Moroccan Medicinal plants as inhibitors against SARS-CoV-2 main protease: Computational investigations

I. Aanouz, A. Belhassan, K. El-Khatabi, T. Lakhlifi, M. El-ldrissi and M. Bouachrine

Faculty of Science, Molecular Chemistry and Natural Substances Laboratory, Moulay Ismail University of Meknes, Meknes, Morocco;
EST Khenifra, Sultan Moulay Sliman University, Khenifra, Morocco

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ABSTRACT

The new Corona-virus, recently called the severe acute respiratory syndrome Coronavirus (SARS-CoV-2) appears for the first time in China and more precisely in Wuhan (December 2019). This disease can be fatal. Seniors, and people with other medical conditions (diabetes, heart disease ...), may be more vulnerable and become seriously ill. This is why research into drugs to treat this infection remains essential in several research laboratories. Natural herbal remedies have long been the main, if not the only, remedy in the oral tradition for treating illnesses. Modern medicine has known its success thanks to traditional medicine, the effectiveness of which derives from medicinal plants. The objective of this study is to determine if the components of natural origin have an anti-viral effect and which can prevent humans from infection by this coronavirus using the most reliable method is molecular docking, which used to find the interaction between studied molecules and the protein, in our case we based on the inhibitor of Coronavirus (nCoV-2019) main protease. The results of molecular docking showed that among 67 molecules of natural origin, three molecules (Crocin, Digitoxigenin, and β-Eudesmol) are proposed as inhibitors against the coronavirus based on the energy types of interaction between these molecules and studied protein.

HIGHLIGHTS

- Determine natural compounds that can have an anti-viral effect and which can prevent humans from infection by this coronavirus;
- Molecular docking to find interaction between the molecules studied and the receptor of COVID-19;
- The synthesis of these molecules and the evaluation of their in vitro activity against SARS-Cov-2 could be interesting.

Introduction

Coronaviruses are non-segmented positive-sense RNA viruses which are part of the family Coronaviridae distributed in humans (Richman et al., 2016), and the subfamily Orthocoronaviridae, there are four genera of coronaviruses; Betacoronavirus, Alpha coronavirus, Gamma coronavirus and Deltacorona virus (Schwartz & Graham, 2020).

