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In vitro potential antiviral SARS-CoV-19- activity of natural product thymohydroquinone and dithymoquinone from *Nigella sativa*

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ABSTRACT

Inflammation, oxidation, and compromised immunity all increase the dangers of COVID-19, whereas many pharmaceutical protocols may lead to increased immunity such as ingesting from sources containing vitamin E and zinc. A global search for natural remedies to fight COVID-19 has emerged, to assist in the treatment of this infamous coronavirus. *Nigella sativa* is a world-renowned plant, an esteemed herbal remedy, which can be used as a liquid medicine to increase immunity while decreasing the dangers of acute respiratory distress syndrome. Thymoquinone (TQ), dithymoquinone (DTQ) and thymohydroquinone (THQ), are major compounds of the essential oil contained in *N. sativa*. A current study aims to discover the antiviral activity of two compounds, Thymohydroquinone and Dithymoquinone, which are synthesized through simple chemical procedures, deriving from thymoquinone, which happens to be a major compound of *Nigella sativa*. A half-maximal cytotoxic concentration, "CC50", was calculated by MTT assay for each individual drug, The sample showed anti-SARS-CoV-2 activity at non-cytotoxic nanomolar concentrations in vitro with a low selectivity index (CC50/IC50 = 31.74/23.15 = 1.4), whereby Dimthymoquinone shows high cytotoxicity.

1. Introduction

The severe acute respiratory syndrome, coronavirus-2 (SARS-CoV-2), known internationally as the cause of COVID-19, also known as the coronavirus, was declared a pandemic in March of 2020. The main goal, world-wide, has been to discover a drug with enough potential antiviral activity to face this pandemic and overcome it; a drug having limited side effects, unlike synthetic drugs which are known to have a variety of aftereffects. With that specific and lofty ambition, our team set out for collective information regarding the potential of natural plants as possible antivirals, and published some papers in regards to the activity of medicinal plants which were previously used to treat SARS-CoV-19, due to their similarity and host receptor. Regrettably, SARS-CoV-19 has infected thousands of people whereby every single country has suffered huge losses. Moreover, the death rate with COVID-19 is higher than that of SAES-CoV and MERS-CoV-19 combined. Therefore, our ambitious task has proven quite difficult.

Seeds of *Nigella sativa* L. (also commonly known as black cumin or

black seed) are widely used in traditional Islamic medicine and for culinary purposes worldwide. *Nigella* seed oil is becoming popular within the Islamic world and beyond. Composition of *Nigella* seed oil is known to be location-dependent. We investigated the composition of *Nigella* seed oil prepared by solvent- or by the cold press-extraction of *Nigella* seeds grown in Morocco. Oil extraction yield was 37% and 27% when solvent or cold press extraction methods were used, respectively. In terms of its oil major components, the composition of *Nigella* seed oil from Morocco is similar to that of other Mediterranean countries, who are commonly known for their *Nigella* seed-oil quality [1].

The antibacterial activity of the essential oil of *N. sativa* was determined against a panel of strains of bacteria. The GC-MS analysis showed that the major constituents of the oil were monoterpene hydrocarbons and phenolic monoterpenes, whereby the results of antibacterial activity confirmed the possibility of using *Nigella sativa* essential oils or some of their components in biological and pharmaceutical preparations [2].

To fight against coronavirus by using the constituents of *Nigella sativa* L., one important question is posed; Which parts are the most

Abbreviations: Hydrothymoquinone, HTQ; Thymoquinone, TQ; Dimmer of Thymoquinone, DTQ; SARSCoV, Severe Acute Respiratory Syndrome - corona virus; TLC, thin layer chromatography.

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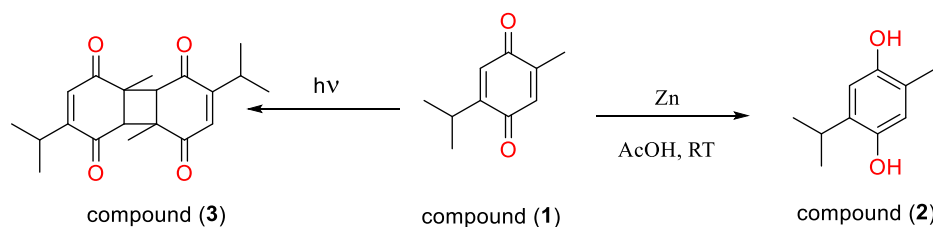
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important, the essential oil or the actual seed?

