Intelligent Pharmacy xxx (xxxx) xxx

Contents lists available at ScienceDirect

Intelligent Pharmacy

journal homepage: www.keaipublishing.com/en/journals/intelligent-pharmacy

Computational studies demonstrating dithymoquinone of *Nigella sativa* as a potential anti-dengue agent: Short review

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ARTICLE INFO

Keywords:
Dengue
DENV
Anti-DENV
Nigella sativa
Dithymoquinone

ABSTRACT

Dengue is acute tropical infectious illness, which is spread by mosquitoes, has presented a significant threat to public health worldwide. Unfortunately, there are no drugs that have been clinically proven to be effective at treating or preventing dengue. The development of some drugs is significantly hampered by our incomplete understanding of dengue pathogenesis. This short review provides a brief description of potential action against DENV of dithymoquinone to develop an anti-DENV inhibitor. In-vitro, in-vivo and clinical trials are required to establish the effectiveness and safety of dithymoquinone as an anti-dengue therapy, even though computational studies have demonstrated antiviral activity against DENV.

1. Introduction

The dengue virus (DENV) is a flavivirus that spreads throughout tropical and subtropical regions of the world through the bite of female Aedes mosquitoes. This infection causes bone-breaking fever, commonly called dengue fever and it causes over 400 million infections and 22,000 deaths worldwide each year.[1] The virus has four antigenically different serotypes, DENV -1, -2, -3, and -4, which can all result in infections that are clinically significant.[2] The virus is successfully spread by alternating cycles of viral replication in each host and is mostly carried out through encounters between mosquitoes and humans, its natural hosts. According to Torres et al,[3] primary dengue virus exposure frequently causes mild classical dengue or asymptomatic infections, but it can also sometimes result in the more serious dengue hemorrhagic fever/dengue shock syndrome (DHF/DSS). Lifelong immunity to a single serotype acquired from primary infection is believed to occur, but cross-protection against other serotypes is only momentary and incomplete.[2] Dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS) are caused by different serotypes of DENV.[4] Although the condition is quite common, there is no FDA-approved treatment for it. Thus, the development of antiviral drugs is crucial to the combat against DENV.

DENV-1 through DENV-4 are the four antigenically different serotypes of the virus. Long-term immunity to one serotype and short-term immunity to the other three serotypes, lasting around six months, result from a primary infection.[5] A single-stranded positive-sense RNA genome, enclosed in a virus, encodes three structural proteins: envelope, pre-membrane, and capsid. Additionally, seven non-structural proteins are encoded: NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 (Fig. 1).[4] Throughout the viral infection cycle, these proteins serve a variety of purposes. Viral propagation and host immune evasion are caused by non-structural proteins. A prospective therapeutic target, the NS5 protein is crucial to viral RNA replication because its deletion from the viral genome prevents replication. The N-terminal end of this protein contains the methyltransferase (Mtase) domain, whereas the C-terminal end is home to the RNA-dependent RNA polymerase (RdRp) domain. García-Ariza et al[1] state that the latter is in charge of shielding the RNA at the 5′ end of newly formed viral genomes.

Nigella sativa (black seeds) is a medicinal plant of the Ranunculaceae

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Production and Hosting by Elsevier on behalf of KeAi

https://doi.org/10.1016/j.ipha.2024.02.006
Received 29 January 2024; Received in revised form 14 February 2024; Accepted 16 February 2024
Available online xxxx

Please cite this article as: Roney M, Mohd Aluwi MFF, Computational studies demonstrating dithymoquinone of *Nigella sativa* as a potential anti-dengue agent: Short review, Intelligent Pharmacy, https://doi.org/10.1016/j.ipha.2024.02.006

