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Black seeds (*Nigella sativa*) for the management of dengue viral disease: insight into the evidence and POM analyses for the identification of antiviral pharmacophore sites: a review

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ABSTRACT

Background & Aim: The number of dengue virus (DENV) infection cases has increased dramatically over the past two decades, with an estimated 3.9 billion cases across the globe potentially at risk. Patients with a DENV infection are managed symptomatically and by supportive care since there is no approved antiviral drug yet for its management. On the other hand, *N. sativa* has been highlighted as a potential antiviral, particularly against DENV.

Experimental: Hence, the anti-DENV potential of *N. sativa* is analyzed in this review using major databases, including Medline/PMC/PubMed, Scopus, EBSCO, EMBASE, Google Scholar, and Science Direct. Moreover, the Petra/Osiris/Molinspiration (POM) bioinformatics platform-2019 was used to analyze a series of compounds (1-15) identified in *N. sativa* (black seeds) to identify those with promising antiviral pharmacophore sites.

Results: Preliminary research showed the potential of *N. sativa* in the control of *Aedes aegypti* mosquitoes and the enhancement of platelet counts. In addition, several clinical, animal, *in vitro* and *in vivo* studies have demonstrated the antiviral, immunomodulatory and anti-inflammatory properties of *N. sativa*. Furthermore, calculation of the physico-chemical properties of *N. sativa* compounds using POM analyses indicated that dithymoquinone possesses potential antiviral activity with two (O, O') pharmacophore sites.

Recommended applications/industries: As a result, *N. sativa* can be employed as an adjuvant/supportive therapy in the management of DENV infection in the early stages of the illness. Furthermore, *N. sativa* can be a source of new lead anti-DENV drugs.

1. Introduction

Dengue viral disease (DVD) is a mosquito-borne viral illness caused by the dengue virus (DENV) that belongs to the Flaviviridae family and flavivirus genus. DENV infection is endemic in Asia, Latin America and other tropical and subtropical areas of the globe. According to the World Health Organization (WHO) from 2000-2019, the number of dengue cases increased dramatically over the last two decades, with an astounding eightfold increase in the reported dengue cases. Sadly, ~40000 people die annually from severe dengue (WHO,2021). Currently, there are four different serotypes of DENV, DENV-1, DENV-2, DENV-3, and DENV-4. Consequently, a person may contract DENV infection four times in his lifetime (Niu *et al.*, 2020; Rathore *et al.*, 2021; Murugesan and Manoharan, 2019). Dengue-infected mosquitoes may continue transmitting DENV to healthy people throughout their entire life (Ferreira-de-Lima and Lima-Camara, 2018).

The genome of DENV comprises a positive-sense, single stranded RNA (ssRNA) encoded by 3 structural proteins, including capsid (C) protein, membrane (M) protein, and envelope (E) protein, and seven nonstructural (NS) proteins, including NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5 (Lafridi *et al.*, 2023; Reddy *et al.*, 2018; Tay and Vasudevan, 2018). The C protein is involved primarily in viral assembly through the interaction between the viral RNA genome and the ER membrane. The E protein recognizes the host cell receptor and is also involved in viral fusion to the endosomal membrane during cell entry, whereas the function of the M protein has not been fully identified (Nasar *et al.*, 2020; Chew *et al.*, 2017; Nanaware *et al.*, 2021). NS1 protein is detected at higher levels in patients' sera, and hence, it is employed in dengue diagnostic assays. In the early stages of DENV infection, the interaction between NS4A and NS4B proteins is most likely mediated by the NS1 protein for viral replication. NS1 protein has been identified to mediate vascular leakage (Dengue hemorrhagic fever (DHF)/Dengue Shock Syndrome (DSS) through the activation of macrophages and disruption of endothelial cells (Glasner *et al.*, 2018). The NS2A protein participates in the recruitment of viral RNA, structural proteins, and protease enzymes to the site of virion assembly, whereas the NS2B protein is reported to be a cofactor for the viral protease enzyme and has been found to take part in the viral replication process (Shrivastava *et al.*, 2020). The NS3 protein exerts RNA-triphosphatase (RTP), nucleoside triphosphatase (NTPase), NS3 protease, and NS3 helicase enzymatic activities during viral replication. NS4A and NS4B proteins are involved in the formation of replication complexes, whereas NS5 protein is the largest flaviviral protein that is involved in the suppression of the host antiviral response (Obi *et al.*, 2021).

Dengue viral disease (DVD) was initially categorized by the WHO as dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS). However, the WHO has reclassified DVD as nonsevere (dengue without warning signs and dengue with warning signs) and severe dengue fever (SDF) (Hadinegoro *et al.*, 2012; Ajlan *et al.*, 2012). The most at-risk populations for severe dengue include infants and elderly individuals, together with risk factors such as secondary dengue infection and comorbidities such as diabetes, renal diseases, asthma, and heart diseases (Sangkaew *et al.*, 2021; Htun *et al.*, 2021; Naaraayan *et al.*, 2021; Tsheten *et al.*, 2021; Halstead *et al.*, 2019).

Secondary dengue infection (subsequent infection with different serotypes of DENV) may lead to DHF and DSS. The pathogenesis of DENV infection depends on the viral factors NS1 of DENV and the genome of DENV, together with host factors such as antibody-dependent enhancement (ADE), anti-NS1 antibodies, and T cells (Lai *et al.*, 2020). Anti-NS1 antibodies bind to NS1 proteins, followed by the activation of cellular signal transduction pathways and stimulation of the release of numerous inflammatory mediators, including interleukin-6 (IL-6), interleukin-8 (IL-8), and monocyte chemoattractant protein-1 (MCP-1) (Shukla *et al.*, 2020). Moreover, viremia is increased, and target immune cells are activated by ADE via facilitated viral uptake and infection of Fcγ receptor–bearing cells during secondary dengue infection (Castillo *et al.*, 2019). In addition, memory T cells are activated during secondary dengue infection, which results in the release of interferon-γ (IFN- γ) and tumor necrosis factor (TNF)-α, which is followed by the activation of macrophages and monocytes, leading to the release of cytokines (IL-6, IL-8, TNF-α) and other inflammatory mediators, such as histamine, platelet-activating factor, and leukotrienes, causing enhanced vascular permeability and DHF (Berger *et al.*, 2000). T lymphocytes are a major source of cytokines. Moreover, they are distinguished by the presence of cell surface molecules such as CD4 and

CD8. T lymphocytes with CD4 are known as helper T (Th) cells, which release proinflammatory cytokines during immune responses and are further subdivided into Th1 and Th2 cells. The major Th1 cytokine is IFN-γ, whereas Th2 cytokines include IL-4, IL-5, IL-13 and IL-10 (Bhatt *et al.*, 2021). Dysregulation of the cytokine cascade is associated with the pathogenesis of DHF. The Th1 cytokine response of DF is shifted to the Th2 cytokine response (secretion of IL-10 resulting in the disturbance of a fine balance between cytokines leading to cytokine storm) during DHF (increased vascular permeability resulting in plasma leakage and contracted intravascular volume). Patients with DHF and neurological manifestations were observed to have significantly higher levels of IL-6 and IL-8 (Li *et al.*, 2017).