In humans, especially common colds affecting children and adults, are called mild illnesses. However, there are two zoonotic coronaviruses which are cited: The Middle East Respiratory Syndrome Coronavirus (MERS-CoV) and Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) which can especially affect the respiratory system through serious infections. In addition, the latter have the same characteristics:
| No | Compound                  | Plant                          | %   |
|----|---------------------------|-------------------------------|-----|
| 1  | Estragol                  | Foeniculum Vulgare            | 5.29|
| 2  | β-Phellandren             | Nerium Oleander               | 4.84|
| 3  | β-Eudesmol                | Lauris Nobilis L              | 2.39|
| 4  | Amorphan                  | Nerium Oleander               | 8.11|
| 5  | α-Terpinyl acetate        | Lauris Nobilis L              | 10.49|
| 6  | α-Terpeneol               | Myrtus Communis L             | 3.84|
| 7  | Myrtenyl Acetate          | Myrtus Communis L             | 25.05|
| 8  | β-Thujone                 | Sauge Officinal               | 3-8.5|
| 9  | Camphe                    | Sauge Officinal               | 4.5-24.5|
| 10 | α-Thujone                 | Sauge Officinal               | 18-43|
| 11 | Isoamyl-2-methyl butyrate | Ammi Visnaga L               | 27.68|
| 12 | Cis- linalool oxide       | Ammi Visnaga L               | 2.14|
| 13 | α-Terpinene               | Ammi Visnaga L               | 3.97|
| 14 | Amyl valerate             | Ammi Visnaga L               | 9.98|
| 15 | Amyl isobutyrate          | Ammi Visnaga L               | 16.04|
| 16 | Allyl-methyl disulfide    | Allium Sativum               | 1.71|
|    |                           | Thymelea Tartonraira          | 2.96|
|    |                           | Artemisia Vulgaris            | 2.58|
| 17 | Limonene                  | Foeniculum Vulgare            | 8.14|
|    |                           | Nerium Oleander               | 5.01|
| 18 | Diallyl sulfide           | Allium Sativum                | 0.66|
| 19 | Diallyl disulfide         | Allium Sativum                | 14.30|
| 20 | Diallyl trisulfide        | Allium Sativum                | 46.52|
| 21 | Tricyclene                | Thymelea Tartonraira          | 7.11|
|    |                           | Thymelea Tartonraira          | 3.1 |
| 22 | β-Carophyllene            | Eugenia Caryophyllus          | 5-14|
| 23 | D-Camphor                 | Thymelea Tartonraira          | 37.92|
|    |                           | Artemisia Vulgaris            | 16.72|
| 24 | Camphene                  | Thymelea Tartonraira          | 14.66|
|    |                           | Artemisia Vulgaris            | 8.35|
| 25 | Borneol                   | Thymelea Tartonraira          | 2.45|
|    |                           | Artemisia Vulgaris            | 19.65|
| 26 | α-Pinene                  | Thymelea Tartonraira          | 10.98|
|    |                           | Nerium Oleander               | 10 |
| 27 | 1-8 Cineol                | Thymelea Tartonraira          | 8.39|
|    |                           | Myrtus Communis L             | 43.03|
|    |                           | Nerium Oleander               | 6.58|
|    |                           | Artemisia Vulgaris            | 3.23|
|    |                           | Eugenia Caryophyllus          | 80 |
| 28 | Eugenol                   | Lauris Nobilis L              | 2.15|
|    |                           | Eugenia Caryophyllus          | 6.6 |
| 29 | Acetyl-eugenol            | Lauris Nobilis L              | 3.98|
|    |                           | Nerium Oleander               | 2.39|
|    |                           | Nerium Oleander               | 3.22|
| 30 | Sabinene                  | Lauris Nobilis L              | 3.15|
|    |                           | Mentha Rotundifolia           | 85.47|
| 31 | Pulegone                   | Lauris Nobilis L              | 4.10|
|    |                           | Artemisia Vulgaris            | 6.77|
|    |                           | Lauris Nobilis L              | 8.78|
|    |                           | Ammi Visnaga L                | 22.71|
| 32 | Methyl - Eugenol          | Lauris Nobilis L              | 6.95|
|    |                           | Lavandula Stoechas            | 6.94|
| 33 | Linalool                  | Lauris Nobilis L              | 30.52|
| 34 | Fenchone                  | Lauris Nobilis L              | 4.69|
|    |                           | Cinnamomum Cassia             | 90.08|
|    |                           | Allium Cepa L                 | 8.09|
|    |                           | Thymelea Tartonraira          | 6.86|
| 35 | 2-carboxylic acid, 3- methyl thiophene | Allium Cepa L | 8.96|
| 36 | Eucalyptol                | Lauris Nobilis L              | n.d |
|    |                           | Cinnamomum Cassia             | 4.69|
| 37 | Trans-Cinnamylacetat      | Cinnamomum Cassia             | 90.08|
|    |                           | Allium Cepa L                 | 8.09|
| 38 | Trans-Cinnamaldehyde      | Allium Cepa L                 | 8.66|
| 39 | Undecane                  | Allium Cepa L                 | 8.96|
|    |                           | Thymeus Broussonetti          | 22.56|
|    |                           | Thymeus Broussonetti          | 8.99|
| 40 | Undecane-2,6-dimethyl     | Allium Cepa L                 | 28.69|
|    |                           | Crocus Sativus L              | n.d |
|    |                           | Thymeus Broussonetti          | 11 |
| 41 | 2-carboxylic acid, 3- methyl thiophene | Allium Cepa L | n.d |
| 42 | Safranal                  | Thymeus Broussonetti          | 22.56|
|    |                           | Thymeus Broussonetti          | 8.99|
| 43 | pi-Cymene                 | Crocus Sativus L              | 11 |
|    |                           | Thymeus Broussonetti          | 8.99|
| 44 | Picrocrocin               | Crocus Sativus L              | 28.69|
|    |                           | Thymeus Broussonetti          | 11.25|
| 45 | Nerium                   | Crocus Sativus L              | N.D |
|    |                           | Crocus Sativus L              | N.D |
|    |                           | Nerium Oleander               | 5.12|
| 46 | γ-Terpinene                | Nerium Oleander               | 5.82|
| 47 | Dodecan                   | Allium Cepa L                 | 5.82|
| 48 | Digitoxigenine            | Nerium Oleander               | 11.25|
| 49 | Crocin                    | Crocus Sativus L              | N.D |
| 50 | Crocetin                  | Crocus Sativus L              | N.D |
| 51 | Galarene                  | Nerium Oleander               | 5.12|
| 52 | 1,2,4-Trithiolane, 3, 5-dimethyl | Allium Cepa L | 5.82|

