Discoveries Journals
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DISCOVERIES REPORTS 2024, 7: e42
DOI: 10.15190/drep.2024.1

REVIEW Article

Evaluation of Phytochemicals of Nigella sativa as Natural Antagonists of Salmonella typhi OmpF: Pharmacokinetic Analysis and Molecular Docking

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Submitted: Mar 03, 2023; *Revised:* Oct 02, 2023*; Accepted:* Jan 28, 2023; *Published:* Jan xx, 2024
Citation: Mehmood H, Ali U, Bashir I, Sarwar M, Ijaz F, Sohail H. Evaluation of Phytochemicals of Nigella sativa as Natural Antagonists of Salmonella typhi OmpF: Pharmacokinetic Analysis and Molecular Docking. *Discoveries Reports* 2024; 7: e42. DOI: 10.15190/drep.2024.1

ABSTRACT

Life threatening conditions such as typhoid fever and acute severe gastro-enteritis are caused by Salmonella typhi and Salmonella enteritidis. The injudicious use of antibiotics has resulted in the development of resistance to antibiotics amongst various bacterial strains of Salmonella. The Outer membrane porin F (OmpF) is a surface molecule shown to be involved in the multiple drug resistance of these bacteria. Due to increasing drug resistance, there is a need to discover new antibiotics to treat S. typhi. Nigella sativa is an annual flowering plant that is known to exhibit multiple pharmacologic properties including vast anti-microbial properties also against Salmonella species.

This study utilized *in-silico* analytic tools such as molecular docking and adsorption, distribution, metabolism, excretion (ADME) analysis to find out the efficacy of phytochemicals as natural antagonists against Salmonella typhi OmpF and compared them with the commercially available azithromycin and ciprofloxacin.

The phytochemicals of N. sativa exhibited optimum pharmacologic properties comparable to the commercially available azithromycin and ciprofloxacin. The best binding scores were obtained by Nigellidine i.e. -5.98 kCal/mol, higher compared to Azithromycin and Ciprofloxacin, followed by Nigellicine i.e. -5.52 kCal/mol.

To conclude, the phytochemicals of N. sativa especially Nigellicine and Nigellidine show a good inhibitory potential against the Salmonella typhi OmpF and have optimum pharmacokinetic properties thus exhibiting a significant potential to be used as drugs against Salmonella typhi infection. Further *in vitro* and animal-based studies are required to better evaluate the pharmacologic potential of these phytochemicals in Salmonella infection.

Keywords

OmpF, N. sativa, in-silico, molecular docking, antibiotic, Nigellicine, Nigellidine.

Abbreviations

Outer-membrane protein F (OmpF); Extensively Drug Resistant (XDR); Python Molecule Viewer (PMV); Molecular Graphics Library (MGL); Protein Data Bank (PDB); Grid Parameter File (GPF); Dock Parameter File (DPF); Lamarckian Genetic Algorithm (LMA); Adsorption, Distribution; Metabolism, Excretion, and Toxicity (ADMET);

Topological Surface Area (TPSA); Blood Brain Barrier (BBB); Gastrointestinal (GI); Cytochrome P (Cyp).

INTRODUCTION

Salmonella are gram-negative, motile, non-spore forming rods belonging to the family *Enterobacteriaceae*. They are transmitted in humans through feco-oral route and are pathogenic. Life threatening conditions such as typhoid fever and acute severe gastro-enteritis leading to sepsis are caused by *Salmonella typhi* and *Salmonella enteritidis*[1]. The incidence is higher in extremes of age and in immunocompromised people with a disease burden of almost 21 million new cases and 200,000 deaths reported every year[2].

The injudicious use of antibiotics has resulted in the development of resistance to antibiotics amongst various bacterial strains of *Salmonella* which has contributed to increased disease severity and left the physicians with limited treatment options. An epidemic of Extensively Drug Resistant Typhoid (5372 XDR Typhoid) was reported in Pakistan (2016-18) where the isolates of *Salmonella typhi* were resistant to all recommended antibiotics for typhoid. These strains were found susceptible only to Azithromycin and Ciprofloxacin. Thus, this left the treating physicians to use an extensive antimicrobial therapy with a greater urge on preventive measures such as vaccination, improved sanitation and improvising water and food safety measures[3].

H58 lineage of *Salmonella* is highly associated with multiple drug resistance and declining susceptibility to fluoroquinolones[4]. As a result of this emerging drug resistance in these highly pathogenic bacteria, there is a great urge to discover and develop therapeutic agents effective against these species.

