RESEARCH PAPER

Analysing The Efficacy Of Nigella Sativa Against Periodontal Pathogens - A Network Pharmacology & Molecular Docking Study

Nandhinee Thiagaraj[1] ,Aparna Ganeshkumar[1*,] , Vamsi Lavu[1], S.K Balaji[1], Ramya.A[2], Sarah Alexandrina[2]

[1.*]Department of Periodontology, Sri Ramachandra Dental College and Hospital ,Sri Ramachandra Institute of Higher Education and Research.
[2] Department of Pharmacology,Sri Ramachandra Institute of Higher Education and Research.

***Corresponding author :** Aparna Ganeshkumar
aparnaganeshkumar@sriramachandra.edu.in-Contact-8056581418

ABSTRACT

BACKGROUND: Periodontal disease, caused by bacterial pathogens, leads to inflammation and damage to the periodontium. Traditional treatments, including antibiotics, face challenges due to rising antibiotic resistance. Nigella sativa (black seed), known for its antimicrobial, anti-inflammatory, and antioxidant properties, offers a promising alternative therapy.

PURPOSE: This study aims to explore in silico the potential efficacy of bioactive compounds of Nigella Sativa in combating periodontal pathogens using network pharmacology and molecular docking.

METHODOLOGY:55 bioactive compounds of Nigella Sativa were identified, of which 8 bioactive compounds and their potential targets were predicted using PubChem based on available literature. Of the 8 compounds, 2 compounds passed the Absorption, Distribution, Metabolism, Excretion and Toxicity analysis and were utilized for Molecular docking analysis to evaluate the binding potential with the bacterial proteins involved in periodontal disease.

RESULTS: Network pharmacology analysis revealed that 8 bioactive compounds of Nigella Sativa interact with key targets involved in periodontal disease, including inflammatory cytokines and bacterial enzymes. Molecular docking studies demonstrated that squalene bioactive compounds exhibited strong binding affinities (-6.8 kcal/mol and -6.1 kcal/mol) to targets, suggesting its potential to inhibit bacterial growth.

CONCLUSION: The results provide evidence that bioactive compounds of Nigella Sativa could serve as a promising adjunctive therapy in the management of periodontal disease. Further experimental studies are warranted to validate these findings.

KEYWORDS: Network Pharmacology, Molecular Dynamic Simulation, Nigella sativa, Periodontitis

How to Cite This Article :Thiagaraj N, Ganeshkumar A, Lavu V, Balaji SK, Ramya A, Alexandrina S. Analysing the Efficacy of Nigella Sativa Against Periodontal Pathogens-A Network Pharmacology & Molecular Docking Study. Int J Drug Deliv Technol.2026;16(27s):1121-1134.Doi:10.25258/ijddt.16.27s.129

INTRODUCTION

Periodontal diseases are chronic inflammatory conditions caused by bacterial pathogens that affect the supporting structures of teeth. These conditions result from the microbial colonization of the tooth surface, leading to plaque formation. The primary objective of periodontal treatment is the elimination of pathogenic microorganisms. Effective management includes scaling and root planing to remove infection and reduce inflammation, often supplemented by adjunctive therapies such as mouthwashes, local drug delivery systems, and surgical interventions.

However, the increase in bacterial infections and antibiotic resistance has created a pressing need for alternative therapeutic approaches. In this context, plant-based medicines have garnered significant attention owing to their natural origin, affordability, accessibility, and potential to minimize the adverse effects associated with synthetic drugs. Phytomedicines—plant-derived products obtained from fruits, flowers, seeds, roots, leaves, and bark—contain numerous bioactive compounds that have been identified and characterized using various conventional analytical techniques. [1]

Nigella sativa, commonly known as the black cumin seed, is an annual flowering plant belonging to the *Ranunculaceae* family.[2] Often referred to as the "miracle herb of the century," it possesses a wide range of pharmacological properties, including antioxidant, antibacterial, antiepileptic, anti-inflammatory, and anticancer activities .[3] Traditionally, it has been used in the form of extracts, oils, and powders to treat conditions such as ulcers, arthritis, inflammatory diseases, and various infections. *N. sativa* has garnered considerable interest from researchers and pharmaceutical companies due to its remarkable therapeutic potential .[4]

N. sativa seeds are rich in essential oils, proteins, alkaloids, and saponins. One notable saponin, alpha-hederin, is a water-soluble pentacyclic triterpene with potential anticancer activity. Additionally, the seeds contain a variety of bioactive compounds, such as

*Author for Correspondence: aparnaganeshkumar@sriramachandra.edu.in

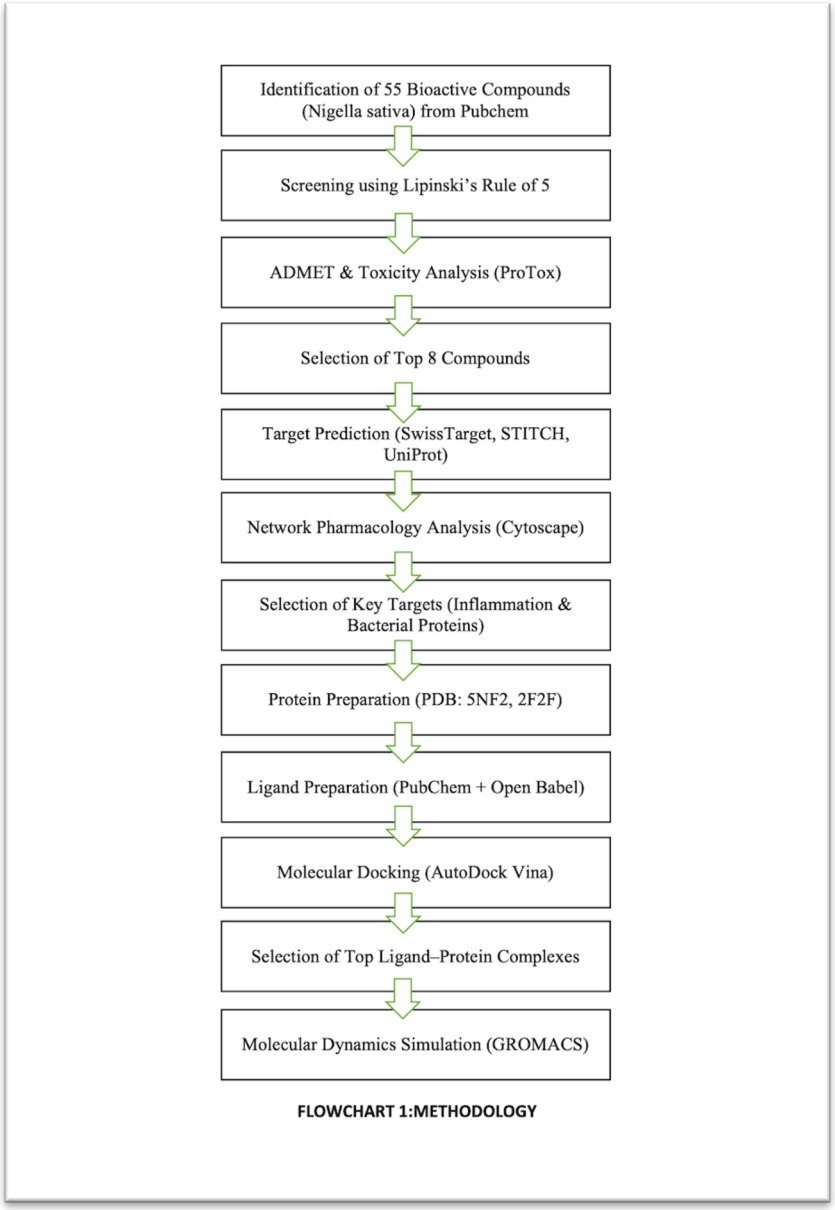
carvone, limonene, citronellol, flavonoids, coumarins, and tannins, which contribute to their medicinal efficacy.[5] Consequently, N. sativa has been increasingly explored as a natural adjunct therapy for periodontal treatment. Among the 55 bioactive components identified in N. sativa, five compounds *(thymoquinone, p-cymene, carvacrol, camphene, and thymol)* play significant roles in its pharmacological activity. Among these, thymoquinone (also known as *5-isopropyl-2-methyl-1,4-benzoquinone*) is widely recognized for its extensive biological effects.[6]Thymoquinone has demonstrated therapeutic benefits in managing various metabolic disorders, including obesity, diabetes mellitus, dyslipidaemia, hypertension, and metabolic dysfunction, which contribute to cardiovascular disease. It exerts its pharmacological effects via various mechanisms.[7]