(continued)
et al., 2020; Huang et al., 2020), was reported in Wuhan, the coronavirus (COVID-19; previously known as 2019-nCoV) (Wu et al., 2020). In late December 2019, an outbreak of a new disease, the coronavirus (COVID-19; previously known as 2019-nCoV) (Wu et al., 2020; Huang et al., 2020), was reported in Wuhan, the capital from Hubei province and a large city of around 11 million people in the central region of the People's Republic of China (Zhu, 2020), which subsequently affected 26 countries around the world. China immediately declared the epidemic to the World Health Organization (WHO) and also shared the sequence information with the international community after the discovery of the causative agent. WHO has done its part by coordinating the development of the diagnosis; issue guidelines for patient monitoring, sample collection and treatment; and provide up-to-date information on the epidemic (Munster et al., 2020). To date, the primary source of infection has been pneumonia patients infected with COVID-19. Transmission of respiratory droplets is the main route of transmission, which can also be transmitted by contact (G. O. of N. H. Committee & Office of State Administration of Traditional Chinese Medicine, 2020). The steps for preparing ligands and proteins for docking were done in the Autodock 1.5.4 tools from MGL Tools package employing default settings (Morris et al., 1998), a grid box \((x = -26,283, y = 12,599, z = 58,965)\) at 1 Angstrom spacing, the bioactive conformations were simulated employing Autodock vina (Trott & Olson, 2010). For autodock vina study, an extended PDB format, termed PDBQT, is used for cordonnante files, which includes atomic partial charges and atom types. Torsion angles were calculated to assign the flexible and non-bonded rotation of molecules. The results were subsequently analyzed using Discovery studio 2016 (Pilot, 2016) and PyMol (Delano, 2002). The crystal structure of Coronavirus (COVID-19) was downloaded from the protein databank (http://www.rcsb.org), and its original ligand and water were eliminated, then all compounds from our study were made a selection of plants based on two principles: the first one is oral efficacy, this means that the majority of Moroccan plants should be absorbable by the oral route, the second one is the compatibility of traditional use, then we have used molecular docking study to selected potential compounds that could have an anti-coronavirus effect by fighting their energy and type of interactions in studied enzyme.