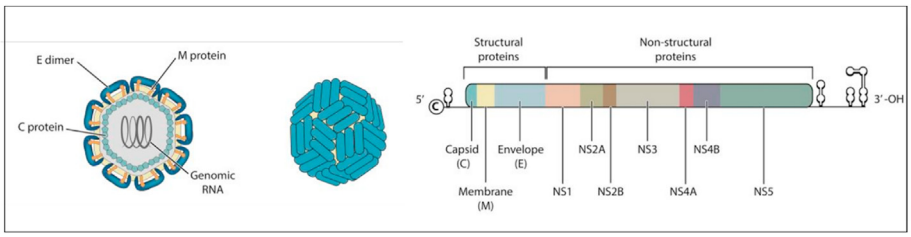

Fig. 1. Structure of Dengue virus.[6]

family which contains of thymoquinone, thymohydroquinone, dithymoquinone, p-cymene, carvacrol, and thymol, among other substances. Numerous health benefits have been discovered for this plant, including diuretic, antihypertensive, antidiabetic, immunomodulatory, anticancer, analgesic, antibacterial, anticancer, anti-inflammatory, and antioxidant effects[7] as well as positive effects on blood pressure, cholesterol.[8] It has been used for decades to treat a wide range of conditions, such as rheumatism, diarrhea, bronchitis, asthma, and skin problems. It is also used for digestive, anti-diarrheal, immune system support, and liver tonic properties.[9] *N. sativa* is used as a medicinal plant in several traditional medical systems, including as Ayurveda, Unani, Siddha, and Tibb. *N. sativa* contains three major benzoquinone molecules: Thymoquinone, thymohydroquinone, and dithymoquinone.

Dithymoquinone (Fig. 2) is one of the most important pharmacologically active constituents/quinone/benzoquinone in *N. sativa* which is the conjugate of two thymoquinone. The IUPAC designation for Dithymoquinone is 4*b*,8*b*-dimethyl-3,7-di(propan-2-yl)-4*a*,8*a*-dihydrobiphenylene-1,4,5,8-tetrone. Furthermore, the chemical formula for is $C_{20}H_{24}O_4$, and its molecular weight is 328.4 g/mol. Two isopropyl groups are connected to two aromatic rings in the position of 2 and 6 as well as four ketones are attached in the position of 1, 4, 5 and 8. It has been demonstrated to have cytotoxic, antifungal, antioxidant, and antiviral properties.[10,11]

The management of DENV infection mostly involves supportive therapy, symptomatic care, and preventive measures, as there are currently no licensed antiviral drugs to treat the virus. Preventive measures include things like protecting oneself from mosquito bites, reducing mosquito populations, and informing people about diseases that mosquitoes may transmit. The severe stage of a DENV infection is treated with a paracetamol named Acetaminophen.[12] Dithymoquinone can be used to treat DENV infection, although no antiviral drugs are approved for this application. Computational research suggests that dithymoquinone may have antiviral properties and be a good supplemental medication for early-stage DENV infection. It is important to note that the use of dithymoquinone to treat DENV infection is still in its early stage.

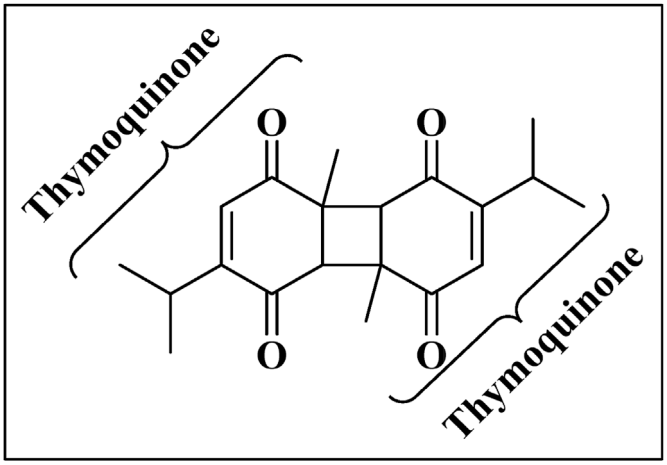

Fig. 2. Structure of dithymoquinone.