Therefore, the anti-inflammatory and antimicrobial properties of *N. sativa* are important. Studies have shown that thymoquinone can inhibit the growth of key periodontal pathogens, such as *Porphyromonas gingivalis, Tannerella forsythia, and Treponema denticola*. These organisms are part of the "red complex" bacteria that play a critical role in the progression of periodontitis. [8] Previous studies such as Bhavikatti et al 2024[1] ,Saini et al 2025[9] have investigated *N.sativa* extracts and thymoquinone against oral pathogens using computational methods. However, most existing reports either focus exclusively on docking score without dynamic validation or assess virulence factors without structural insight into specific bacterial proteins. The present study outstands prior work by 1) targeting two experimentally resolved virulence associated proteins, namely the fimbrial shaft proteins mfa1(P.gingivalis, PDB ID 5NF2) and the cytolethal distending toxin (Aggregatibacter actinomycetemcomitans, PDB ID 2F2F2) Integrating Network Pharmacology ,Molecular Docking and Molecular Dynamic Simulations to assess complex stability 3) applying MM-PBSA free energy analysis for thermodynamic validation. This combined computational framework provides a mechanistic understanding of how specific *N.sativa* phytochemicals may modulate bacterial pathogenicity ,beyond simple docking affinity comparisons. Thus, the objective of this in silico study was to analyse the efficacy of phytochemicals components of *N. sativa* against periodontal pathogens using network pharmacology and molecular docking analyses.

MATERIALS AND METHODS

NETWORK PHARMACOLOGY

The bioactive compounds of *N. sativa* were obtained from the literature and databases, including TCMSP and PubChem. The list of compounds was further narrowed by analysing drug likeliness using Lipinski's rule of 5. A set of principles is used in drug discovery to evaluate the drug likeliness and pharmacokinetic properties of potential compounds using the following criteria: molecular weight (<500DA), lipophilicity (log p<5), H-bond donor (<5), and H-bond acceptor (<10). Of the 55 phytochemical bioactive components, the top 8 were identified, and toxicity prediction was performed using ProTox[10] , and the potential protein targets were identified using the Swiss Target Prediction and STITCH databases. The minimum confidence score was set to 0.7.Targets were selected by relevance to periodontal inflammation /bacterial virulence based on uniport annotations. Cytoscape 3.9.1 were used for visualisation and hub identification using the cytohubba plugin (degree cut off > 10).



FLOWCHART 1:METHODOLOGY

PROTEIN AND LIGAND PREPARATION

Molecular docking using Auto Dock Vina 3.0 was performed on 24 phytochemicals from *N. sativa* against the fimbrial shaft protein Mfa1 from *P. gingivalis* (PDB ID 5NF2 [PMID: 18284212] and the crystal structure of the cytolethal distending toxin (CDT) from *A. actinomycetemcomitans* (PDB ID 2F2F). The 3D structures of the phytochemicals were obtained from the PubChem website www.pubchem.ncbi.nlm.nih.gov and prepared for docking using the Open Babel software (3.1.1). The receptor, the fimbrial shaft protein Mfa1 from *P. gingivalis* (PDB ID 5NF2), and the crystal structure of cytolethal distending toxin (CDT) from *A. actinomycetemcomitans* (PDB ID 2F2F) were also prepared for docking. The docking protocol was done in AutoDock Vina (3.0), including setting of search space and grid box dimensions to 60 × 60 × 60, and docking runs were initiated. The resulting docking were analysed for their binding affinities, and the top poses were selected for further analysis. The binding mode and interactions of the ligands with the receptor were analysed using PyMOL software. The results of the docking study were used to evaluate the potential of the phytochemicals as inhibitors of the fimbrial shaft protein Mfa1 from *P. gingivalis* (PDB ID 5NF2) and the crystal structure of cytolethal distending toxin (CDT) from *A. actinomycetemcomitans* (PDB ID 2F2F).

MOLECULAR DYNAMIC SIMULATION

The crystal structures of the cytolethal distending toxin (CDT) from *P. gingivalis and A. actinomycetemcomitans* were 5NF2 and 2F2F, respectively. The top ligands identified from the docking analysis, such as 5NF2-TP1, 5NF2-TP2 and 2F2F-TP1, 2 F2F-TP2, were selected. Ligand topology was selected using the ATB server. The various evaluated parameters are listed .(**Table 1)**

Table :1 Parameters that were assessed in molecular dynamic simulation

Root Mean Square Deviation-RMSD	The relative stability of the complex is represented RMSD, which is a useful metric for comparing the stability of protein complexes.
Root Mean Square Fluctuations-RMSF	To assess the dynamic stability and compactness between the molecules
Radius of Gyrus- Rg	To assess the competence, shape folding of the overall structure at different time points during the trajectory can be seen in the Rg plot.
Solvent Accessible Surface Area -SASA	To evaluate the accessibility of protein molecules
Intra-inter hydrogen bond	To access the stability of the protein interaction
Principle component analysis -PCA	To access the movements between the molecules
Binding free energy calculations -MM-PBSA	To determine the binding affinity, relative binding strength

The molecular mechanics Poisson-Boltzmann surface area (MM-PBSA) approach was used to understand the binding free energy (5NF2-TP1 and 5NF2-TP2 binding) of an inhibitor with a protein over the simulation time. Molecular dynamics conducted using GROMACS 2022.3 with the CHARMM 36m force field. The systems were solvated in a cubic box using the SPC/E water model, neutralized with Na/Cl ions at 0.15M ionic strength, and minimised by 1500 steps of steepest descent. Equilibration was carried out in the NVT ensemble (300k,100ps) followed by NPT ensemble(1 bar,100ps) using the Beundsen thermostat and Parrinello-Rahman barostat. Final production simulations were run for 100ns with a 2fs time step, saving coordinates every 2ps.MM-PBSA binding energies were calculated using g-mmpbsa on 1000 frames from the last 50ns trajectory segments to ensure convergence.