Additionally, acute disseminated encephalomyelitis, neuromyelitis optica, optic neuritis, myelitis, encephalopathy and Guillain− Barre syndrome may occur as a result of autoimmune reactions (Carod-Artal *et al.*, 2019; Somkijrungroj *et al.*, 2019). Patients with DENV infection may develop ophthalmic manifestations, including blurred vision, eyestrain, central scotoma, retroocular pain, diplopia, photopsia, foreign body sensation, uveitis, subconjunctival hemorrhage, retinal vasculitis, retinal edema, optic neuritis, and others (Vijitha *et al.*, 2021; Dissanayake *et al.*, 2018). The hepatic manifestations of DENV infection include tender hepatomegaly, elevated liver enzymes, right hypochondrial pain, and acute liver failure (Gurugama *et al.*, 2018), whereas the renal manifestations of DENV infection include mild electrolyte imbalance and acute kidney injury (Eswarappa *et al.*, 2019; WHO, 2021). To prevent complications and mortality, close observation is recommended for 24-48 hours in patients with severe dengue. In the convalescent phase, the plasma leakage subsides, and the patient begins to reabsorb extravasated intravenous fluids. Pleural and abdominal effusions and neurological manifestations are also reported in this phase (Muller *et al.*, 2017; Chen *et al.*, 2018; WHO, 2021).

The preventive measures against DENV infection include personal protection from mosquito bites, taking steps to control mosquitoes indoors and outdoors, educating the community on mosquito-borne DENV infection, and active mosquito and DENV surveillance (Rather *et al.*, 2018; Maideen *et al.*, 2020). No antiviral drug has yet been approved to treat DENV infection, and patients in the febrile phase of DENV infection can be managed symptomatically using paracetamol (acetaminophen). Consequently, we intend to review the potential use of black seeds (*Nigella sativa* or *N. sativa*) in the management of DENV infection.

2. Materials and Methods

To link between DENV and the use of *N. sativa*, we extensively searched the terms “Dengue Virus Infection”, “Dengue Fever”, “Dengue Hemorrhagic Fever”, “Dengue Shock Syndrome”, “Black seeds”, “Nigella sativa”, “Kalonji”, “Thymoquinone”, “Antiviral”, and “Anti-inflammatory” in major databases, including Medline/PMC/PubMed, Scopus, EBSCO, EMBASE, Google Scholar, and Science Direct. The most significant publications reported in English were included, while those with duplicate information were excluded. Moreover, the Petra/Osiris/Molinspiration (POM) bioinformatics platform-2019 was used to analyze a series of compounds (1-15) reported in *N. sativa* (black seeds). The computational POM analyses were performed for the identification of the compounds from *N. sativa* with potential antiviral pharmacophore sites.

3. *N. Sativa* in the management of DENV virus

Most cases with DENV infection depend mainly on supportive care, as there is no specific antiviral therapy approved yet. *N. sativa* has been reported to have efficient antiviral, immunomodulatory and anti-inflammatory properties; thus, we expected that it can be employed as a potential herbal candidate or adjuvant therapy to manage DENV infection. *N. sativa* has been a popular prophetic medicine since the Prophet Muhammad (PBUH) stated that “There is a cure for every ailment except death, in the black seeds”, and *N. sativa* is mentioned in the Holy Bible as “Curative black seed” (Maideen *et al.*, 2021a). Traditionally, *N. sativa* has been used in the treatment of several conditions for decades. Furthermore, numerous clinical studies have demonstrated the efficacy of *N. sativa* in the management of diabetes (Maideen *et al.*, 2021b), hypertension (Maideen *et al.*, 2021c), dyslipidemia (Maideen *et al.*, 2021d), obesity (Adam *et al.*, 2016) and other chronic conditions. In addition, *N. sativa* has been identified to have hepatoprotective (Noorbakhsh *et al.*, 2018; Ostadpoor *et al.*, 2021; Aktaş *et al.*, 2021;

Erboga *et al.*, 2016), nephroprotective (Cascella *et al.*, 2017; Dera *et al.*, 2020; Farkhondeh *et al.*, 2017), cardioprotective (Xiao *et al.*, 2018; Ran *et al.*, 2021; Alhibshi *et al.*, 2019), neuroprotective (Farkhondeh *et al.*, 2018; Kanter *et al.*, 2005), and gastroprotective (Abdel-Sater *et al.*, 2018) effects.

Several phytochemical (Kawsar *et al.*, 2008a, 2008b, 2008c, 2009, 2010a, 2010b; Matsumoto *et al.*, 2011) analyses of *N. sativa* revealed the presence of 27-40% of fixed oils such as linoleic acid, oleic acid, myristic acid, and palmitic acid, 28.5-33.7% carbohydrates, 16-19% proteins such as arginine, glutamic acid, leucine, and lysine, 5.5-8.9% soluble dietary fibers, 1.79-3.74% minerals such as copper, zinc, phosphorous, and iron, 0.5-1.5% volatile oil including thymoquinone (TQ), *p*-cymene, dithymoquinone (DTQ, nigellone), thymohydroquinone, carvacrol, and thymol, alkaloids such as nigellimine, nigellimine-*N*-oxide, nigellicine, nigellidine, nigellidine-4-*O*-sulfite, methyl nigellidine, higenamine, and nigeglanine, saponins such as α-hederin, and hederagenin derivatives, phenolic compounds such as rutin, quercetin, kaempferol, pyrogallol, gallic acid, and apigenin, and others (Fig. 1).

