellicine emerged as the most active molecule, exhibiting a binding energy of -8.2 kcal/mol for HSP90AA1 (PDB: 3O0I), -7.6 kcal/mol for AKT1 (PDB: 4EKL), -6.2 kcal/mol for IL6 (PDB: 4ZS7), and -7.1 kcal/mol for ALB (PDB: 6M4R).



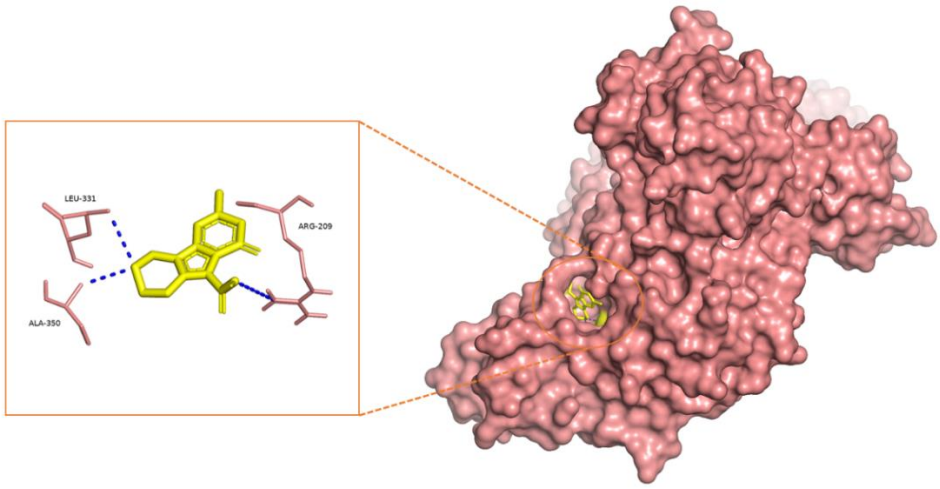
4ekl



3O0I



4ZS7



6M4R

Figure 6A-D. Binding modes of ligands, and receptors by molecular docking simulation. Docking pose and surface representation of Nigellicine, and AKT1 (PDB: 4EKL). Docking pose and surface representation of Nigellicine, and HSP90AA1 (PDB: 3O0I). Docking pose and surface representation of standard drug Nigellicine, and IL6 (PDB: 4ZS7) and Docking Pose and surface representation of ALB (PDB: 6M4R).

Table 7. Docking scores of *Nigella Sativa* compounds and potential targets.

Compound	Binding Energy (kcal/mol)			
	AKT1 (PDB: 4EKL)	IL6 (PDB: 4ZS7)	ALB (PDB: 6M4R)	HSP90AA1 (PDB: 3O0I)
Nigellicine	**-7.6**	**-6.2**	**-7.1**	**-8.2**
Nigellimine	-6.5	-5.3	-6.7	-7.9
Thymoquinone	-6.5	-5.1	-6.7	-7.5
Myristicin	-6	-5.2	-6.2	-7.4
Cymene	-5.9	-4.9	-6	-7.3
Thymol	-6.2	-5	-6	-7.3
Carvone	-6.1	-5	-5.8	-7.2
Linolenic acid	-5.7	-4.3	-5.4	-7

Discussion

In contrast to the conventional paradigm of "one drug, one target" in drug design, network pharmacology adopts a perspective of multi-targeted therapy to explore the intricate relationships between pharmaceuticals and diseases [17]. This unconventional approach leverages principles from systems biology, including network analysis, connectivity, and redundancy. Researchers have increasingly turned to network pharmacology studies [18-19] as a means to elucidate how medications interact with yet-to-be-identified signaling pathways. The Network Pharmacology framework [20] introduces novel insights into the systemic interplay between therapeutic targets and the entirety of a disease, rendering it a potent and promising tool for elucidating disease mechanisms at the systemic level and identifying potential bioactive ingredients. In the present study, a pioneering network was constructed to delve into the molecular processes associated with *Nigella sativa*.

The compliance of the components with drug-likeness (DL) and oral bioavailability (OB) specifications was ascertained. *Nigella sativa* underwent further investigation, with emphasis on identifying bioactive targets related to colorectal cancer and their integration into a disease-centric network. The constructed network elucidated protein-protein interactions and various pathways, underscoring the potential of *Nigella sativa* bioactives in colorectal cancer inhibition. Key genes implicated in colorectal cancer, notably AKT1, IL6, ALB, HSP90AA1, ESR1, PTGS2, PPARG, MAPK3, GSK3B, and ERBB2, were identified through protein-protein interaction (PPI) analysis. Subsequent Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses unveiled additional pathways, diseases, and disorders associated with these selected genes. Results from the GO enrichment study suggested a significant preventive effect of bioactives in colorectal cancer. Pathway analysis utilizing the KEGG database provided supportive evidence for the therapeutic potential of *Nigella sativa* in colorectal cancer treatment. IL-

17 signaling, TNF signaling, Estrogen signaling, MAPK signaling, and Apoptosis emerged as significant nodes in the network. The findings indicated that *Nigella sativa* influenced multiple signaling pathways, suggesting its applicability beyond prostate cancer, stomach cancer, and Th17 cell deficiency. Colorectal cancer, along with cancers of the colon, pancreas, endometrium, prostate, melanoma, bladder, lungs (small and large cell), liver, and stomach, demonstrated susceptibility to the anticancer effects of *Nigella sativa*. Validation of target accuracy was conducted through an additional docking port assessment. The evaluation of the components' affinity for their respective targets provided immediate insights into the structure-activity relationship.

Conclusions

This computational investigation utilizes network pharmacology and docking analysis to systematically explore the pharmacological mechanisms underlying the preventative and therapeutic effects of *Nigella sativa* in colorectal cancer. Emphasis is placed on elucidating the significant contributions of network pharmacology in unraveling the intricate mechanisms associated with *Nigella sativa*. The insights gained from this study may extend to benefit our understanding of analogous mechanisms in other related cancer types. As the current study has been conducted in line with the principle of network pharmacology but it is necessary to carry out validation study.

Abbreviations: FAP, Familial adenomatous polyposis; HNPCC, Hereditary nonpolyposis colorectal cancer; MW, Molecular Weight; OB, Oral bioavailability; DL, Drug-likeness; BBB, Blood Brain Barrier; KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene Ontology; 3D, Three Dimensional; PDB, Protein Data Bank; CAS, Chemical Abstracts Service; PPI**,** Protein Protein Interaction; DPED, Duke's Phytochemical and Ethnobotanical Databases; NS, *Nigella sativa*; CC, Colorectal Cancer; MD, Molecular Docking; SWP, Swiss Target Prediction.

Ethics approval and consent to participate

As this study does not involve animal and patient experiments, the ethical approval and consent to participate are not applicable.

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Consent for publication

All authors agreed with the content and that all gave explicit consent to submit and publish.

Competing interests

The authors declare no competing interests

Conflicts of interest

There are no conflicts to declare.

Author contribution

All authors have carefully reviewed and given their approval for final version of the manuscript. The all authors contributed equally.

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Data availability

All data generated or analyzed during this study are included in this published article.

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