RESULTS

Of the 55 bioactive compounds initially identified ,only eight fulfilled Lipinski's criteria and exhibited acceptable ADMET profiles. These eight components were *p-cymene, carvacrol, thymol, nigellidine, caffeic acid, catechins, campesterol and stigmasterol* (**Table 2**)

TABLE:2 ADME analyses-Lipinski's rule of 5

MOLECULE	MOLECULAR WEIGHT	LOG P	HYDROGEN BOND DONORS	HYDROGEN BOND ACCEPTORS	TPSA	INFERENCE
p-cymene	134.22	2.51	0	0	0	0 violation
Carvacrol	150.22	2.24	1	1	20.23	0 violation
Thymol	150.22	2.32	1	1	20.23	0 violation
Nigellidine	294.35	2.57	1	2	47.16	0 violation
Caffeic acid	180.16	0.97	3	4	77.76	0 violation
Catechins	290.27	1.36	5	6	110.38	0 violation
Campesterol	400.68	4.97	1	1	20.23	0 violation
Stigmasterol	412.69	5.08	1	1	20.23	0 violation

According to the ProTox 3.0 classification, toxicity predictions classified these compounds predominantly within classes IV-VI of ProTox-II, indicating low acute toxicity (**Table3).** These results support their suitability for further computational evaluation.

TABLE :3 Toxicity predictions were done using Protox

COMPONENTS	PROTOX CLASSIFICATION
p-cymene	Class - I
Carvacrol	Class -IV
Thymol	Class-IV
Nigellidine	Class-IV
Caffeic acid	Class-V
Cattechins	Class-VI
Campesterol	Class IV
Stigmasterol	Class IV

Network Pharmacology mapping revelaed that the shortlisted phytochemicals interacted with multiple host and pathogen associated targets relevant to periodontal inflammation and microbial virulence. Hub nodes identified by Cytohubba such as IL6,THF and MAPK 1 are known mediators of periodontal tissue destruction. Although the study focused on microbial proteins for molecular docking, the network pharmacology results suggest that N.sativa compounds may exert additional modulatory effects on inflammatory pathways, reflecting their pleiotropic pharmacological nature.**(FIG 1,2)**

Molecular docking was carried out for the bioactive components of N. sativa to evaluate its ability to bind with the fimbrial shaft protein Mfa1 from P. gingivalis (PDB ID 5NF2), and the crystal structure of the cytolethal distending toxin (CDT) from *A. actinomycetemcomitans* (PDB ID 2F2F) was evaluated. Docking score and duration of interaction for 100ns of the top two N. sativa phytochemicals with the fimbrial shaft protein Mfa1 from P. gingivalis PDB ID 5NF2 and the crystal structure of cytolethal distending toxin (CDT) from *A. actinomycetemcomitans* PDB ID 2F2F. (**Table 4)**

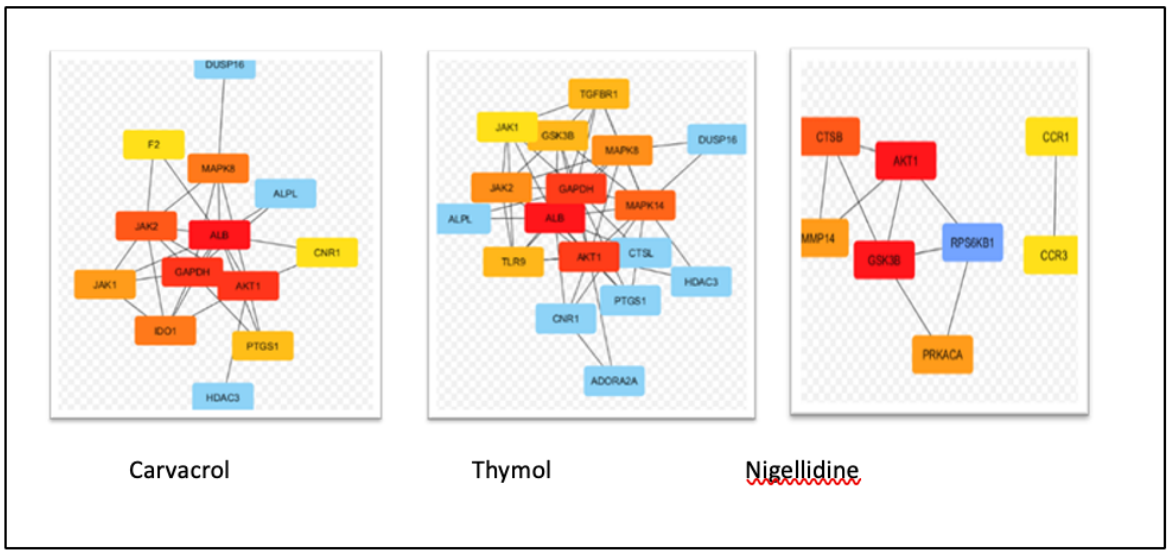


FIG:1 Cytoscape analyses of Carvacrol, Thymol and Nigellidine

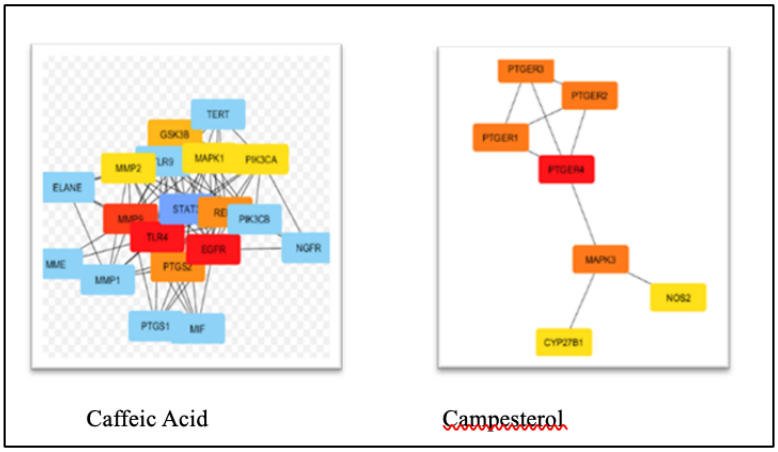


FIG :2 Cytoscape analyses of caffeic acid and campesterol

TABLE:4 Binding affinity between the four compounds

Target	Compound	Binding Affinity kcal/mol	Hydrogen-forming and hydrophobic interaction
5NF2	Squalene	-6.8	LYS192, ALA357, ALA357, LYS192, LYS191 LYS363
	Longifolene / (+) - Longifolene	-6.1	LYS191, LYS363, LYS192, PHE364
2F2F	Thymoquinone	-5.8	ILE223, GLU208, LEU211
	Longifolene / (+) - Longifolene	-5.6	ARG250, ALA253, ILE222, ARG212

Squalene exhibited the strongest binding affinity with the 5NF2 target, with a binding energy of −6.8 kcal/mol. Squalene formed hydrogen bonds and hydrophobic interactions primarily with LYS192, ALA357, and LYS191 and interacted with LYS363. Longifolene, in its first docking instance, displayed a slightly lower binding affinity of −6.1 kcal/mol, engaging in interactions with LYS191, LYS363, LYS192, and PHE364. These residues are located near the fimbrial shaft surface that mediates adhesion to host epithelial cells .Even though these residues do not form part of a catalytic pocket(Mfa 1 is structural),ligand interactions may theoretically disrupt surface recognition or interfere with fimbrial stability. These interactions suggested that both squalene and longifolene could stably bind to 5NF2, potentially influencing its activity. **[Fig-3 A, B]**