### Table 1. Continued.

| N° | Compound               | Plant                | %  |
|----|------------------------|----------------------|----|
| 53 | Trans-Anethole         | Foeniculum Vulgare   | 53.2 |
| 54 | Undecan,2methyl        | Allium Cepa L        | 3.59 |
| 55 | 6-Isopropenyl-4,8a-Dimethyl-1,2,3,5,6,7,8,8a-Octahydronephthalene-2,3-diol | Lavandula Stoechas | 4.56 |
| 56 | α-Terpinene            | Myrtus CommunisL     | 2.90 |
| 57 | β-Ocimene              | Artemisia Vulgaris   | 2.21 |
| 58 | Bicyclogermacrene      | Artemisia Vulgaris   | 3.18 |
| 59 | Acetat Bomyl           | Lavandula Stoechas   | 8.86 |
| 60 | Cubenol                | Lavandula Stoechas   | 2.55 |
| 61 | D-Fenchol              | Lavandula Stoechas   | 6.62 |
| 62 | Geranyl Acetat         | Myrtus CommunisL     | 2.85 |
| 63 | Germacrene D           | Artemisia Vulgaris   | 3.82 |
| 64 | 2-Methyl-9-(prop-1-en-3-ol-2-yl)(Bicyclo[4.4.0]dec-2-en-4-yl) | Lavandula Stoechas | 4.50 |
| 65 | Mycrene                | Artemisia Vulgaris   | 3.8  |
| 66 | Thymol                 | Thymus Broussoneti   | 63.09 |
| 67 | Viridifloral           | Lavandula Stoechas   | 6.10 |

In our study we made a selection of plants based on two principles: the first one is oral efficacy, this means that the majority of Moroccan plants should be absorbable by the oral route, the second one is the compatibility of traditional use, then we have used molecular docking study to selected potential compounds that could have an anti-coronavirus effect by fighting their energy and type of interactions in studied enzyme.

### 2. Materials and methods

#### 2.1. Data set

For this study we have selected 67 compounds extracted from different aromatic and medicinal plants, Table 1 shows the origin for each studied compound and the percentage present in each plant. These molecules were considered to molecular docking study.

#### 2.2. Molecular docking

Molecular docking analysis was used to study the binding affinity and the type of interactions between all compounds (67 molecules) and the target (Coronavirus (2019-nCoV) main protease). The steps for preparing ligands and proteins for docking protocol were done in the Autodock 1.5.4 tools from MGL Tools package employing default settings (Morris et al., 1998), a grid box \((x = -26,283, y = 12,599, z = 58,965)\) at 1 Angstrom spacing, the bioactive conformations were simulated employing Autodock vina (Trott & Olson, 2010). For autodock vina study, an extended PDB format, termed PDBQT, is used for cordonnante files, which includes atomic partial charges and atom types. Torsion angles were calculated to assign the flexible and non-bonded rotation of molecules. The results were subsequently analyzed using Discovery studio 2016 (Pilot, 2016) and PyMol (Delano, 2002). The crystal structure of Coronavirus (2019-nCoV) main protease (PDB entry code: 6lu7) was downloaded from the protein databank (http://www.rcsb.org), and its original ligand and water were eliminated, then all compounds from our study were made a selection of plants based on two principles: the first one is oral efficacy, this means that the majority of Moroccan plants should be absorbable by the oral route, the second one is the compatibility of traditional use, then we have used molecular docking study to selected potential compounds that could have an anti-coronavirus effect by fighting their energy and type of interactions in studied enzyme.

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data set were docked in the active site of the studied protein. The preparation of the PDB file was done using Discovery Studio 2016 (Pilot, 2016).