Nigella sativa (Black cumin/Kalvanji) is a flowering plant that has been extensively used in traditional and folk medicine to treat various ailments since about 2000 years. It was used as an appetizer, diuretic, expectorant, purgative, carminative, sudoriferous, stimulant and sedative. Historically, the seeds of this plant were used in Arabic culture to treat various skin and gastrointestinal pathologies, jaundice, arthritis, conjunctivitis, hemorrhage, gynecological abnormalities, arthritis, cough, fever, respiratory pathologies, headache, and paralysis. A large number of *in vitro* studies and clinical trials published in recent years describe various pharmacological properties of *N. sativa* including anti-microbial, anti-cancer, anti-histaminic, anti-inflammatory, gastroprotective, hepatoprotective, anti-tussive, cardio-protective, augmentation of fertility and lactation, anti-dermatophyte, and antiviral properties[5]. Some studies[6-8] have also shown the anti-microbial efficacy of phytochemicals of N. sativa against *Salmonella* species.

OmpF is an integral membrane protein that is involved in the transport of ions, nutrient molecules, and export of waste products[9, 10]. Various studies have reported that alteration and mutations in OmpF structure is associated with drug resistance in Salmonella typhimurium[11-13].

Molecular docking uses binding affinities to predict protein-ligand interactions, conformations, and orientations and is a widely used screening tool in drug discovery and drug design[14]. Using the same, we intend to evaluate the efficacy of phytochemicals of N. sativa against the Ompf protein target of *Salmonella typhi*. This would help to understand the mechanism of action at the molecular level and might help to develop new anti-microbial therapy against *S. typhi.*

MATERIALS AND METHODS

3D structure of OmpF of *S. typhi* was retrieved from Protein Data Bank (ID: 4KR4). It was opened in Python Molecular Viewer (PMV, MGL tools), and the already attached inhibitor Ampicillin, and HETATM were removed. The molecule was checked for missing atoms and was repaired. The identical A and B chains were removed. Chain C was selected as the target molecule. Water molecules were removed, polar hydrogens were added. Kollman charges were added, Gasteiger charges were computed and the residual charges were spread over the molecule. The finally processed target molecule was saved in pdbqt format.

The chemical structures of phytochemicals of N. sativa and commercially available drugs for typhoid fever were retrieved from the PubChem Database. Energy minimization and ligand preparation was done using Avogadro software[15] and the ligands were saved in pdb format for further analysis.

The computational studies were carried out using Autodock 4[14] (The Scripps Research Institute) with the extension suite to the PMVs. The finally processed target molecule and ligand were imported. Then, the grid parameter file (gpf) and docking

parameter file (dpf) were prepared. 50 independent runs were set using Lamarckian genetic algorithm (LMA). The resulting docking conformations were analyzed using Discovery Visual Studio and Python Molecule Viewer for the binding scores with the least binding scores referring to the best affinity to the target molecule.

The drug-likeness of the phytochemicals of *N. sativa* based on adsorption, distribution, metabolism, excretion, and toxicity (ADMET) properties was computationally evaluated using the online SwissADME platform[16].

RESULTS

In this study, a total of 8 compounds were docked against Salmonella OmpF protein, out of which 6 were phytochemicals from Nigella sativa and 2 were commercially available drugs used in Salmonella infection i.e. Azithromycin and Ciprofloxacin. The best binding scores were obtained by Nigellidine i.e. -5.98 kCal/mol, followed by Nigellidine i.e. -5.52 kCal/mol, which are both higher binding energies than obtained by Azithromycin and Ciprofloxacin. Table 1 summarizes the binding energies and interactions of all the compounds docked in this study. Figure 1 gives 2D illustrations of the molecular interactions of all the tested compounds with OmpF.

The molecular and pharmacokinetic properties of the compounds were analyzed using SwissADME. The analyzed parameters included the lipophilicity, solubility, topological polar surface area (TPSA), Gastrointestinal (GI) absorption, Blood Brain Barrier (BBB) permeability, skin permeability, inhibition of various cytochromes, p-glycoprotein and bioavailability score. All the phytochemicals exhibited significant pharmacologic properties comparable to the commercially available azithromycin and ciprofloxacin. The results are summarized in Table 2 for all the compounds docked and analyzed in this study.

DISCUSSION

Bacterial infections such as *Salmonella* are becoming increasingly difficult to treat because of the bacteria developing resistance against commonly used antibiotics[4]. Our results from molecular docking of various phytochemicals of nigella suggest that these compounds bind well to the OmpF of *Salmonella* and exhibit a significant binding score and thus have a good inhibitory potential. These binding scores are

Table 1. Binding Energy, Inhibitory Potential (kI), Interactive Residues, and Bond Length of the compounds docked in the current study.