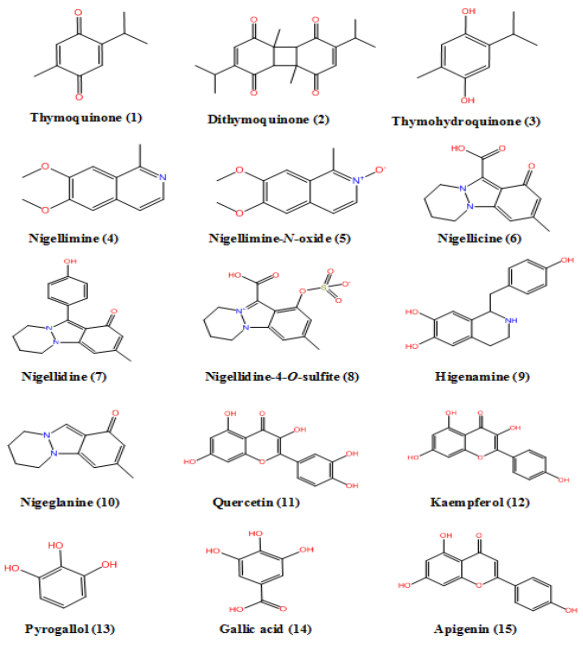


Fig. 1. Molecular structures of the bioactive compounds of *N. sativa* L. (Salehi *et al.*, 2021).

4. *N. sativa* for vector control

Dengue fever is a vector-borne disease that is transmitted mainly by *Aedes aegypti* and *Aedes albopictus* mosquitoes. *Aedes albopictus* mosquitoes serve as a secondary vector of dengue fever. A study demonstrated the larvicidal activity of *N. sativa* oil against *Aedes aegypti* mosquitoes (Raj *et al.*, 2015). Another study demonstrated the potential activity of thymoquinone, styrol, and p-cymene in disturbing the behavior of *Aedes aegypti* mosquitoes (Fahma *et al.*, 2019). Moreover, studies on mosquitoes fed a TQ-glucose mixture revealed that TQ can counter mosquito-borne diseases through the stimulation of an immuno-control approach to limit vector reproduction within infected persons (Ahmed *et al.*, 2008; 2010).

5. *N. sativa* to enhance platelet counts

Dengue hemorrhagic fever is associated with thrombocytopenia, which may occur due to the suppression of bone marrow, destruction of existing platelets, and production of antibodies against platelets (Boo *et al.*, 2019). A study involving the supplementation of an aqueous extract of *N. sativa* seeds for 12 days in albino rats resulted in enhanced platelet counts (Saadia *et al.*, 2017), indicating the potential of *N. sativa* to increase the number of platelets.

6. *N. sativa* as a potential antiviral

Dengue virus (DENV) belongs to the Flaviviridae family and flavivirus genus. Several clinical, animal, *in vivo*, and *in vitro* studies have reported the antiviral efficacy of *N. sativa* against various viruses, including human immunodeficiency virus (HIV) (Maideen *et al.*, 2021), severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) (Maideen *et al.*, 2021), hepatitis C virus (HCV), Newcastle disease virus (NDV), avian influenza (H9N2), murine cytomegalovirus (MCMV), Peste des petits ruminants (PPR) virus, broad bean mosaic virus (BBMV), zucchini yellow mosaic virus (ZYMV), and papaya ring spot virus (Maideen *et al.*, 2020).

7. Immunomodulatory effects of *N. sativa*

Innate immune cells, including dendritic cells, mast cells, Langerhans cells, macrophages, and monocytes, respond first to DENV infection and trigger the release of cytokines (IFN-γ, TNF-α) and chemokines along with innate immune responses, including the type 1 interferon response, complement activation, RNA interference, autophagy, and apoptosis (Uno *et al.*, 2018, Tremblay *et al.*, 2019; Malavige *et al.*, 2020). Interestingly, the severity of DENV infection increases in vulnerable individuals due to aberrant immune responses (significantly higher levels of proinflammatory cytokines, immunosuppressive cytokines (IL-10), chemokines and proinflammatory and immunosuppressive lipid mediators) in the initial stages of illness, resulting in endothelial dysfunction and cytokine storms (John *et al.*, 2019). Moreover, adaptive immune responses are initiated by activated dendritic cells infected by DENV by presenting antigen to $CD4^+$ and $CD8^+$ T cells. Furthermore, preexisting antibodies neutralize DENV infection during a secondary homologous infection, whereas preexisting, subneutralizing antibodies may lead to increased viral replication, cytokine storms, and increased vascular permeability during secondary heterologous infection (Wilken *et al.*, 2020; Mahdy *et al.*, 2009).

The immunomodulatory potential of *N. sativa* has been demonstrated through various clinical, animal, *in vitro* and *in vivo* studies. In a clinical study, treatment of 36 female rheumatoid arthritis patients with 500 mg of *N. sativa* capsules two times daily for 2 weeks caused a significant reduction in the disease severity scores of 28 joints (DAS28), Ritchie Articular Index (RAI), morning stiffness, and white blood cell (WBC) counts (Gheita *et al.*, 2012). Similarly, supplementation with 500 mg of *N. sativa* oil capsules two times daily for 4 weeks in 40 female patients with rheumatoid arthritis resulted in a significant reduction in DAS28. WBC counts and visual analog scale (VAS) for pain also showed an improvement in the number of swollen joints along with a decrease in the duration of morning stiffness (Gheita *et al.*, 2012). A randomized, double-blinded, placebo-controlled trial of 43 female rheumatoid arthritis patients treated with a single gram of *N. sativa* capsules daily for 2 months demonstrated a significant reduction in DAS28, whereby the percentage of $CD8^+$ T cells showed a significant elevation of the $CD4^+/CD8^+$ ratio as well as the percentage of $CD4^+CD25^+$ T cells (Kheirouri *et al.*, 2016). Moreover, a clinical trial of 25 blood transfusion-dependent children with beta-thalassemia supplemented with a 2 g daily dose of *N. sativa* powder over a course of 3 months resulted in a significant enhancement of cell-mediated immunity via elevated $CD4^+$ T cells and $CD8^+$ T cells (El-Shanshory *et al.*, 2019). The regulatory effects of *N. sativa* on immunity might be mediated through nuclear factor kappa B (NF-kB) signaling pathways (Niu *et al.*, 2021).