Many reviews were conducted on plants and their secondary metabolites, which have shown activity against SARS-CoV. Numerous scientific reports on the potential of plants and secondary metabolites against SARS-CoV infection, whereby many of the compounds had been studied through computational studies [4-6]. confirmed that the primary host receptor for SARS-CoV-2 is the human angiotensin-converting enzyme 2 (ACE2).

Ye et al. overviewed the existing knowledge about 7 human coronaviruses (HCoVs), with a focus on the history of their discoveries as well as their zoonotic origins and interspecies transmissions. They also compared and contrasted the different HCoVs from a perspective of virus evolution and genome recombination [7].

Lo et al. reported their experience on the evaluation of SARS-CoV-2 RNA shedding in clinical specimens and clinical features of all 10 patients in Macau, and recommended the assessment of both fecal and respiratory specimens for enhancing diagnostic sensitivity because there were not any specific antiviral drugs available for the treatment of this sudden and lethal disease. Many drugs have been used in this therapy. Yang et al. [8] reported that greater than 85% of COVID-19 patients in China have been receiving Traditional Chinese Medicine (TCM) treatment, and presented strong clinical evidence showing the beneficial effect of TCM in the treatment of these patients [8].

Zhou and Zhaopointed out the great importance of using therapeutic neutralizing antibodies (NABs) to control the spread and re-emergence of SARS-CoV-2 and assert that the development of NABs should therefore be a high priority in future considerations [9].

The two main components of black seed essential oil, Thymoquinone (TQ) and Thymohydroquinone (THQ), were investigated for their antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella flexneri*, *Salmonella typhimurium*, *Salmonella enteritidis* and *Staphylococcus aureus* [10,11].

Thymohydroquinone (2), also referred to as Dihydrothymoquinone or Hydrothymoquinone, is the phenolic hydroquinone derivative of Thymoquinone, Thymohydroquinone is the first reductive product of Thymoquinone, which is a significant co-product of the essential oil in *Nigella sativa*.

Several studies on the biological activity of Thymoquinone have been discussed in literature; oxidative stress, hepatoprotection, the anti-tumor proliferation, the anti-inflammatory, the hypertension, the antimicrobial, neuropathy pain, gastroenterological, the kidney, renal, and the heart [12-14]. In contrast to Thymoquinone, there is no concern over Dihydrothymoquinone or hydrothymoquinone.

N. sativa seeds contain 36–38% fixed oil and low concentrations of some unusual unsaturated fatty acids. Different components were characterized from the oil: the major ones were TQ (28–57%) and *para*-cymene (7–15%), whereas low concentrations of the dimeric form of TQ (Dithymoquinone), and only a minimal quantity of Dihydrothymoquinone (DHTQ) were detected in the oil [15]. DHTQ could be formed in the body after TQ ingestion, following the action of reductases, as reported for DT-diaphorase, which catalyzes reduction of TQ to DHTQ in different organs [16].

Thymoquinone (TQ), the main, active ingredient of black seed oil, possesses antioxidant, anti-inflammatory, antiviral, antimicrobial, immunomodulatory and anticoagulant activities [17].

Using POM Theory, a relatively new computational method for virtually screening many compounds from natural source, we screened 20 compounds derived from *Nigella sativa*, *Artemisia herba alba* and *thymus*. The choice of these plants, as previously discussed, was made based on their role in traditional medicine for curing a variety of diseases, with special focus on influenza, fever, and colds. Unfortunately, only a few of these plants have been screened against SARS-CoV, with very limited references reporting these plants as a possible antiviral source. From amongst these selected plants, we choose only 5 compounds, Thymole, Thymoquinone, Dihydrothymoquinone (DHQ) and Dithymoquinone (DTQ) and Artemisinin (ARTM) all show promising results. So, we began the work protocol by studying in vitro, antiviral activity of these compounds against COVID-19.

The aim of this current study is to understand and emphasize the potential antiviral activity of Thymohydroquinone (THQ), and dithymoquinone (DTQ), whereby many papers study the effect of *Nigella sativa* as antiviral [1-18]. However, there is not a single study of antiviral compounds derived from this plant, documenting antiviral activity against SARS-CoV-19.