### Table 2. Flavor agents docking results.

| No. | Compound | Binding Energy (Kcal/mol) |
|-----|----------|--------------------------|
| 1   |          | -4.7                     |
| 2   |          | -4.7                     |
| 3   |          | -7.1                     |
| 4   |          | -5.8                     |
| 5   |          | -5.7                     |
| 6   |          | -6.1                     |
| 7   |          | -5.9                     |
| 8   |          | -5.6                     |
| 9   |          | -5.6                     |
| 10  |          | -5.6                     |
| 11  |          | -4.8                     |
| 12  |          | -4.4                     |
| 13  |          | -4.9                     |
| 14  |          | -4.3                     |
| 15  |          | -4.3                     |
| 16  |          | -3.2                     |
| 17  |          | -4.6                     |
| 18  |          | -2.9                     |
| 19  |          | -2.9                     |
| 20  |          | -3.3                     |
| 21  |          | -4.6                     |
| 22  |          | -6.1                     |
| 23  |          | -4.7                     |
| 24  |          | -5                      |
| 25  |          | -4.8                     |
| 26  |          | -4.8                     |
| 27  |          | -5.1                     |
| 28  |          | -5.5                     |
| 29  |          | -5.5                     |
| 30  |          | -5                      |
| 31  |          | -5.1                     |
| 32  |          | -5.2                     |
| 33  |          | -5.1                     |
| 34  |          | -4.4                     |
| 35  |          | -5.3                     |
| 36  |          | -5.3                     |
| 37  |          | -5.4                     |
| 38  |          | -5.1                     |
| 39  |          | -3.9                     |
| 40  |          | -4.5                     |
| 41  |          | -4.1                     |
| 42  |          | -5.3                     |
| 43  |          | -5                      |
| 44  |          | -6.8                     |
| 45  |          | -6.9                     |
| 46  |          | -4.9                     |
| 47  |          | -3.9                     |
| 48  |          | -7.2                     |
| 49  |          | -8.2                     |
| 50  |          | -6.2                     |
| 51  |          | -6.1                     |
| 52  |          | -3.1                     |
| 53  |          | -4.5                     |
| 54  |          | -4                      |
| 55  |          | -6.4                     |
| 56  |          | -4.9                     |
| 57  |          | -4.3                     |
| 58  |          | -6.1                     |
| 59  |          | -5.5                     |
| 60  |          | -5.9                     |
| 61  |          | -5                      |
| 62  |          | -5.1                     |
| 63  |          | -5.9                     |
| 64  |          | -6.1                     |
| 65  |          | -4.1                     |
| 66  |          | -4.9                     |
| 67  |          | -5.8                     |

### 3. Results and discussion

Totally, we have docked 67 components, Table 2 shows the binding affinity of natural compounds toward main protease, and Table 3 mentioned the 11 top flavor agent docking result based on binding energy.

Table 2 shows the obtained results from the molecular docking study carried out on all the molecules present in different medicinal plants (67 molecules), by giving the interaction energy for each compound, there is a difference in energy between each ligand and nCOV-19 main protease.

By comparing all studied molecules with Chloroquine on the basis of the interaction energy criterion. Knowing that the energy value of interaction of the molecule referred (Chloroquine) is (-6 kcal/mol), 11 molecules which have a good interaction with the studied enzyme are mentioned in Table 2. For example, the Crocin at interaction energy equal to (-8.2 kcal/mol), Digitoxigenin at a value of (-7.2 kcal/mol), and β-Eudesmol at a value of (-7.1 kcal/mol). The 3D binding mode of these compounds is shown in Figures 1 and 2.

From a biological or pharmacological point of view, these first three molecules which are proposed as inhibitors of Coronavirus main protease are molecules having a significant antiviral power and according to bibliographical research and experiments which have already done, the results found for each molecule of natural origin is as following:

Crocin is an important compound in Crocus Sativus L, it has the capacity to inhibit the replication of HSV before and after the entry of virions in Vero cells. Crocin could be a promising anti-HSV and anti-HIV agent for herbal medicine against viral infections (Soleymani et al., 2018).

Digitoxigenin: represents 11.25% of the quantity present in Nerium Oleander, the derivatives of these molecules are used as antiviral and anti-cancer inhibitors (Boff et al., 2019).

β-Eudesmol despite its low amount of Laurus Nobilis L which contains only 2.39%, but this compound has a good interaction with the target, and it has significant antibacterial and antiviral power (Astani et al., 2011).