8. Anti-inflammatory potential of *N. sativa*

Patients with severe dengue exhibit significantly higher levels of inflammatory cytokines, chemokines and lipid parameters (Malavige *et al.*, 2020) and significantly higher levels of inflammatory markers such as C-reactive protein (CRP), serum amyloid P (SAP), ferritin, TNF-α and IL-1β (Patra *et al.*, 2021). Numerous clinical, animal, *in vitro* and *in vivo* studies have demonstrated the anti-inflammatory activity of *N. sativa*. A randomized, double-blinded, placebo-controlled trial of 42 rheumatoid arthritis patients treated with 2 capsules of *N. sativa* (500 mg) daily for 8 weeks led to a significant reduction in DAS28, malondialdehyde (MDA), and nitric oxide (NO) with an increase in the serum levels of IL-10 (Hadi *et al.*, 2016). In another randomized, double-blinded trial of 52 older patients with osteoarthritis, the topical application of *N. sativa* oil twice daily for 21 days caused better pain relief when compared to diclofenac gel (Azizi *et al.*, 2019). Moreover, an *in vitro* analysis of preadipocytes treated with *N. sativa* oil revealed a significant reduction in IL-6 levels and an inhibition of IL-1β activity combined with higher antioxidant activity (Bordoni *et al.*, 2021).

Several reports have focused on the anti-inflammatory and antiviral properties of *N. sativa* and TQ as promising therapeutic agents to target contemporary inflammatory and infectious diseases, including COVID-19 (Wendling *et al.*, 2021).

9. *N. sativa* as a source of novel antiviral compounds

9.1. Bioinformatics POM analyses and identification of new pharmacophore sites

POM theory (Petra/Osiris/Molinspiration), which was invented by Taibi Ben Hadda and his team in collaboration with the American NCI and TAACF, was employed in this study to predict compounds from *N. sativa* with new potential pharmacophoric sites against DENV. POM theory uses very important descriptors, including (i) the geometry of pharmacophore sites (*cis* or *trans*), (ii) the 3D complementary interaction of the drugs with specific biotargets, and (iii) the electrostatic drug/biotarget interaction. A series of compounds 1-15 identified from *N. sativa L.* was analyzed by the Petra/Osiris/Molinspiration (POM) bioinformatics platform-2019 (Fig. 2).

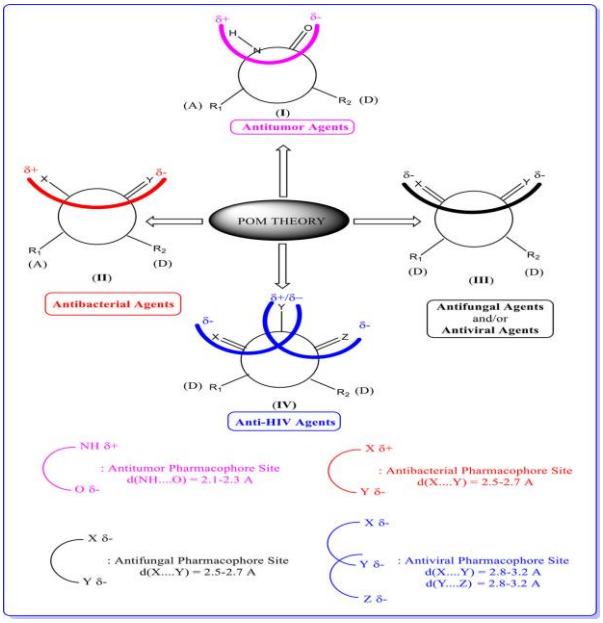


Fig. 2. Organigram of the identification of pharmacophore sites based on POM theory.

By using POM (Petra/Osiris/Molinspiration) theory, it has become easier to identify and optimize most of the antibacterial (Rbaa *et al.*, 2021; Bhat *et al.*, 2021), antifungal (Youssoufi *et al.*, 2015; Khana *et al.*, 2017), and antiviral (Lafridi *et al.*, 2022; Hadda *et al.*, 2021) pharmacophore sites, one by one, based on their different physicochemical parameters and their different electronic charge repartitions of corresponding heteroatoms.

9.2. Osiris analysis

In this analysis, the physical and chemical properties of the tested compounds were used to identify the type of pharmacophore site. The pharmacokinetic properties and bioactivity score analysis are shown in Fig. 2 and Table 1. The toxicity risks (mutagenicity, tumorgenicity, irritation, reproductive toxicity) and physicochemical properties (ClogP, solubility (ClogS), drug-likeness (DL), drug-score (DS), etc.) of compounds 1-15 were also calculated (Table 1). The method consists of fragment-based contributions and correction factors. All the calculated results were presented in color codes where the red color signifies high risks with undesired effects such as mutagenicity or poor intestinal absorption, and the green color suggests drug-conforming behavior.

Seven structures (1, 3, 11-15) were predicted to be mutagenic, while two compounds (11, 13) and one compound (13) were predicted to be tumorigenic and have reproductive side effects, respectively (Table 1 and Fig. 3). Regarding the irritant effects, all the compounds are at low risk, except compound 13. Osiris calculations confirmed that all the selected compounds of the (1-15) series possess low to moderate side effects. However, an encouraging parameter of the drug scores in this series 1-15 (22% < DS < 92%) was identified in comparison to various standard drugs cited in the literature. This high bioactivity is probably due to the good and mixed lipo/hydro solubility, which has a direct effect on their bioavailability. However, because of the superior metabolic transformations that exist in these molecules, dithymoquinone was predicted to be an efficient antiviral agent with two (O, O') pharmacophore sites (Bhat *et al.*, 2023; Hadda *et al.*, 2020).