2. Material and methods

2.1. Material and equipment

Thymoquinone, solvent, acetone, ethanol, glacial acetic acid and TLC, HPLC-grade methanol were purchased from Sigma-Aldrich.

FT IR Spectra (Brüker Tensor 27 with ATR configuration), ^1H NMR (Brüker Avance 400 NMR spectrometer), UV-Visible spectra (Varian Cary® 50 Scan spectrometer in a 1.0 cm square cuvette).

2.2. Synthesis of compounds (1, 3)

Thymoquinone was used for synthesis two compound HTQ and DTQ.

2.3. Synthesis hydrothymoquinone HTQ (2)

Thymoquinone (0.50 g, 3.1 mmol) was dissolved in glacial acetic acid (10.0 mL). Zinc (0.75 g) powder was added, and the reaction mixture was stirred at room temperature for 12 h. The progress of the reaction was monitored by TLC. After completion of the reaction, acetic acid was concentrated under vacuum and neutralized by Na_2CO_3 (2 M solution, 50 mL). Then, dichloromethane (50 mL) was added, and the organic materials were partitioned between the two phases. The organic layer was separated, dried over anhydrous MgSO_4 , and evaporated under reduced pressure, the separated compound was then purified by column chromatography using silica gel as the stationary phase and hexane:ethyl acetate (9:1) mobile phase. White solid, 288 mg, yield 60%. was obtained [16].

2.4. Characterization of compound 2 HTQ

The obtained FTIR spectra of the bending alkane vibration band, the bending $\delta(\text{C}-\text{H})$ vibration of the resulting absorption band is displayed here [19]. The strong band appeared at 1231 cm^{-1} due to stretching vibrations of $\nu(\text{C}-\text{O})$ of aromatic ether. The two medium absorption

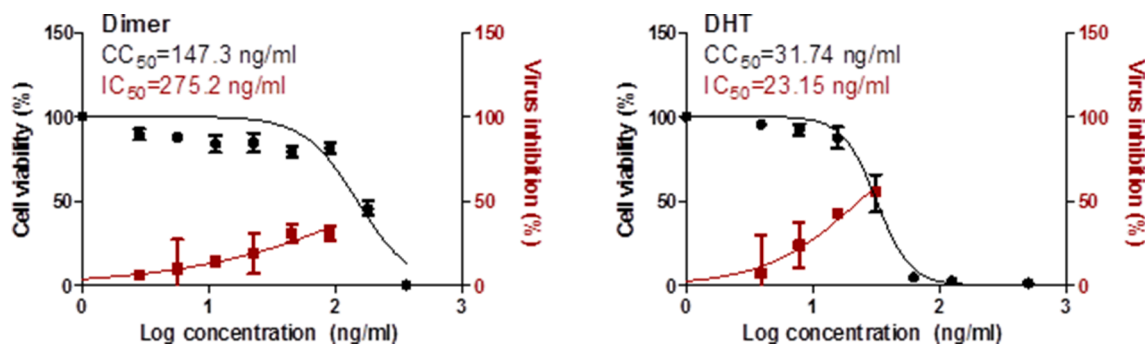


Fig. 1. Cytotoxic effect of compounds, Dimer (3) and DHT (2). Inhibitory concentration (IC_{50}) values were calculated by nonlinear regression analysis of Graph pad prism software (version 5.01) by plotting log inhibitor versus normalize response (variable slope).

bands at 1124 cm^{-1} and 900 cm^{-1} attributed to bending vibration of δ ($C=C$) of α,β -unsaturated alkene., broad band appears at 3300 cm^{-1} corresponding stretching vibrations of νOH . 1H NMR ($CDCl_3$, 500 MHz, δ in ppm): 1.04–1.06 (d, $j = 1.6$, 6H), 1.98 (s, CH_3), 3.3 (m, 1H, CH), 6.42 (d, CH, $j = 2.4$), 8.35 (d, CH, $j = 2.4$). MS detection show a peak at m/z 165 [M–H] corresponding to the formation of HTQ. Also the absence of peak at m/z 163 [M–H] relayed to TQ confirmed the complete reaction which also confirmed by comparison the compound by authentic sample on TLC System ethyl acetate:hexane (1:9). All spectral data confirmed the presence of pure HTQ compound, and its purity was confirmed by HPLC.