Additional research, including in-vitro, in-vivo and clinical studies will be needed to verify the safety and efficacy of dithymoquinone.

2. Dithymoquinone as anti-DENV inhibitor

One of the biggest threats to public health in developing nations is vector-borne illnesses. The main vector for spreading illnesses carried by vectors and infuriating people in public places is the mosquito. The three most common diseases that impact humans are malaria, filaria, and dengue, which are transmitted by infected Anopheles, Culex, and Aedes mosquitoes and are most deadly in tropical and subtropical regions.[13] Female mosquitoes of the species Aedes aegypti and A. albopictus are the primary carriers of the dengue virus, which has four distinct serotypes (DENV-1-4) and is a member of the *Flaviviridae* family and genus Flavivirus.[14] The enormous genetic variability of the dengue serotypes has been brought to light by the identification of several genotypes within each serotype. The escalation of urbanisation, commerce, travel, and other factors has led to a notable rise in the incidence of dengue and other illnesses in recent times. There is currently no effective medication or vaccination to treat dengue. Particularly in tropical and sub-tropical nations, they present a difficult public health issue and have a substantial social and economic effect.[14,15]

The results of the molecular docking and molecular dynamic simulation studies suggest that dithymoquinone could be a successful inhibitor against the DENV. This compound displayed hydrogen bond, hydrophobic, electrostatic, and pi-sulfur interactions in the active site of NS3 protease. This compound exhibited three hydrogen bonds with the residues of Lys74, Leu76, and Trp83 with the binding energy of -7.3 ± 0.09 kcal/mol. Furthermore, this compound showed the best pharmacokinetics profiles. The MD simulation study revealed that dithymoquinone exhibited a stable complex with NS3 protease and a free binding energy of -11.74 kcal/mol.[16] The physico-chemical properties of dithymoquinone showed two (O, O′) pharmacophore sites using the POM study, which indicated that it could be an antiviral drug. Consequently, dithymoquinone can be utilised as a therapeutic or supplementary medicine in the early stages of the disease to control DENV infection.[17]

Dithymoquinone met all parameters of pharmacokinetics and druggability as well as this molecule exhibited two hydrogen bonds with the residues of Gly148 and Lys180 in the active site of NS5 protease of DENV with the binding energy of -43.6164 kcal/mol. According to the DFT study, dithymoquinone showed the most reactive and higher stability with the NS5 protease. In addition, the MD simulation analysis revealed that dithymoquinone complex exhibited stable form and free binding energy of -53.51 kJ/mol.[4] According to,[12] dithymoquinone shown moderate action as an ion channel modulator, kinase inhibitor, protease inhibitor, nuclear receptor ligand, GPCR ligand, and enzyme inhibitor. Furthermore, this compound showed the best pharmacokinetics profiles without any violations in ADMET and drug-likeness studies. Dithymoquinone had a binding energy of -7.2 kcal/mol and interacted with the His51, Gly153, Pro132, and Leu128 residues in the active site of NS2B/NS3 protease of DENV. Furthermore, this compound also had a binding energy of -7.5 kcal/mol and interacted with Val687, His52, and Pro692 residues in the active site of NS5 protease of DENV.[12] MMGB/PBSA-based study demonstrated that dithymoquinone had a

binding energy of -11.74 kcal/mol in the NS2B/NS3 protease of DENV.[23] The promise activity of dithymoquinone as a lead molecule for dengue antiviral development is revealed by the results of these research, which are helpful in the hunt for phytochemicals that are effective against the DENV.