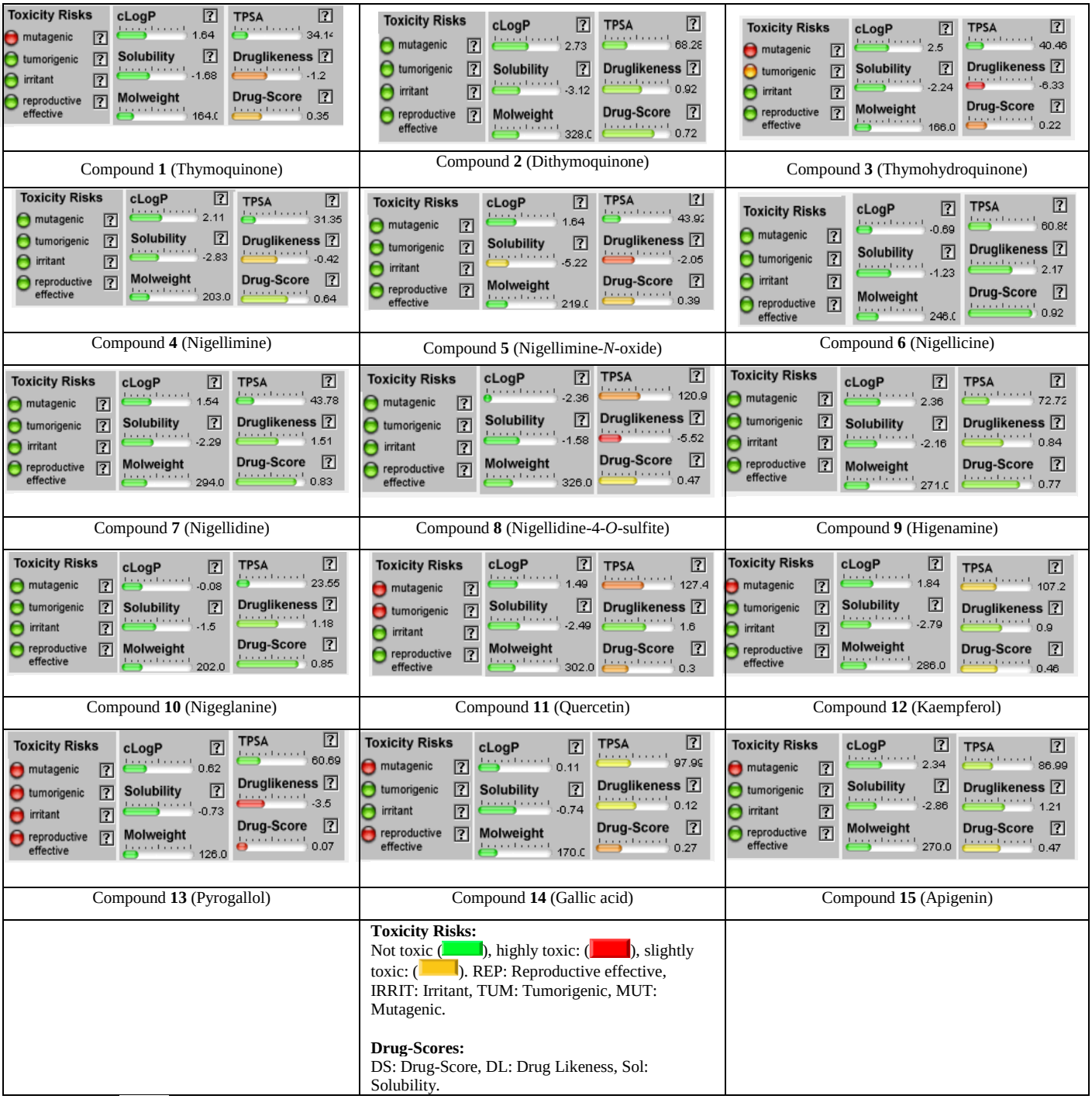


Fig. 3. Osiris calculations of drug-likness and drug-scores of selected compounds (1-15) from *N. sativa*.

Table 1. Osiris calculations of drug-likness and drug-scores of compounds (1-15) reported from *N. sativa*.

Compound	MW (g/mole)	Toxicity risks[a]				Drug score calculations[b]			
		MUT	TUM	IRRI	REP	cLog*P*	cLog*S*	DL	DS
1	164.20					1.64	-1.68	-1.2	0.35
2	328.41					2.73	-3.12	0.92	0.72
3	166.22					2.5	-2.24	-6.33	0.22
4	203.24					2.11	-2.83	-0.42	0.64
5	219.24					1.64	-5.22	-2.05	0.39
6	246.27					-0.69	-1.23	2.17	0.92
7	294.35					0.79	-3.7	-0.52	0.58
8	374.42					-0.5	-3.03	-5.03	0.43
9	271.32					2.36	-2.16	0.84	0.77
10	202.26					-0.08	-1.5	1.18	0.85
11	302.24					1.49	-2.49	1.6	0.3
12	286.24					1.84	-2.79	0.9	0.46
13	126.11					0.62	-0.73	-3.5	0.07
14	170.12					0.11	-0.74	0.12	0.27
15	270.24					2.34	-2.86	1.21	0.47

[a] Not toxic: ( ), highly toxic: ( ), slightly toxic: ( ). REP: Reproductive effective, IRRIT: Irritant, TUM: Tumorigenic, MUT: Mutagenic. [b] DS: Drug Score, DL: Drug Likeness, Sol: Solubility.

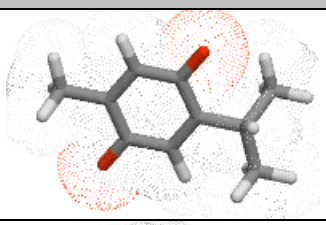
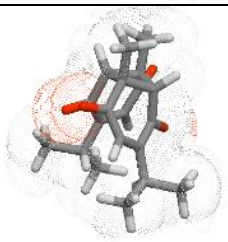
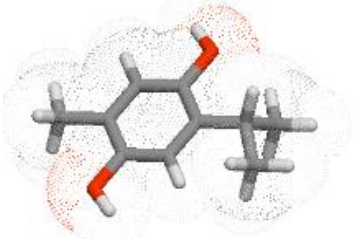
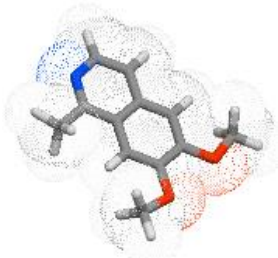
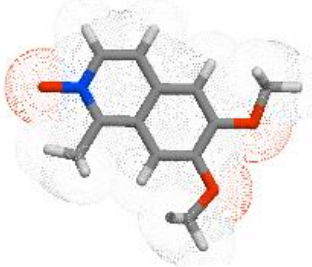
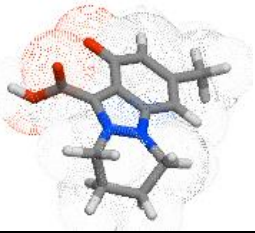
9.3. Molinspiration analysis