Sr. No	Compound Name	Binding Energy (kCal/mol)	kI	Interactive Residues	Bond Length (A)
1.	CID 10281 Thymoquinone	-4.71	351.17 uM	C 114, C 108, C 112	1.81, 3.80, 4.12
2.	CID 10364 Carvacrol	-4.03	1.11 mM	C 114, C 111, C 112	2.07, 2.94, 3.99
3.	CID 2764 Ciprofloxacin	-5.6	78.77 uM	C 130, C 101, C 108, C 121, C 108	3.57, 3.00, 2.38, 2.14, 3.22
4.	CID 21368 Nigelline	-3.49	2.77 mM	C 110, C 111, C 114, C 101, C 108	3.57, 2.87, 3.15, 1.93, 2.86
5.	CID 136828302 Nigellidine	-5.98	41.03 uM	C 111, C 101, C 129, C 108	3.27, 2.37, 1.96, 3.71
6.	CID 11402337 Nigellicine	-5.52	89.31 uM	C 111, C 114, C 20, C 108	2.84, 2.00, 2.67, 3.39
7.	CID 7463 P-Cymene	-3.39	1.31 mM	C 108, C 112	4.04, 3.63
8.	CID 447043 Azithromycin	-5.91	46.65 uM	C 101, C 42	3.45, 4.55

*Bolded blue font shows a conventional Carbon-Hydrogen covalent bond, Bolded black font shows a conventional Hydrogen bond, non- bolded font suggests a bond of lesser strength (e.g. Pi-alkyl or Pi-anion bond), CID (Chemical Identity Number PubChem), C refers to Carbon.

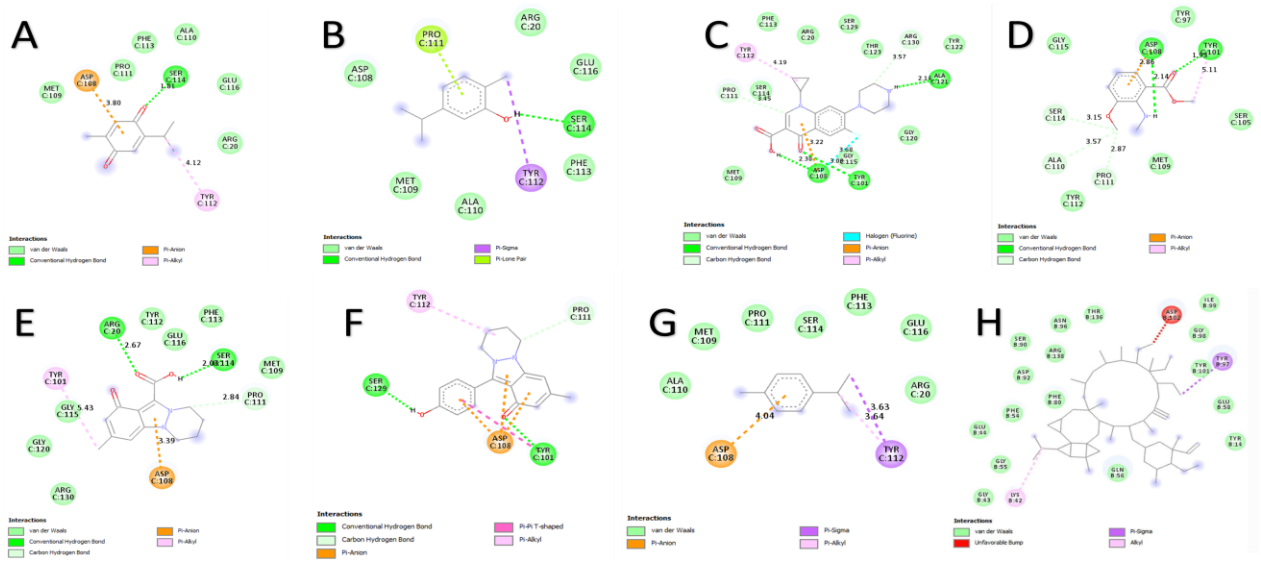

Figure 1. *Molecular interactions of the docked compounds with OmpF*. (A) Thymoquinone with OmpF. (B) Carvacrol with OmpF. (C) Ciprofloxacin with OmpF. (D) Nigelline with OmpF. (E Nigellidine with OmpF. (F) Nigellicine with OmpF. (G) p-cymene with OmpF. (H) Azithromycin with OmpF.

higher in comparison the commercially available drugs against *Salmonella* infection i.e., azithromycin and ciprofloxacin. The alkaloids of N. sativa, viz. Nigelline, Nigellicine, and Nigellidine make covalent and hydrogen bonds with the binding site of OmpF (as shown in the results section) and thus have a greater potency to inhibit the compound. They also have a low inhibitory concentration (kI) thus further advocating their therapeutic potential against *Salmonella* OmpF. Our results thus supplicate the previous studies which have also shown the efficacy of N. sativa oil against various strains, including the drug-resistant strains of *Salmonella*[6, 7, 8].