2.5. Synthesis of Dithymoquinone DTQ (3)

In a 500 mL glass beaker, compound 1 (0.50 g) was dissolved in 5.0 mL acetone. The bright yellow solution was gently rotated along the inner surfaces of the beaker until complete evaporation occurred, into a thin, crystalline layer. The resulting thin layer (solid state) of 1 was exposed to UV lamp (345 nm max) in a fume hood at room temperature. The reaction was found to be greater than 99% complete after 8 h. The photodimerization reaction was monitored by TLC. Crude product was dissolved in a small amount of DCM, loaded on silica gel, and then purified by column chromatography using silica gel as the stationary phase and hexane:ethyl acetate (9:1) mobile phase. Compound (DTQ) was dissolved in a minimal volume of ethyl acetate, transferred to a smaller Erlenmeyer flask, and then evaporated to dryness over gentle heat. Crystallization of compound was performed using ethanol to give fine, pale yellow needle-like crystals, ultra-pure water and cold 2-propanol, re-centrifuged and lyophilized overnight to dryness. The method was followed as the method described by Elsharqawy et al., [20].

2.6. Characterization of compound 3 DTQ

Compound 3, 110 mg, 22% yield, m.p. $200.5\text{ }^\circ\text{C}$, UV_{max} 250 nm and UV_{min} 380 nm. IR (solid state): 3060 cm^{-1} (vinylic $C=C-H$ stretch), $2969\text{--}2872\text{ cm}^{-1}$ ($C-H$ stretch of aliphatic groups), 1H -NMR (600 MHz), δ 6.70 (s, 2H), 3. (s, 2H), 3.12–3.06 (septet, $j = 6.6, 2H$), 1.22 (s, 6H), 1.16–1.13 (2d, $j = 7.2, 6.6, 12H$). ESIMS: 329 [M + 1].

2.7. Cytotoxicity assay

To assess the half maximal cytotoxic concentration (CC_{50}), stock solutions of the test compounds were prepared in 10% DMSO in ddH_2O and diluted further to the working solutions with DMEM. The cytotoxic activity of the extracts was tested in VERO-E6 cells by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method with minor modifications. Briefly, the cells were seeded in 96 well-plates ($100\text{ }\mu\text{l}$ /well at a density of 3×10^5 cells/ml) and incubated for 24 h at $37\text{ }^\circ\text{C}$ in 5% CO_2 . After 24 h, cells were treated with various concentrations of the tested compounds in triplicates. 24 h later, the

supernatant was discarded, and cell monolayers were washed with sterile 1x phosphate buffer saline (PBS) 3 times and MTT solution ($20\text{ }\mu\text{l}$ of 5 mg/mL stock solution) was added to each well and incubated at $37\text{ }^\circ\text{C}$ for 4 h followed by medium aspiration. In each well, the formed formazan crystals were dissolved with $200\text{ }\mu\text{l}$ of acidified isopropanol (0.04 M HCl in absolute isopropanol = 0.073 mL HCl in 50 mL isopropanol). Absorbance of formazan solutions was measured at λ max 540 nm with 620 nm as a reference wavelength using a multi-well plate reader. The percentage of cytotoxicity compared to the untreated cells was determined with the following equation.

The plot of % cytotoxicity versus sample concentration was used to calculate the concentration which exhibited 50% cytotoxicity (CC_{50}) [21].

2.8. Inhibitory concentration 50 (IC_{50}) determination

In 96-well tissue culture plates, 2.4×10^4 Vero-E6 cells were distributed in each well and incubated overnight at a humidified $37\text{ }^\circ\text{C}$ incubator under 5% CO_2 conditions. The cell monolayers were then washed once with 1x PBS and subjected to virus adsorption (hCoV-19/Egypt/NRC-03/2020 (Accession Number on GSAID: EPI_ISL_430820)) for 1 h at room temperature (RT). The cell monolayers were further overlaid with $100\text{ }\mu\text{l}$ of DMEM containing varying concentrations of the test compounds. Following incubation at $37\text{ }^\circ\text{C}$ in a 5% CO_2 incubator for 72 h, the cells were fixed with $100\text{ }\mu\text{l}$ of 4% paraformaldehyde for 20 min and stained with 0.1% crystal violet in distilled water for 15 min at RT. The crystal violet dye was then dissolved using $100\text{ }\mu\text{l}$ absolute methanol per well and the optical density of the color was measured at 570 nm using Anthos Zenyth 200rt plate reader (Anthos Labtec Instruments, Heerhugowaard, Netherlands). The IC_{50} of the compound is that required to reduce the virus-induced cytopathic effect (CPE) by 50%, relative to the virus control.