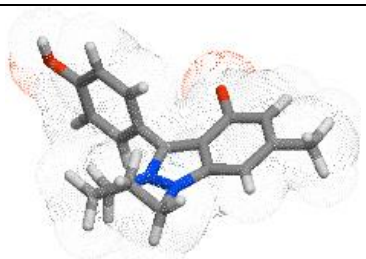
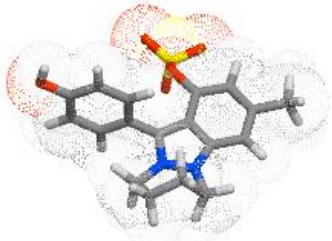
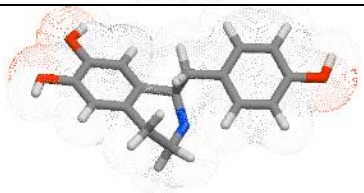
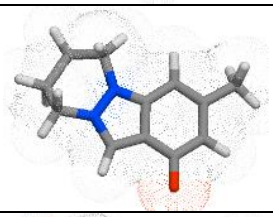
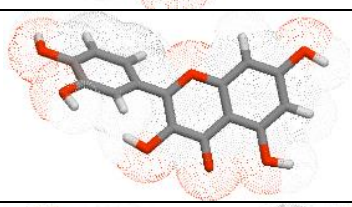
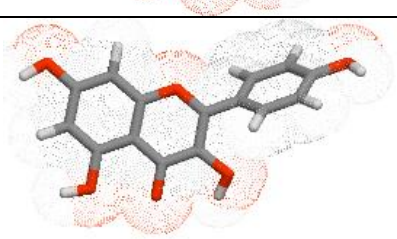
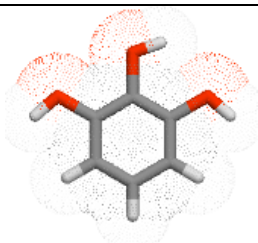
The molinspiration program was used to determine the drug score and drug likeness of selected compounds **1-15** (Tables 2, 3). It appears that no compound exhibits any violation of Lipinski rule of five (NV = 0). More interestingly, most of the compounds **1-15** show various and positive drug scores, which is very encouraging to test their actual activity against the target enzymes (drug score > 10%) (Hosen *et al.*, 2023a, 2023b).

The drug score data pertaining to thymidine analogs were computed, and it was observed that the hydrophilic nature of these analogs was ascertained based on their cLogP value. There is an indication that a high cLogP value is linked to inadequate absorption or permeation, and it is recommended that the value be below 5. The findings indicate that all of the analogs exceeded the established threshold (5.18 < cLogP< 6.31) and that these analogs exhibited suboptimal cLogP values, falling below the requisite cLogP< 5). The assessment of a compound's potential as a drug candidate involves the computation of a drug score (DS), which integrates drug-likeness, cLogP, TPSA, and molecular weight derived from an antifungal ($O^{\delta-}$, $O^{\delta-}$) pharmacophore. The hypothesis site is supported by atomic charge analysis, as reported in Hosen *et al.*, (2022).

Conversely, the set of uridine derivatives underwent treatment with the aim of discerning their pharmacophoric locations. The determination of the pharmacophore site classification of these compounds was based on their chemo-physical, atomic charge, and geometric characteristics. This was accomplished through the utilization of the Petra, Osiris, and Molinspiration (POM) platform. The analysis of pharmacokinetic properties and bioactivity scores revealed that a limited number of compounds exhibited negligible adverse effects. Nevertheless, it is worth noting that only a limited number of derivatives exhibited favorable bioavailability, as indicated by a cLogP value of less than 5. The subsequent installments exhibited reduced bioavailability due to decreased solubility. This phenomenon can be attributed to the significant level of alkylation. The derivatives were subjected to Molinspiration calculations, which revealed that the series exhibited a moderate to good level of inhibition toward multiple biotargets. The pharmacophore sites exhibiting antifungal properties ($O1^{\delta-}$ --- $O2^{\delta-}$) have been validated through atomic charge calculations of compounds. It was observed that these compounds exhibit a greater degree of efficacy as antifungal and antiviral agents than antibacterial agents (Munia *et al.*, 2022; Kawsar *et al.*, 2022, 2023).

Table 2. Molinspiration prediction of Drug-Scores of compounds (1-15).

Compd	Structure	Molecular proprieties	Drug scores
1		cLogP 1.90 TPSA 34 MW 164 nOHNH 0 nviolations 0 volume 161	GPCR ligand -1.40 Ion channel modulator -0.31 Kinase inhibitor -1.27 Nuclear receptor ligand -1.47 Protease inhibitor -1.45 Enzyme inhibitor -0.40
2		cLogP 1.70 TPSA 68 MW 328 nOHNH 0 nviolations 0 volume 311	GPCR ligand -0.18 Ion channel modulator -0.09 Kinase inhibitor -0.48 Nuclear receptor ligand 0.14 Protease inhibitor -0.10 Enzyme inhibitor 0.10
3		cLogP 3.26 TPSA 41 MW 166 nOHNH 2 nviolations 0 volume 167	GPCR ligand -0.92 Ion channel modulator -0.44 Kinase inhibitor -1.06 Nuclear receptor ligand -0.54 Protease inhibitor -1.17 Enzyme inhibitor -0.46
4		cLogP 1.89 TPSA 31 MW 203 nOHNH 0 nviolations 0 volume 192	GPCR ligand -0.47 Ion channel modulator -0.17 Kinase inhibitor -0.40 Nuclear receptor ligand -0.75 Protease inhibitor -0.64 Enzyme inhibitor -0.12
5		cLogP 0.48 TPSA 44 MW 219 nOHNH 0 nviolations 0 volume 200	GPCR ligand -0.09 Ion channel modulator 0.16 Kinase inhibitor 0.01 Nuclear receptor ligand -0.57 Protease inhibitor -0.20 Enzyme inhibitor 0.27
6		cLogP 1.65 TPSA 64 MW 246 nOHNH 1 nviolations 0 volume 218	GPCR ligand -0.15 Ion channel modulator -0.00 Kinase inhibitor -0.18 Nuclear receptor ligand -0.29 Protease inhibitor -0.57 Enzyme inhibitor 0.20