The analysis of pharmacokinetic properties of the compounds show that the phytochemicals of N. sativa have optimal properties to be used as pharmacological agents. For instance, an XLGOP3 score (which is an indicator of lipophilicity) between -0.7 and +5.0, a molecular weight between 150-500 g/mol, a topological surface area (TPSA, an indicator of polarity) between 20 and 130 A^2, a log S value (indicator of solubility) less than 6.0, fraction of carbons in sp^3 (cp3 score) higher than 0.25 and number of rotatable bonds less than 9; suggest a stable, and orally bioavailable compound[16]. All the phytochemicals of N. sativa analyzed in this study were in this optimal range and thus have a significant potential to be used as a drug. Furthermore, all the phytochemicals except p-cymene show a high gastrointestinal absorption and a brain-blood-barrier permeability. Also, the analysis of the inhibitory potential of these compounds against various cytochromes and p-glycoprotein also show comparable properties to ciprofloxacin and azithromycin (as shown in the Table 2).

Previous studies have reported that Ethanol and n-hexane extracts of the N. sativa exhibit significant dose-dependent antimicrobial activity against various gram-negative and gram-positive strains including *E. coli, B. cereus, B. subtilis, S. epidermidis, K. pneumonia, S. typhimurium*. Furthermore, methanol and water extracts of *N. sativa* have been reported to have remarkable activity against *S. pyogenes, P. aeruginosa, K. pneumonia,* and *P. vulgaris*[17,18], thus implying the inhibitory potential of phytochemicals of N. sativa against *Salmonella typhi* infections. Further *in vitro* and animal-based studies are required to better evaluate the pharmacologic potential of these phytochemicals in *Salmonella* infection.

Limitations

As with all computational studies, our study also carries certain limitations. Though chemical interactions simulated using molecular docking provide a good screening and exclusion basis to drug discovery and drug design but their potential efficacy, interactions, and side effects in humans and animals always remain questionable for which *in-vitro*, animal based, and human based studies are recommended.

Table 2. *Druglike properties analysis* and Absorption, Distribution, Metabolism and Excretion (ADME) profiling of the docked compounds using SwissADME. CYP refers to the cytochrome P, p-gp refers to the p-glycoprotein, BBB refers to the blood brain barrier.

Properties	1	2	3	4	5	6	7	8
Mol. Weight	164.2 g/mol	150.22 g/mol	331.34 g/mol	195.22 g/mol	294.35 g/mol	246.26 g/mol	134.22 g/mol	748.98 g/mol
Rotatable bonds	1	1	3	4	1	1	1	7
TPSA	34.14	20.32	74.57	47.56	47.16	64.23	0.00	180.08
XLGOP3	2.20	3.49	-1.08	2.58	2.93	1.39	4.10	4.02
Fraction of Csp3	0.40	0.40	0.41	0.30	0.28	0.38	0.40	0.97
Log S	-2.18	-3.31	-1.32	-2.73	-3.95	-2.55	-3.63	-6.55
Solubility Class	Soluble	Soluble	Soluble	Soluble	Soluble	Soluble	Soluble	Poorly soluble
GI absorption	High	High	High	High	High	High	Low	Low
BBB Permeability	Yes	Yes	No	Yes	Yes	Yes	Yes	No
Log Kp	-5.74 cm/s	-4.74 cm/s	-9.09 cm/s	-5.66 cm/s	-6.02 cm/s	-6.82 cm/s	-4.21 cm/s	-8.01 cm/s
P-gp substrate	No	No	Yes	No	Yes	No	No	Yes
CYP1A2 inhibitor	No	Yes	No	Yes	Yes	No	No	No
CYP2C19 inhibitor	No	No	No	No	No	No	No	No
CYP2C9 inhibitor	No	No	No	No	No	No	No	No
CYP2D6 inhibitor	No	No	No	No	Yes	No	Yes	No
CYP3A4 inhibitor	No	No	No	No	No	No	No	No
Lipinski Druglikeness	Yes	Yes	Yes	Yes	Yes	Yes	Yes 1 viol	No 2 viol
Bioavailability score	0.55	0.55	0.55	0.55	0.55	0.85	0.55	0.17

CONCLUSION

The phytochemicals of N. sativa especially Nigellicine and Nigellidine show a good inhibitory potential against the *Salmonella typhi* OmpF and have optimum pharmacokinetic properties thus have a significant potential to be used as drugs against *Salmonella typhi* infection. Further *in vitro* and animal-based studies are required to better evaluate the pharmacologic potential of these phytochemicals in *Salmonella* infection.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

We acknowledge the support of our institute CMH Lahore Medical College, Lahore. No funding of any kind was taken for this study.

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