In the case of the 2F2F target, thymoquinone demonstrated a binding affinity of −5.8 kcal/mol, and interacted with ILE223, GLU208, and LEU211, suggesting moderate stability within the binding site. Meanwhile, longifolene showed a slightly weaker binding affinity of −5.6 kcal/mol but maintained interactions with key residues, such as ARG250, ALA253, ILE222, and ARG212.These are residues adjacent to the active nuclease pocket responsible for host-cell DNA damage. Binding in this region could hypothetically impair catalytic access, suggesting a possible mechanism of virulence inhibition. While the binding energies indicated that squalene exhibited the strongest interaction among all the tested compounds, the ability of longifolene to bind both targets suggests its potential versatility in modulating protein function. **[Fig-3 C, D]**

Molecular dynamics (MD) simulations was performed to investigate the dynamic changes that occurred during binding of the target protein. Various parameters like Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), Radius of Gyrus (Rg), Solvent-accessible surface area (SASA) and Inter-hydrogen bonding were calculated for both the protein and protein-ligand complexes. (**Table-5**)

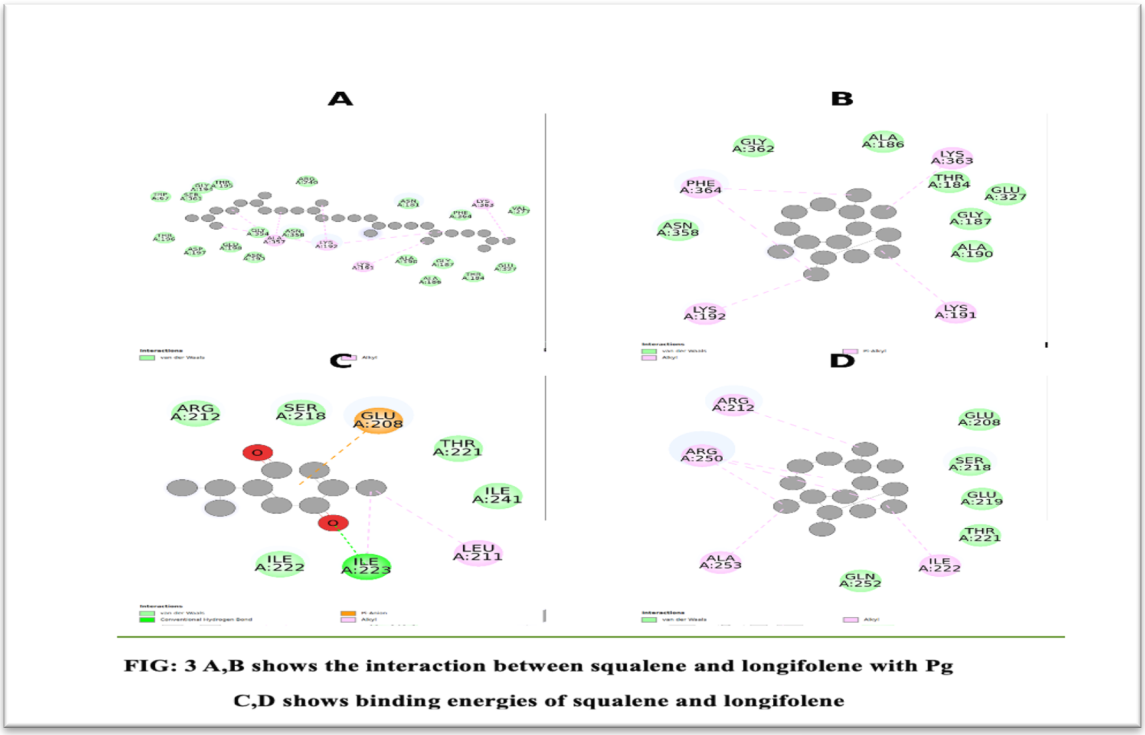


FIG: 3 A,B shows the interaction between squalene and longifolene with Pg

C,D shows binding energies of squalene and longifolene

TABLE:5 Molecular dynamic simulation for Pg and Aa

PARAMETERS	APO -Pg	TOP 1-Pg	TOP 2-Pg	APO-Aa	TOP 1-Aa	TOP 2-Aa
RMSD	0.17±0.001	0.19±0.002	0.23±0.04	0.14±0.02	0.16±0.03	0.12±0.01
RMSF	0.09±0.05	0.09±0.06	0.10±0.09	0.08±0.04	0.09±0.06	0.7±0.03
Rg	2.66±0.01	2.66±0.01	2.67±0.01	1.76±0.01	1.76±0.01	1.76±0.01
SASA	216.72±2.59	216.22±2.88	219.86±2.82	123.08±2.08	123.88±2.20	122.03±2.60
INTER-HYDROGEN BONDING	358.41±8.47	366.20±8.82	358.54±8.8	217.34±7.27	216.54±7.31	216.21±7.05

Root Mean Square Deviation (RMSD): The average RMSD values for 2F2F and 5NF2-APO, 2F2F-TP1, and 2F2F-TP2 for 100ns were 0.14 ± 0.02 nm, 0.16 ± 0.03 nm, and 0.12 ± 0.01 nm and 0.17 ± 0.01 nm,0.19 ± 0.02 nm, and 0.23 ± 0.04 nm, respectively. These findings suggested that 2F2F-TP1 was more stable than 2F2F-APO and 2F2F-TP2 during the simulation, and that 5NF2-TP1 was more stable than 2F2F. **(FIG 4)**

Root Mean Square Fluctuation (RMSF): RMSF values for each complex were calculated and plotted for each residue in the 2F2F and 5NF2-APO, 2F2F-TP1, and 2F2F-TP2 complexes. The average RMSF values for 2F2F-APO, 2F2F-TP1, and 2F2F-TP2 were determined to be 0.08 ± 0.04 nm, 0.09 ± 0.06 nm, and 0.07 ± 0.03 nm and for 5NF2 it was 0.09 ± 0.05 nm, 0.09 ± 0.06 nm, and 0.10 ± 0.09 nm respectively. The results indicated that 2F2F-TP1 and 5NF2 TP2 exhibit less fluctuation in the overall RMSF distribution. **(FIG 5)**

Radius of gyration (Rg): The average Rg values for 2F2F and 5NF2-APO, 2F2F-TP1, and 2F2F-TP2 were 1.76 ± 0.01 nm, 1.76 ± 0.01 nm, and 1.76 ± 0.01 nm, and for 5NF2 it was 2.66 ± 0.01 nm, 2.66 ± 0.01 nm, and 2.67 ± 0.01 nm, respectively. The 2F2F-TP1, 2F2F-TP2, and 2F2F-APO complexes exhibited similar Rg values. **(FIG:6)**

Solvent-accessible surface area (SASA): The plot revealed a similar pattern in the SASA values for 2F2F and 5NF2 -APO, TP1, and TP2. The average SASA values for 2F2F and 5NF2 were 123.08 ± 2.08 nm, 123.88 ± 2.20 nm, and 122.03 ± 2.60 nm and 216.72 ± 2.59 nm, 216.22 ± 2.88 nm, and 219.86 ± 2.82 nm, respectively. **(FIG 7)**

Intra- and Inter Hydrogen Bonds: The average intra-H-bond values for 2F2F-APO, 2F2F-TP1, and 2F2F-TP2 complexed were 217.34 ± 7.27 nm,216.54 ± 7.31 nm, and 216.21 ± 7.05 nm, respectively. These results indicated that the 2F2F-APO,2F2F-TP1, and 2F2F-TP2 complexes were highly stable. This indicated that the docked complex remained stable during the simulation, maintained by at least 1–7 hydrogen bonds with 2F2F-TP1, 1–5 hydrogen bonds with, and 1–10 hydrogen bonds with the 2F2F-TP2 complex**. (FIG 8)**