7		miLogP -3.06 TPSA 52 MW 294 nOHNH 1 nviolations 0 volume 270	GPCR ligand 0.12 Ion channel modulator 0.36 Kinase inhibitor -0.23 Nuclear receptor ligand -0.50 Protease inhibitor -0.28 Enzyme inhibitor 0.23
8		miLogP -3.72 TPSA 95.48 MW 374.42 nOHNH 1 nviolations 0 volume 310.52	GPCR ligand 0.10 Ion channel modulator 0.26 Kinase inhibitor -0.24 Nuclear receptor ligand -0.40 Protease inhibitor -0.16 Enzyme inhibitor 0.21
9		miLogP 2.00 TPSA 72.71 MW 271 nOHNH 4 nviolations 0 volume 248	GPCR ligand 0.25 Ion channel modulator 0.23 Kinase inhibitor -0.24 Nuclear receptor ligand -0.15 Protease inhibitor -0.04 Enzyme inhibitor 0.15
10		miLogP 1.84 TPSA 27 MW 202 nOHNH 0 nviolations 0 volume 191	GPCR ligand -0.35 Ion channel modulator -0.12 Kinase inhibitor -0.19 Nuclear receptor ligand -0.90 Protease inhibitor -0.92 Enzyme inhibitor -0.08
11		miLogP 1.68 TPSA 131 MW 302 nOHNH 5 nviolations 0 volume 240	GPCR ligand -0.06 Ion channel modulator -0.19 Kinase inhibitor 0.28 Nuclear receptor ligand 0.36 Protease inhibitor -0.25 Enzyme inhibitor 0.28
12		miLogP 2.17 TPSA 111 MW 286 nOHNH 4 nviolations 0 volume 232	GPCR ligand -0.10 Ion channel modulator -0.21 Kinase inhibitor 0.21 Nuclear receptor ligand 0.32 Protease inhibitor -0.27 Enzyme inhibitor 0.26
13		miLogP 0.73 TPSA 61 MW 126 nOHNH 3 nviolations 0 volume 108	GPCR ligand -2.18 Ion channel modulator -1.43 Kinase inhibitor -2.19 Nuclear receptor ligand -2.24 Protease inhibitor -2.40 Enzyme inhibitor -1.53

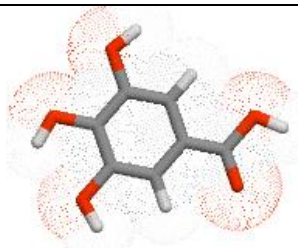
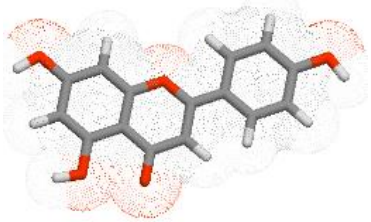
14		miLogP 0.59 TPSA 98 MW 170 nOHNH 4 nviolations 0 volume 135	GPCR ligand -0.77 Ion channel modulator -0.26 Kinase inhibitor -0.88 Nuclear receptor ligand -0.52 Protease inhibitor -0.94 Enzyme inhibitor -0.17
15		miLogP 2.46 TPSA 91 MW 270 nOHNH 3 nviolations 0 volume 224	GPCR ligand -0.07 Ion channel modulator -0.09 Kinase inhibitor 0.18 Nuclear receptor ligand 0.34 Protease inhibitor -0.25 Enzyme inhibitor 0.26

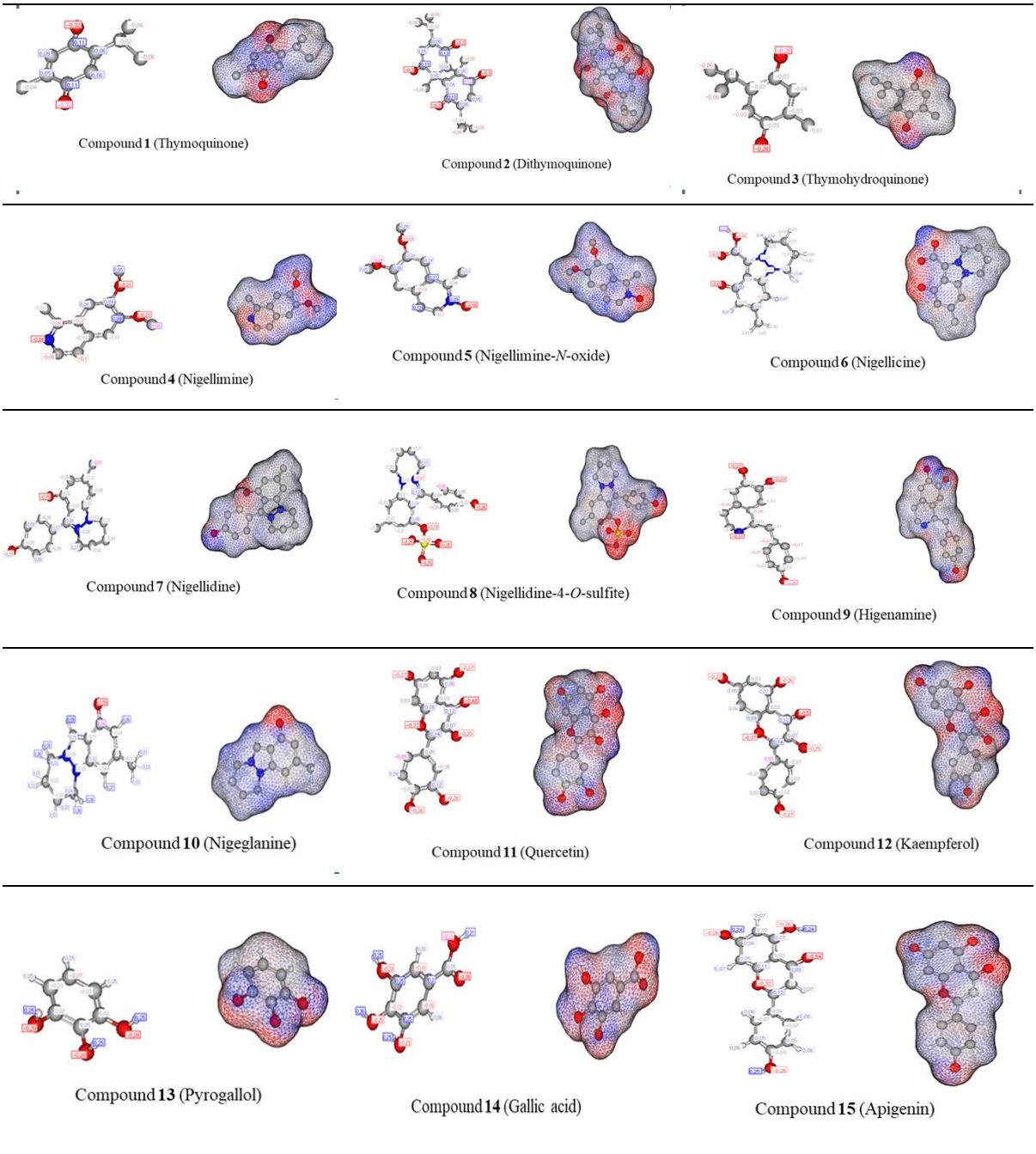
Table 3. Molinspiration calculations of drug-likeness and drug-scores of compounds (1-15).