Ribavirin and Quercetin have shown in-vitro and in-silico antiviral activity against DENV. According to,[18] Ribavirin showed the anti-dengue activity with the IC_{50} value of 6.66 μM. This has been studied for its potential as an anti-dengue drug through in silico molecular docking with RdRp protease of Dengue viruses. Ribavirin showed the binding energy of -20.8740 kcal/mol with the formation of 9 hydrogen bonds at the active site residue of Gly536, Asp663, Ser710, Asp664, Asp533, Lys689 was docked with dengue virus RdRp.[19] On the other hand, Quercetin exhibited significant antiviral activity against dengue virus type-2, with an IC_{50} of 35.7 μg mL-1.[20] Quercetin 3-(6″-(E)-p-coumaroylsophoroside)-7-rhamnoside, as potent inhibitors of dengue polymerase, which is essential for viral replication. Molecular dynamics simulations and binding free energy calculations were used to validate the stability of the polymerase-lead complex, with interactions involving residues Trp795, Arg792, and Glu351 being crucial for the complex's stability.[21] Furthermore, Quercetin was demonstrated the binding affinity of -5.393 kcal/-mol with DENV-2 NS5 protein.[22] Additionally, dithymoquinone has been identified as a potential drug candidate with antiviral activity and promising drug likeness, suggesting its potential as an inhibitor against the NS3 protease of the dengue virus. Overall, these studies suggest that dithymoquinone has the potential to be developed as anti-dengue agents, and molecular docking has been a valuable tool in identifying and validating these compounds.

Various chemical constituents of *N. sativa* have been identified, including dithymoquinone, -pinene, -pinene, thymoquinone, thymohydroquinone, thymol, nigellicine, carvacrol, *p*-cymene, nigellicine, nigllimine, nigellidine, alpha-hederin, and limonene. Among them, thymoquinone, dithymoquinone and thymohydroquinone are the major phytoconstituents of *N. sativa* which showed the binding affinity towards the DENV. Thymoquinone and hymohydroquinone showed the binding affinity of -5.6 kcal/mol, where dithymoquinone showed the binding affinity of -7.2 kcal/mol towards the DENV NS2B/NS3 protease.[12] Furthermore, Thymoquinone and hymohydroquinone showed the binding affinity of -24.1278 and -28.8107 kcal/mol, respectively, where dithymoquinone showed the binding affinity of -43.6164 kcal/mol towards the DENV-3 NS5 MTase protein.[4] These finding suggested that dithymoquinone showed the best activity against DENV among the major compounds of *N. sativa*.

Dithymoquinone, a substance present in *N. sativa*, has demonstrated possible antiviral efficacy against dengue virus. Its potential as an inhibiting molecule against dengue virus NS2B/NS3 protease and NS5 polymerase has been demonstrated by computational simulations, indicating that it might be a promising lead drug for dengue antivirals. It's crucial to remember that more in-vitro, in-vivo and clinical trials would be necessary to determine the efficacy of dithymoquinone as an antiviral treatment for dengue. Since there are currently no antiviral medications for dengue, research into the development of therapeutic agents, including possible antivirals, is ongoing. Consequently, even though dithymoquinone appears promising, more investigation—including in-vitro, in-vivo and clinical trials—is required to assess its viability as a future dengue therapy suggestion.

3. Conclusion

Several computational studies have demonstrated the potential of dithymoquinone as an anti-dengue agent. These studies suggest that dithymoquinone could be an attractive candidate for the creation of innovative, unique, and effective dengue medications. Furthermore, dithymoquinone can bind to the active site of DENV protein, suggesting a potential novel drug candidate for dengue treatment. Additionally, dithymoquinone passed the crucial “druggability" test and met all five parameters of Lipinski's requirements for physiochemical consistency. However, more research is needed to ascertain the efficacy and safety of dithymoquinone as a dengue agent. Even though the computational research appears encouraging, safety and effectiveness of dithymoquinone as an anti-dengue medication still need to be established through clinical trials.

Data availability

Not applicable.

Financial support

No.

CRediT authorship contribution statement

Miah Roney: Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Mohd Fadhlizil Fasihi Mohd Aluwi:** Writing – review & editing, Validation, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The authors would like to thanks to the Faculty of Industrial Sciences and Technology, Universiti Malaysia Pahang Al-Sultan Abdullah, Lebuhraya Tun Razak, 26300 Gambang, Kuantan, Pahang Darul Makmur, Malaysia.

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