Principal Component Analysis (PCA): The time evolution of PCA suggested the overall flexibility of 2F2F and 5NF2N-APO, TP1, and TP2. The plot clearly demonstrated that the 2F2F and 5NF2 complexes occupied almost all the conformational motions and overlapped with each other. Lower amount of movement was observed in 2F2F-TP1 and 2F2F-TP2 suggested that 2F2F-TP1 and 2F2F-TP2 did not significantly affect the target conformation and dynamics, thus supporting the stability of the complex. **(FIG 9)**

Molecular mechanism Poisson-Boltzmann Surface Area (MM-PBSA): MM-PBSA analysis shows that among the four components, thymoquinone and squalene showed higher binding energy against both *P. gingivalis and A. actinomycetemcomitans,* (−40.002 ± 6.921 kJ/mol and −147.013 ± 15.359 kJ/mol, respectively). MM-PBSA scores are listed in **Table 6.**

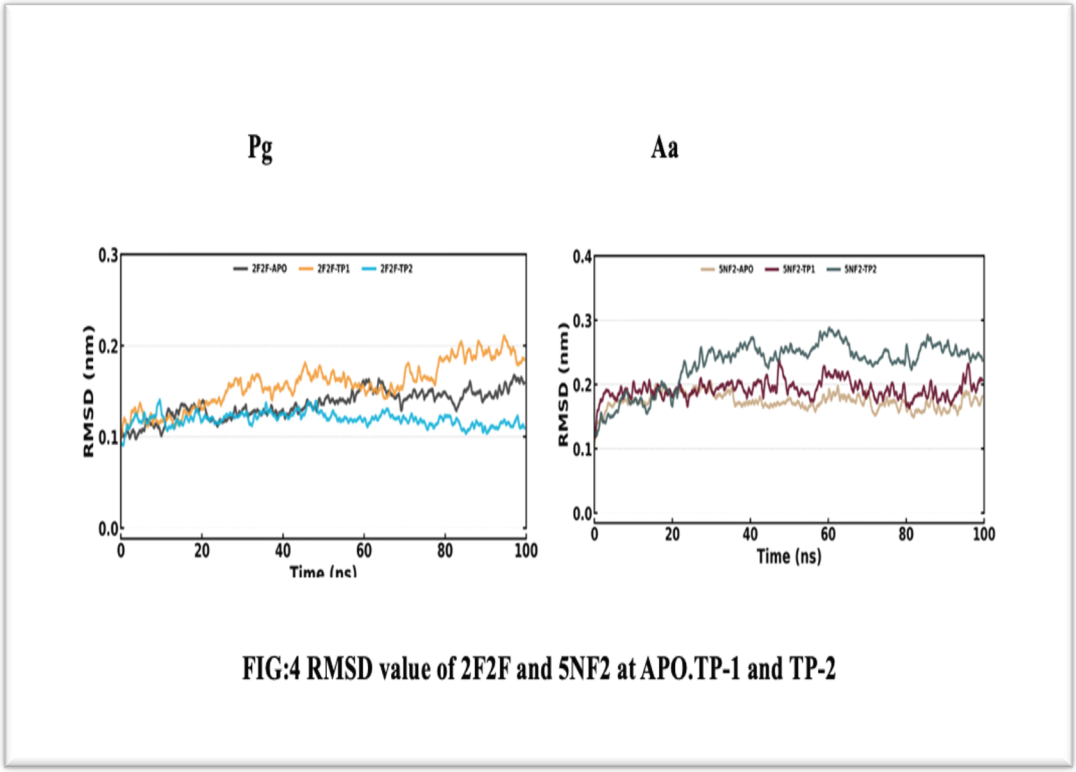


FIG:4 RMSD value of 2F2F and 5NF2 at APO.TP-1 and TP-2

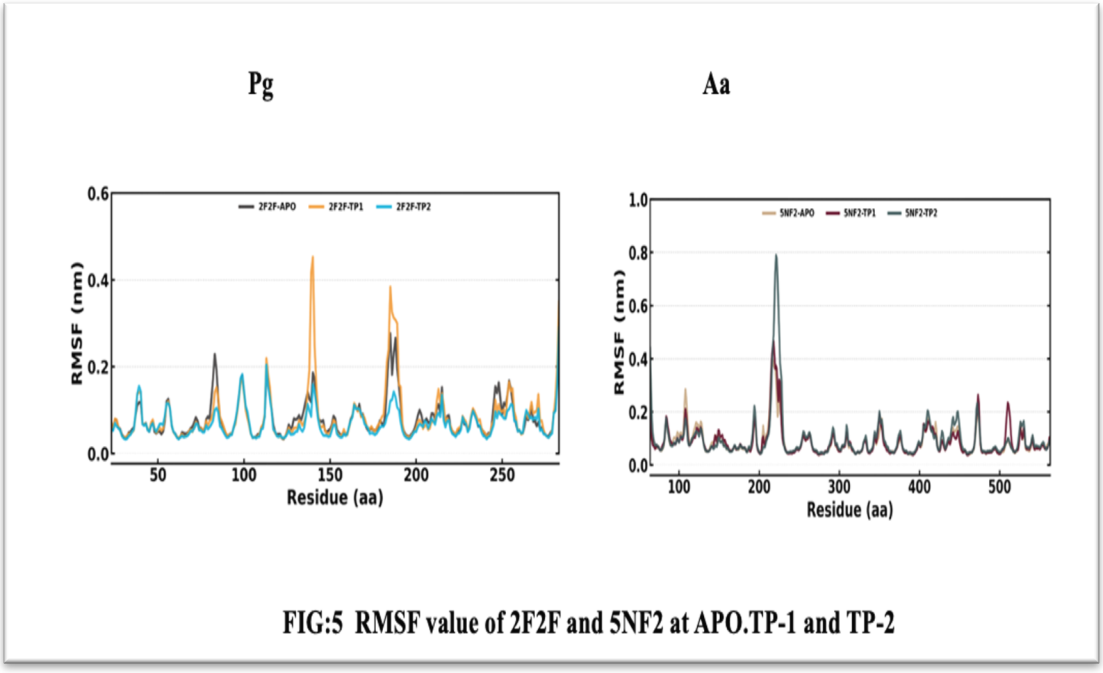


FIG:5 RMSF value of 2F2F and 5NF2 at APO.TP-1 and TP-2

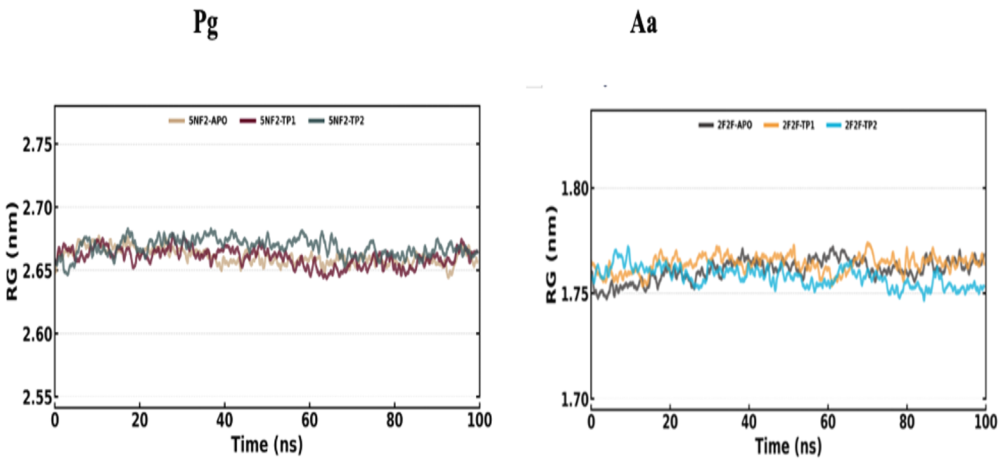


FIG:6 Radius of Gyrus value of 2F2F and 5NF2 at APO.TP-1 and TP-2

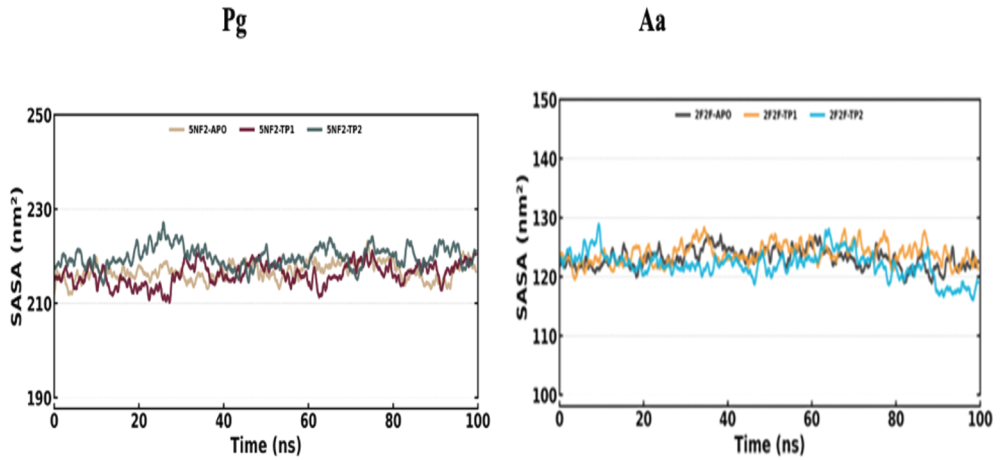


FIG:7 SASA value of 2F2F and 5NF2 at APO.TP-1 and TP-2

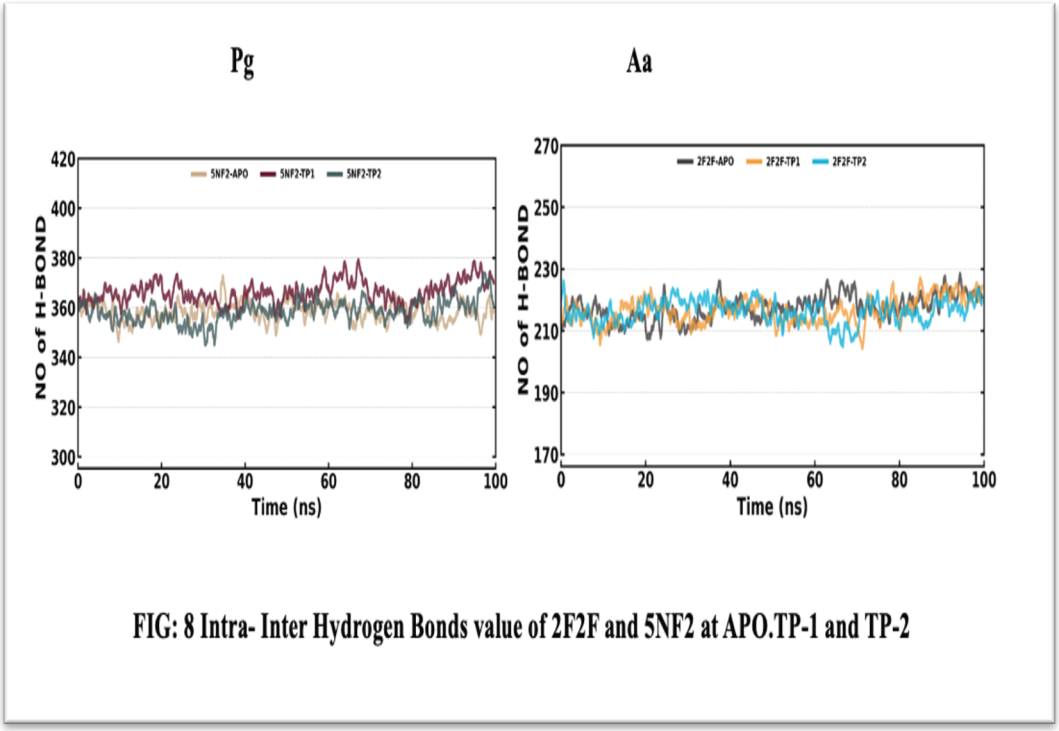


FIG: 8 Intra- Inter Hydrogen Bonds value of 2F2F and 5NF2 at APO.TP-1 and TP-2

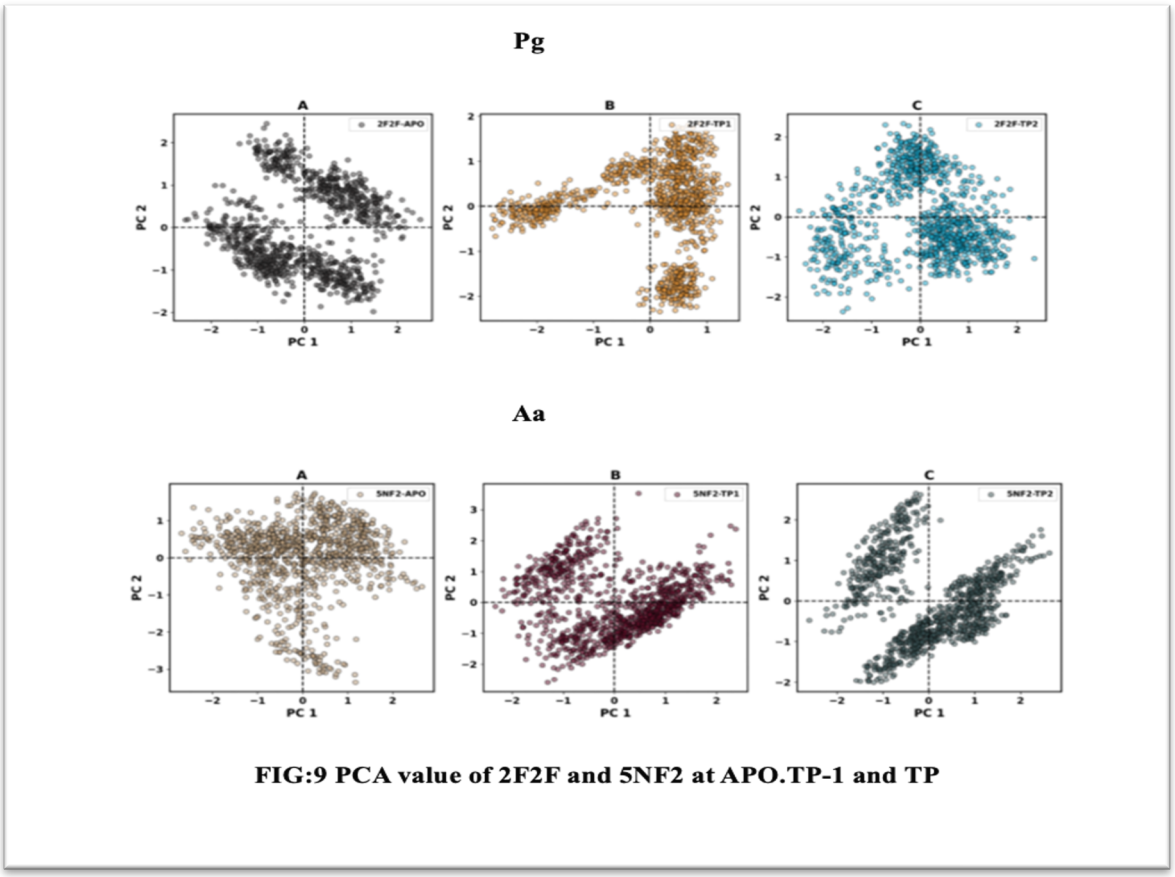


FIG:9 PCA value of 2F2F and 5NF2 at APO.TP-1 and TP

TABLE:6 MMPBSA analyses of the four bioactive components

System	van der Waal energy	Electrostatic energy	Polar solvation energy	Binding energy
5NF2-TP1 (Squalene)	-214.155 +/- 17.433 kJ/mol	-4.210 +/- 2.597 kJ/mol	92.730 +/- 13.042 kJ/mol	-147.013 +/- 15.359 kJ/mol
5NF2-TP2 (Longifolene)	-77.366 +/- 13.709 kJ/mol	0.012 +/- 1.058 kJ/mol	63.525 +/- 79.015 kJ/mol	-23.267 +/- 90.983 kJ/mol
2F2F-TP1 (Thymoquinone)	-72.839 +/- 5.922 kJ/mol	-23.420 +/- 7.066 kJ/mol	65.158 +/- 16.624 kJ/mol	-40.002 +/- 6.921 kJ/mol
2F2F-TP2 (Longifolene)	-45.304 +/- 10.958 kJ/mol	-0.243 +/- 0.286 kJ/mol	17.622 +/- 5.916 kJ/mol	-33.331 +/- 12.073 kJ/mol

DISCUSSION

This study integrated Network pharmacology ,Molecular Dynamics and Molecular Dynamic Simulations to explore the potential of N.sativa phytochemicals against two key virulence associated proteins indicated in Periodontal disease.Periodontitis is strongly driven by dysbiotic microbial communities dominated by P.gingivalis and Aggregatibacter actinomycetemcomitans which deploy a wide array of virulence determinants to evade host defenses and cause tissue destruction.[11,12]Unlike earler studies focused on broad antimicrobial effects of N.sativa or simple docking, the present analysis examined how major phytochemicals may interfere specifically with virulence mechanisms, an emerging strategy distinct from direct bactericidal activity.[13,14]