Compd	Lipinski parameters calculations[a]				Drug-likeness[b]					
	TPSA	NONH	NV	VOL	GPCRL	ICM	KI	NRL	PI	EI
1	34	0	0	161	-1.40	-0.31	-1.27	-1.47	-1.45	-0.40
2	68	0	0	311	-0.18	-0.09	-0.48	0.14	-0.10	0.10
3	41	2	0	167	-0.92	-0.44	-1.06	-0.54	-1.17	-0.46
4	31	0	0	192	-0.47	-0.17	-0.40	-0.75	-0.64	-0.12
5	44	0	0	200	-0.09	0.16	0.01	-0.57	-0.20	0.27
6	64	1	0	218	-0.15	-0.00	-0.18	-0.29	-0.57	0.20
7	52	1	0	270	0.12	0.36	-0.23	-0.50	-0.28	0.23
8	96	1	0	311	0.10	0.26	-0.24	-0.40	-0.16	0.21
9	73	4	0	248	0.25	0.23	-0.24	-0.15	-0.04	0.15
10	27	0	0	191	-0.35	-0.12	-0.19	-0.90	-0.92	-0.08
11	131	5	0	240	-0.06	-0.19	0.28	0.36	-0.25	0.28
12	111	4	0	232	-0.10	-0.21	0.21	0.32	-0.27	0.26
13	61	3	0	108	-2.18	-1.43	-2.19	-2.24	-2.40	-1.53
14	98	4	0	135	-0.77	-0.26	-0.88	-0.52	-0.94	-0.17
15	91	3	0	224	-0.07	-0.09	0.18	0.34	-0.25	0.26

[a] VOL: volume, NONH: number of OH---N or O---NH interactions, TPSA: total molecular polar surface area, NV: number of violations of the Lipinski rule of five. [b] EI: enzyme inhibitor, PI: protease inhibitor, NRL: nuclear receptor ligand, GPCRL: GPCR ligand; ICM: ion channel modulator; KI: kinase inhibitor.

9.4. Atomic charge analysis

The atomic charge of oxygen atoms leads to the identification of potential antiviral ($^{\delta-}$O=C---C=O$^{\delta-}$) pharmacophore sites in favor of dithymoquinone (see the structure of compound 2, (Figs. 4, 5).



The best candidates as antiviral/antifungal agents are compounds with (X---Y) pharmacophore sites where both X and Y heteroatoms are in cisoidal conformation and are negatively charged.

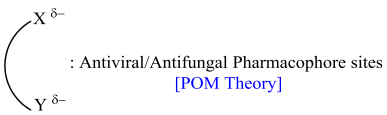


Fig. 4. Atomic charge and identification of potential antiviral ($^{\delta-}$O=C---C=O$^{\delta-}$) pharmacophore sites (see structure of compound 2).

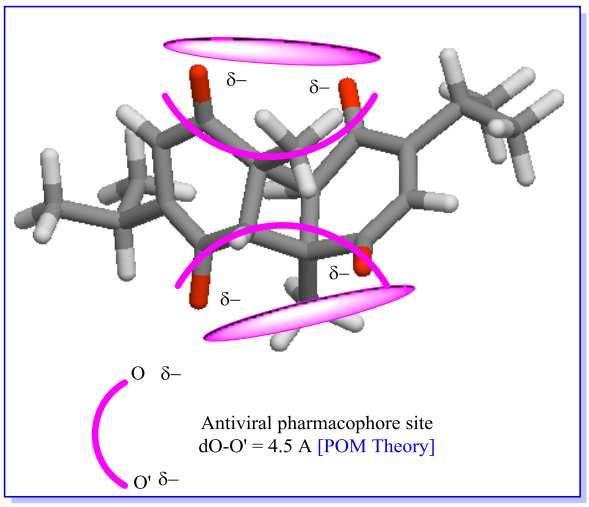


Fig. 5. Atomic charge and identification of two synergetic potential antiviral ($^{\delta-}$O=C---C=O$^{\delta-}$) pharmacophore sites in dithymoquinone (anti-Sars-Cov-2 agent) (Kawsar *et al.*, 2022, 2023).

10. Conclusion and future prospects

Currently, there is no antiviral drug approved for the management of DENV infection; therefore, patients with DENV infection are managed symptomatically along with supportive care. On the other hand, previous research shows the potential of *N. sativa* in the management of DENV infection by vector control of *Aedes aegypti* mosquitoes and the enhancement of platelet levels. In addition, several clinical, animal, *in vitro* and *in vivo* studies have demonstrated the antiviral, immunomodulatory and anti-inflammatory capabilities of *N. sativa*. POM analyses were able to predict that the physicochemical properties of dithymoquinone reported from *N. sativa* can exhibit efficient antiviral activity with two (O, O') pharmacophore sites. Collectively, *N. sativa* may serve as a potential adjuvant therapy/supportive care in the management of patients with DENV infection. Furthermore, dithymoquinone from *N. sativa* can be repurposed for antiviral activity. The potential of *N. sativa* in the management of patients with DENV infection should be further determined by more experimental studies in the future.

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