2.9. Statistical analysis

Statistical tests were carried out using GraphPad Prism 5.01 software. Data are presented as average of means. The IC_{50} and CC_{50} curves represent the nonlinear fit of Normalized Transform of the obtained data.

3. Result and discussion

Thymoquinone was used for synthesis hydrothymoquinone (HTQ) and dithymoquinone (DTQ) to study their antiviral activity.

Compounds HTQ and DTQ were synthesized by the method described in the material and methods part and the identification of compounds was followed by UV, IR, 1H -NMR and ESIMS. The purity of compounds was confirmed by HPLC. And the data were present in supplementary material.

Table 1
Structure of some compounds of *Nigella sativa* for antiviral screening against COVID-19.

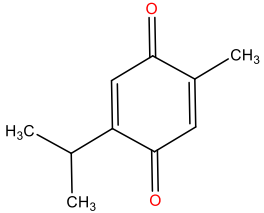
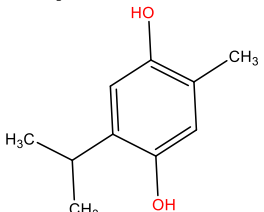
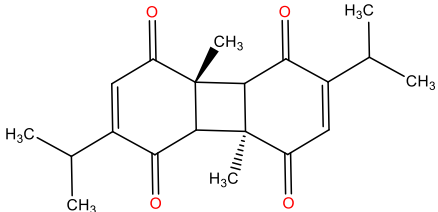
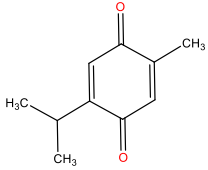
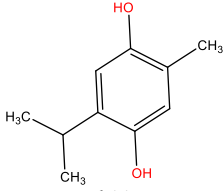
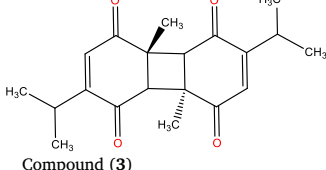
Compound	Name, formula and molecular weight
 <p>Compound (1)</p>	<ul style="list-style-type: none"> Name: Thymoquinone Synonyms: 2-isopropyl-5-methylcyclohexa-2,5-diene-1,4-dione Molecular formula: C₁₀H₁₂O₂ Molecular weight: 164,20 g/mol
 <p>Compound (2)</p>	<ul style="list-style-type: none"> Name: 2,5-Dihydroxy-<i>para</i>-cymene Synonyms: 2-methyl-5-propan-2-ylbenzene-1,4-diol; 2-isopropyl-5-methylbenzene-1,4-diol; Thymoquinol; Thymoquinone; 5-isopropyl-2-methylhydroquinone; 2-Methyl-5-isopropylhydroquinone; 2-Isopropyl-5-methyl-1,4-benzenediol; 2-Methyl-5-isopropylbenzene-1,4-diol; 2-isopropyl-5-methylbenzene-1,4-diol; 2-methyl-5-propan-2-ylbenzene-1,4-diol; 2-methyl-5-propan-2-yl-benzene-1,4-diol; 2-Methyl-5-(1-methylethyl)1,4-benzenediol Molecular formula: C₁₀H₁₄O₂ Molecular weight: 166.22 g/mol Toxicity of compound: LD50 = 25 mg/kg [3] Name: Dithymoquinone Synonyms: (4aR,8aS)-2,6-diisopropyl-4a,8a-dimethyl-4a,4b,8a,8b-tetrahydrobiphenylene-1,4,5,8-tetraone Molecular Formula: C₂₀H₂₄O₄ Molecular weight: 328,17 g/mol
 <p>Compound (3)</p>	

Table 2
Osiris analysis of compounds 1–3.