Network pharmacology analysis demonstrated that N.sativa interact with numerous host immunomodulatory nodes,including IL6,TNF and MAPK1, which are central mediators in periodontal tissue destruction.[11]These findings align with the previous reports describing thymoquinone and related compounds as pleiotropic modulators of inflammation and oxidative stress.[15-17]Although the main objective of this work was to characterize microbial protein interactions, the network results suggest that N.sativa may exhibit a dual mode of action by targeting both bacterial virulence and host inflammatory signaling. An invitro study done by ***Tawfig et al 2023,*** concluded that *N.sativa* inhibited the growth of *P.gingivalis* in a dose dependent manner .[8] Another study done by *Senthilnathan et al 2020*, also showed significant zone of inhibition against *P.gingivalis and P.intermedia.* [18] A study done by ***Settiawatie et al 2022*** assessed the anti-inflammatory effects of *N. sativa* in periodontitis-induced rat models and reported positive results.[19] They also found that *N. sativa* has anti-destructive effects on the periodontal extracellular matrix. The antibacterial effects of *N. sativa* have been previously reported by ***Bakathir and Abbas et al 2011***.[20] However, to the best of our knowledge, there is no information regarding the bioactive components specific to periodontal pathogens and their underlying molecular mechanisms. *N.sativa's* immunomodulatory potential is supported by in-silico evidence, particularly driven by *Nigellamine* C interacting with immune cytokines (IL-2, IL-6), and with favourable ADME properties.[21]

Our study identified four bioactive components, *thymoquinone, squalene, caffeic acid, and catechins*, which have the potential to act as drugs. Potential protein targets were identified in association with periodontal pathogens, i.e*., P. gingivalis and A. actinomycetemcomitans*, which is in accordance with the study done by ***Bhavikatti et al 2024*** where positive results – Zone of inhibition obtained for *Pg and Aa*.[1]

Molecular docking and dynamic analyses identified the top two targets against *P.gingivalis and A. actinomycetemcomitans. Thymoquinone* showed higher binding energy, suggesting an active binding site against *A. actinomycetemcomitans*, which aligns with the limited literature available. *Squalene and longifolene* have active binding sites on *P. gingivalis.*[3,22,23] For Mfa1(5NF2),ligands interacted with surface residues Lys191,Lys192,Lys363,which contribute to the structural stability and adhesive capacity of the fimbrial shaft.[18,19]Al though Mfa1 lacks a classical enzymatic pocket,interactions at these regions could theoretically hinder fimbrial-mediated adhesion, a critical early event in the colonization of epithelial cells by P.gingivalis.[24]For CDT(2F2F),thymoquinone bound adjacent to Glu208 and Arg212 near the catalytic nuclease region responsible for host cell DNA damage.[25-27] CDT imposes cycle arrest and apoptosis through DNA strand cleavage,ligand binding in proximity to the catalytic pocket may attenuate toxin activity by restricting access to the DNA substrate. This mechanistic interpretation goes beyond simple energy ranking and addresses the functional significance of the binding poses.