In our study, we took into account the interaction energies, so the interactions of the 1st level concern the hydrogen bonds, those of the 2nd level concerning the interactions between π systems and cation -π interactions, while the interactions of the 3rd level are hydrophobic contacts and non-specific Van der Waals interactions between aliphatic or aromatic carbon atoms. These interactions are generally spherical with a radius of 4Å and cover most of the ligand. So, for the displacement of ligand in the binding site of our enzyme, the first level of interactions was considerate as the most important, the presence of Hydrogen bond interaction in selected complex explains that we have a good interaction between the three molecules and the studied protein (Table 4, Figure 3 and 4) (Adnan, 2019).
### Table 3. Top compounds docking results.

| No. | Name              | Structure          | Binding Energy |
|-----|-------------------|--------------------|----------------|
| 3   | β-Eudesmol       | ![β-Eudesmol](image1) | -7.1           |
| 6   | α-Terpeneol      | ![α-Terpeneol](image2) | -6.1           |
| 22  | β-Carophyllene   | ![β-Carophyllene](image3) | -6.1           |
| 44  | Picrocrocin      | ![Picrocrocin](image4) | -6.8           |
| 48  | Digitoxigenine   | ![Digitoxigenine](image5) | -7.2           |

(continued)
| N° | Name                                      | Structure | Binding Energy |
|----|------------------------------------------|-----------|----------------|
| 49 | Crocin                                   | ![Crocin Structure] | −8.2           |
| 50 | Crocetin                                 | ![Crocetin Structure] | −6.2           |
| 51 | Calarene                                 | ![Calarene Structure] | −6.1           |
| 55 | Bicyclogermacrene                        | ![Bicyclogermacrene Structure] | −6.1           |
| 58 | 6-Isopropenyl-4,8a-Dimethyl-1,2,3,5,6,7,8,8a-Octahydroronaphthalene-2,3-diol | ![6-Isopropenyl-4,8a-Dimethyl-1,2,3,5,6,7,8,8a-Octahydroronaphthalene-2,3-diol Structure] | −6.4           |
4. Conclusion

Today, the search for new molecules with a preservative power of natural origin is based on ethnobotanical studies which make it possible to carry out inventories of plants in a zone or a country, then on phytochemical and pharmacological studies and well other scientific aspects, so, the importance of the use of these medicinal plants which pushed us to seek and find the molecules which can prevent SARS-CoV-2 infection. Based on molecular docking,

| No | Name | Structure | Binding Energy |
|----|------|-----------|---------------|
| 64 | 2-Methyl-9-(prop-1-en-3-ol-2-yl)bicyclo[4.4.0]deca-2-en-4-ol | ![Structure of 2-Methyl-9-(prop-1-en-3-ol-2-yl)bicyclo[4.4.0]deca-2-en-4-ol](image1.png) | -6.1 |

**Figure 1.** 2D View of the binding conformation of the Crocin inhibitor at the active site of Coronavirus (2019-nCoV) main protease.

**Figure 2.** 2D View of the binding conformation of the Digitoxigenin and β-Eudesmol inhibitors at the active site of Coronavirus (2019-nCoV) main protease.

Table 3. Continued.

| No | Name | Structure | Binding Energy |
|----|------|-----------|---------------|
| 64 | 2-Methyl-9-(prop-1-en-3-ol-2-yl)bicyclo[4.4.0]deca-2-en-4-ol | ![Structure of 2-Methyl-9-(prop-1-en-3-ol-2-yl)bicyclo[4.4.0]deca-2-en-4-ol](image1.png) | -6.1 |
the results is very satisfactory, we have found three molecules among 67 which are very interesting either on the chemical side or on the biological side and therefore we propose these three molecules as inhibitor of SARS-CoV-2 main protease. The synthesis of these molecules and the evaluation of their in vitro and in vivo activity against SARS-Cov-2 main protease could be interesting, before clinical essay.

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Disclosure statement

The authors declare that they have no competing interests.

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