Compound	Toxicity Risks	Solubility	Drug-Score
 <p>Compound (1)</p>	<p>Toxicity Risks</p> <ul style="list-style-type: none"> mutagenic ? tumorigenic ? irritant ? reproductive effective ? 	<p>cLogP 1.64</p> <p>Solubility -1.68</p> <p>Molweight 164.0</p>	<p>TPSA 34.14</p> <p>Druglikeness -1.2</p> <p>Drug-Score 0.35</p>
 <p>Compound (2)</p>	<p>Toxicity Risks</p> <ul style="list-style-type: none"> mutagenic ? tumorigenic ? irritant ? reproductive effective ? 	<p>cLogP 2.5</p> <p>Solubility -2.24</p> <p>Molweight 166.0</p>	<p>TPSA 40.46</p> <p>Druglikeness -6.33</p> <p>Drug-Score 0.22</p>
 <p>Compound (3)</p>	<p>Toxicity Risks</p> <ul style="list-style-type: none"> mutagenic tumorigenic irritant reproductive effective 	<p>cLogP 2.73</p> <p>Solubility -3.12</p> <p>Molweight 328.0</p>	<p>TPSA 68.28</p> <p>Druglikeness 0.92</p> <p>Drug-Score 0.72</p>

3.1. Antiviral assay

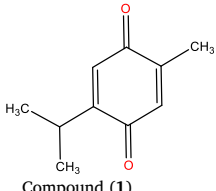
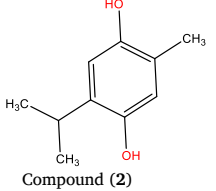
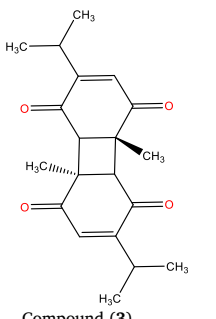
To identify the proper concentrations to define the antiviral activity of the selected drugs, half-maximal cytotoxic concentration “CC₅₀” was calculated by MTT assay for each individual drug. The sample showed anti-SARS-CoV-2 activity at non-cytotoxic nanomolar concentrations in

vitro with low aselectivity index (CC₅₀/IC₅₀, 31.74/23.15 = 1.4). Whereby dithymoquinone shows high cytotoxicity as in Fig. 1.

3.2. POM analyses of compounds 1–3

The three compounds 1–3 were also screened for the in-silico POM

Table 3
Molinspiration analysis of compounds 1–3.

Compound	Molecular Properties	Bioactivity Scores
 <p>Compound (1)</p>	cLogP 1.90	GPCR ligand -1.40
	TPSA 34.14	Ion channel modulator -0.31
	natoms 12	Kinase inhibitor -1.27
	MW 164.20	Nuclear receptor ligand -1.47
	nON 2	Protease inhibitor -1.45
	nOHNH 0	Enzyme inhibitor -0.40
	nviolations 0	
nrotb 1		
 <p>Compound (2)</p>	cLogP 3.26	GPCR ligand -0.92
	TPSA 40.46	Ion channel modulator -0.44
	natoms 12	Kinase inhibitor -1.06
	MW 166.22	Nuclear receptor ligand -0.54
	nON 2	Protease inhibitor -1.17
	nOHNH 2	Enzyme inhibitor -0.46
	nviolations 0	
nrotb 1		
 <p>Compound (3)</p>	cLogP 1.70	GPCR ligand -0.18
	TPSA 68.28	Ion channel modulator -0.09
	natoms 24	Kinase inhibitor -0.48
	MW 328.41	Nuclear receptor ligand 0.14
	nON 4	Protease inhibitor -0.10
	nOHNH 0	Enzyme inhibitor 0.10
	nviolations 0	
nrotb 2		
volume 310.56		

study to calculate various general properties along with the prediction of antiviral bioactivity. Data was analyzed and compared with a standard anti-malaria Artemisinin drug. Osiris and Molinspiration are two cheminformatic based software tools which help in calculation of toxicity risks, molecular properties as well as in the forecasting of bioactivity scores of the screened compounds.