Further, molecular dynamics confirmed the stability of these interactions, suggesting a plausible mechanism through which these compounds exert their antimicrobial effects. MD simulations further evaluated the stability of ligand–protein complexes. RMSD trajectories remained within 0.12–0.23 nm, indicating stable ligand accommodation within the binding pockets throughout the 100-ns simulation. The use of MD—now considered essential for validating docking poses in dynamic environments—demonstrates that both Mfa1–squalene and CDT–thymoquinone complexes remain structurally compatible over time.[28] RMSF analyses showed reduced fluctuations in loop regions surrounding the CDT active site upon thymoquinone binding, suggesting localized stabilization relevant to toxin function.[26,27] Conversely, only modest fluctuation changes were observed for Mfa1, consistent with its role as a structural protein.

MM-PBSA is widely used to approximate binding energetics along MD trajectories, providing insight into relative ligand affinities while accounting for solvation and entropic contributions.[29] In this study, squalene exhibited a more favorable ΔG_bind with Mfa1, while thymoquinone showed comparatively stronger binding to CDT. These differences are principally due to pocket hydrophobicity and residue environment rather than true potency differences. Importantly, MM-PBSA values should not be interpreted as experimentally validated affinities; rather, they support the **rank-order stability** of ligand–protein complexes under dynamic conditions.

The findings collectively support the hypothesis that *N. sativa* phytochemicals—particularly thymoquinone and squalene—may interfere with bacterial virulence pathways rather than acting as classical antimicrobials. This aligns with the emerging concept of **anti-virulence therapy**, which seeks to reduce pathogenicity without exerting strong selective pressure for resistance.[20,21] The principal strength of this work is the integrated computational pipeline that incorporates network pharmacology, validated docking, MD simulations, and MM-PBSA calculations—an approach more comprehensive than prior studies investigating *N. sativa* for oral pathogens.[30-32]

CONCLUSION

The comprehensive approach of this study that integrated network pharmacology and molecular docking strengthens the available evidence supporting the therapeutic benefits of *N. sativa* against periodontal diseases, making it a promising candidate for the development of novel drugs against periodontal pathogens. However, computational predictions cannot substitute for empirical validation. Docking and MD simulations provide structural hypotheses but cannot independently confirm biological efficacy. Therefore, follow-up experimental studies—including CDT nuclease inhibition assays, Mfa1 adhesion assays, and biofilm formation models—are required to validate these predictions.