As our tested compounds 1–3 have a molecular weight of less than 500 g/mol, so they may be highly absorbed because most of the traded drugs, i.e., approximately 80% of them have molecular weights in this range (Table 1). In contrast to compounds 1 and 3 which have a negative drug-score, the dimer drug with a molecular weight of 328 g/mol also shows best drug likeness at 92% with an exceptional drug-score at 72%. The drug likeness of compounds 1 and 2 are -1.2 and -6.33 and their drug-scores are limited to 35% and 22%, respectively (Table 1).

From Molinspiration data (Table 2) it was concluded that the series of tested compounds 1–3 satisfy the rule of Lipinski and behave as a drug and exclusively whereby only the dimer compound 3 has a nuclear receptor ligand along with enzyme inhibition properties. The cLogP value of the compounds 1–3 falls in the standard range, (i.e., less than 5) therefore these compounds may be highly hydrophilic and thus, meet

Table 4
Atomic charge of Oxygen of the antiviral (O,O') pharmacophore sites of 1–3.

Compd.	O1	O2	O3	O4	Distance (Å)	Pharmacophore sites
1	-0.32	-0.32	—	—	O1-O2 5.99	One Medium Antiviral Site
2	-0.28	-0.28	—	—	O1-O2 5.99	One Medium Antiviral Site
3	-0.30	-0.30	-0.30	-0.30	O1-O2 4.12	Two Medium Antiviral
					O3-O4 4.12	plus
					O1-O3 5.98	Two Strong Antiviral
					O2-O4 5.98	Sites

the criteria of market drugs (Table 3).

3.3. Identification of antiviral pharmacophore sites of compounds 1–3

The invention of POM Theory leads us to identify each type of pharmacophore sites with real success, on the basis of semi-empirical data of about 7,000 antibacterial, antifungal, antitumor and antiviral commercial and new drugs. All details of therapeutic applications of POM Theory are given in the literature and the identification of different and various types of pharmacophore sites is well established [23–98].

The Atomic charge calculation of compounds 1–3 (Table 4) show that all oxygen atoms are negatively charged (Fig. 2). The distance between any couple of two oxygen can be obtained after optimization of molecular structure by using the Petra program.

4. Discussion

COVID-19 is considered a critical threat to public health, and what aggravated the situation is that there is no existing antiviral therapy that is clinically approved for the management of this disease. Searching for new drugs for this disease is a global duty for scientists, so the current study concern searching for new drugs from natural plants have a history in the treatment of many diseases, antiviral, antioxidant, antidiabetic and anticancer.

In this study, three proposed anti-COVID-19 supportive drugs/treatments; Thymoquinone (1), hydrothymoquinone (2), and Dithymoquinone (3) have been analysed, and their pharmacophore sites are characterized via bioinformatic POM analysis. Osiris calculations of analysed compounds reveal that Thymoquinone and dihydrothymoquinone are relatively moresafe than hydroxychloroquine. The dithymoquinone is the safest one. The most important antiviral activities of dithymoquinone and dihydrothymoquinone agree with the POM results obtained and the tested Thymoquinone derivatives via a synergic mechanism.

In vitro study the results revealed among two investigated compound, HTQ has anti-SARS-CoV-2 activity at non-cytotoxic nanomolar concentrations in vitro with low aselectivity index (CC₅₀/IC₅₀, 31.74/23.15 = 1.4). Whereby DTQ shows high cytotoxicity, So the compounds isolated from *N.sativia* and *nigella sativa* plant may be used in treatment of CoV-19 after further clinical studies.

Mode of action still has not been identified, however, Molecular docking studies on the TQ showed a notable antiviral activity against a SARS-CoV-19 strain isolated from Egypt, whereby a study revealed that TQ has potent antiviral activity through binding to the receptor. Its binding domain on the spike and envelope proteins of SARS-CoV-19, which may hinder virus entry into the host cell and inhibit its ion channel and pore-forming activity [92]. The authors of this current study suggest the same mechanism of HTQ inhibition, therefore this will be discussed in a future study.