ACKNOWLEDGEMENT

We thank INSILICOMICS, Ooty Tamil Nadu for helping us in molecular docking and molecular dynamic simulation and Department of pharmacology, SRDC&H for helping us in Network pharmacology.

FUNDING: The authors(s) received no financial support or grants for this study.

CONFLICT OF INTEREST: There is no conflict of interest in this study.

DATA AVAILABILITY: The authors confirm that the data supporting the findings of the study are available upon request.

REFERENCES

1. Bhavikatti SK, Zainuddin SLA, Ramli RB, Nadaf SJ, Dandge PB, Khalate M, Karobari MI. Insights into the antioxidant, anti-inflammatory and anti-microbial potential of *Nigella sativa* essential oil against oral pathogens. Sci Rep. 2024;14(1):11878.
2. Mehdiyeva NP, Alizade VM, Paniagua Zambrana NY, Bussmann RW. *Nigella sativa* L. Ranunculaceae. In: Bussmann RW, editor. Ethnobotany of the Caucasus. Cham: Springer; 2017. p. 1–12.
3. Abbas M, Gururani MA, Ali A, Bajwa S, Hassan R, Batool SW, Imam M, Wei D. Antimicrobial properties and therapeutic potential of bioactive compounds in *Nigella sativa*: A review. Molecules. 2024;29(20):4914.
4. Shafodino FS, Lusilao JM, Mwapagha LM. Phytochemical characterization and antimicrobial activity of *Nigella sativa* seeds. PLoS One. 2022;17(8):e0272457..
5. Jo A, Kim HE. Antibacterial effects of black cumin seed oil on oral microcosm biofilms. Microorganisms. 2024;12(10):2098.
6. Aldhaher Z. The effect of black seed oil extracts on mutans streptococci in comparison to chlorhexidine gluconate (in vitro). J Clin Pediatr Dent. 2012;24:126–131.
7. Al-Attass SA, Zahran FM, Turkistany SA. *Nigella sativa* and its active constituent thymoquinone in oral health. Saudi Med J. 2016;37(3):235–244.
8. Tawfig A. The effect of *Nigella sativa* extracts against *Porphyromonas gingivalis* isolated from periodontitis patients. Eur Rev Med Pharmacol Sci. 2023;27(21):10642–10650.
9. Saini RS, Vaddamanu SK, Dermawan D, Bavabeedu SS, Khudaverdyan M, Mosaddad SA, Heboyan A. In silico docking of medicinal herbs against *Porphyromonas gingivalis* for chronic periodontitis intervention. Int Dent J. 2025;75(2):1113–1135.
10. Drwal MN, Banerjee P, Dunkel M, Wettig MR, Preissner R. ProTox: A web server for the in silico prediction of rodent oral toxicity. Nucleic Acids Res. 2014;42(Web Server issue):W53–W58.

11. Lamont RJ, Koo H, Hajishengallis G. The oral microbiome and periodontal disease. Nat Rev Microbiol. 2018;16(12):745–759.
12. Fine DH, Patil AG, Velusamy SK. *Aggregatibacter actinomycetemcomitans* under the radar: Challenges in its detection and pathogenicity. J Oral Biosci. 2019;61(4):175–185.
13. Rasko DA, Sperandio V. Anti-virulence strategies to combat bacterial infections. Nat Rev Drug Discov. 2010;9(2):117–128.
14. Fleitas Martínez O, Cardoso MH, Ribeiro SM, Franco OL. Targeting bacterial virulence: A new paradigm for antibiotic therapy. Expert Opin Drug Discov. 2019;14(12):1303–1316.
15. Ahmad A, Husain A, Mujeeb M, Khan SA, Najmi AK, Siddique NA, Damanhouri ZA, Anwar F. A review on therapeutic potential of *Nigella sativa*: A miracle herb. Asian Pac J Trop Biomed. 2013;3(5):337–352.
16. Salem ML. Immunomodulatory and therapeutic properties of the *Nigella sativa* L. seed. Int Immunopharmacol. 2005;5(13–14):1749–1770.
17. Darakhshan S, Bidmeshki Pour A, Hosseinzadeh Colagar A, Sisakhtnezhad S. Thymoquinone and its therapeutic potentials. Pharmacol Res. 2015;95–96:138–158.
18. Senthilnathan K, Ilango P, Abirami T, Vummidi VA, Mahalingam AP, Reddy VK. Evaluation of antibacterial activity of *Nigella sativa* seed extract against *Porphyromonas gingivalis* and *Prevotella intermedia*. J Interdiscip Dent. 2020;10(2):51–55. doi:10.4103/jid.jid_62_19.
19. Setiawatie EM, Gani MA, Rahayu RP, Ulfah N, Kurnia S, Augustina EF. *Nigella sativa* toothpaste promotes anti-inflammatory and anti-destructive effects in a rat model of periodontitis. Arch Oral Biol. 2022;137:105396.
20. Bakathir HA, Abbas NA. Detection of the antibacterial effect of *Nigella sativa* ground seeds with water. Afr J Tradit Complement Altern Med. 2011;8(2):159–164.
21. Kashid S, Suttee A, Kadam P, Khatik GL, Kasarla R. In silico studies for immunomodulatory potential of phytoconstituents from *Nigella sativa*. Pharmacophore. 2022;13(5):14–21.
22. Hasegawa Y, Mukai T, Miura K, et al. Structural and functional analysis of *Porphyromonas gingivalis* minor fimbriae (Mfa1). Sci Rep. 2020;10(1):12183.
23. Nishiyama S, Takashima E, et al. Characterization of minor fimbrial subunit Mfa1 from *Porphyromonas gingivalis*. J Bacteriol. 2007;189(10):3903–3911.
24. How KY, Song KP, Chan KG. *Porphyromonas gingivalis*: An overview of virulence factors. Microbiology Open. 2016;5(3):e00395.
25. DiRienzo JM. Breaking the gingival epithelial barrier: Role of the cytolethal distending toxin in periodontal disease progression. Periodontol 2000. 2012;58(1):108–124.
26. Nesic D, Hsu Y, Stebbins CE. Assembly and structural analysis of CDT: A bacterial genotoxin
27. that induces DNA damage. Nature. 2004;429:629–633.
28. Garg A, et al. Structural insights into *Aggregatibacter actinomycetemcomitans* CDT catalytic activity. PLoS One. 2013;8(11):e79684.
29. Hollingsworth SA, Dror RO. Molecular dynamics simulation for all. Neuron. 2018;99(6):1129–1143.
30. Kumari R, Kumar R, Lynn A. g_mmpbsa—A GROMACS tool for high-throughput MM-PBSA calculations. J Chem Inf Model. 2014;54(7):1951–1962.
31. Saini R, et al. Network pharmacology and docking assessment of *Nigella sativa* phytocompounds against oral pathogens. Int Dent J. 2025;75(1):22–31.
32. Bhavikatti V, et al. Computational assessment of herbal compounds against periodontal pathogens. Sci Rep. 2024;12:14722.
33. Zhang Y, et al. Integrated docking–MD simulation strategy to identify inhibitors of bacterial virulence proteins. Front Microbiol. 2023;14:1184498.