5. Conclusion

The current study highlights in vitro antiviral studies on two compounds from three major compounds in *Nigella sativa* depending on bioinformatic analysis, which reveals the importance of three major

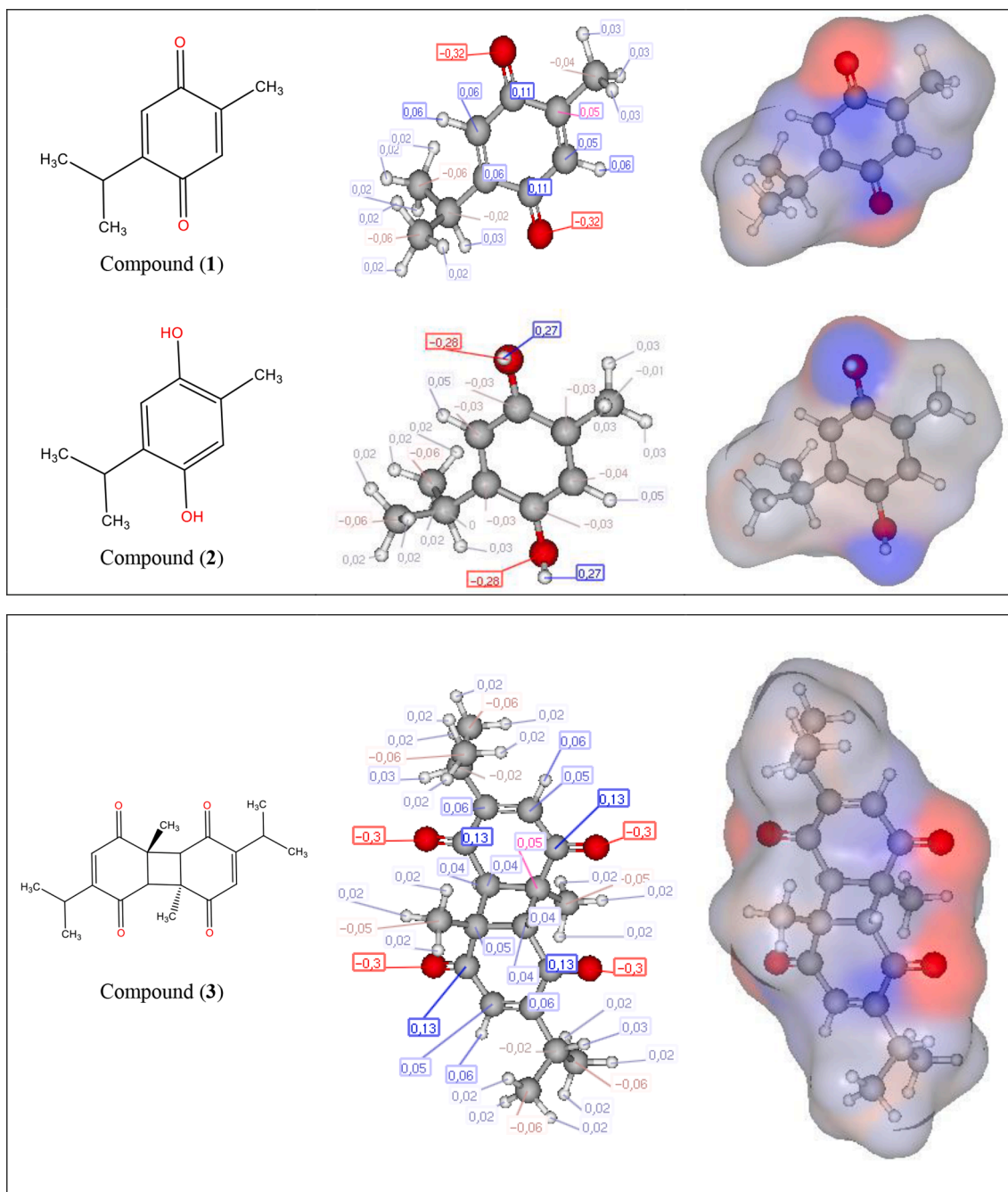


Fig. 2. Atomic charge of compounds 1–3.

compounds (TQ, DTQ, and HTQ), as antiviral agents. *in vitro* antiviral screening on two compounds (DTQ and HTQ) showed the tested drugs exhibited a promising *in vitro* activity against COVID-19, and have promising antiviral activities, further investigations in clinical trials to determine actual *in vivo* activity in the treatment of COVID-19 have recommended.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bioorg.2021.105587>.

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