Biological Chemistry & Chemical Biology

# Evaluation of the Inhibitory Activities of COVID-19 of *Melaleuca cajuputi* Oil Using Docking Simulation

Tran Thi Ai My,[a] Huynh Thi Phuong Loan,[a] Nguyen Thi Thanh Hai,[a] Le Trung Hieu,[a] Tran Thai Hoa,[a] Bui Thi Phuong Thuy,[b] Duong Tuan Quang,*[c] Nguyen Thanh Triet,[d] Tran Thi Van Anh,[e] Nguyen Thi Xuan Dieu,[e] Nguyen Tien Trung,[f] Nguyen Van Hue,[g] Pham Van Tat,[h] Vo Thanh Tung,[i] and Nguyen Thi Ai Nhung*[a]

GC-MS was applied to identify 24 main substances in *Melaleuca cajuputi* essential oil (**TA**) extracted from fresh cajeput leaves through steam distilling. The inhibitory capability of active compounds in the **TA** from Thua Thien Hue, Vietnam over the Angiotensin-Converting Enzyme 2 (ACE2) protein in human body - the host receptor for SARS-CoV-2 and the main protease (PDB6LU7) of the SARS-CoV-2 using docking simulation has been studied herein. The results indicate that the ACE2 and PDB6LU7 proteins were strongly inhibited by 10 out of 24 compounds accounting for 70.9% in the **TA**. The most powerful anticoronavirus activity is expressed in the order: Terpineol (**TA2**) ≈ Guaiol (**TA5**) ≈ Linalool (**TA19**) > Cineol (**TA1**) > β-Selinenol (**TA3**) > α-Eudesmol (**TA4**) > γ-Eudesmol (**TA7**). Interestingly, the synergistic interactions of these 10 substances of the **TA** exhibit excellent inhibition into the ACE2 and PDB6LU7 proteins. The docking results orient that the natural *Melaleuca cajuputi* essential oil is considered as a valuable resource for preventing SARS-CoV-2 invasion into human body.

## Introduction

The SARS-CoV-2 pandemic has been the hottest issue all over the world currently. The number of newly infected and died patients has been increasing day by day.[1] Finding solutions to treat and prevent SARS-CoV-2 is concerned by scientists in the world. In particular, medicine derived from natural and safe herbs is one of the research directions that deserve attention. Given the abundant supply in Vietnam and the specific healing properties of *Melaleuca cajuputi*,[2] we recommend a solution for prevention of SARS-CoV-2 by using cajeput essential oil. *Melaleuca cajuputi* (Figure 1) is one of hundreds species of *Melaleuca* genus (Myrtaceae family) that is commonly grown in Vietnam, Indonesia, and Thailand.[3] *Melaleuca cajuputi* can be used as fuelwood, piles and frame poles in construction. Its flowers attract honey bees.[4] Its timber is used for pulp and paper, fiber and particle board. It can produce qualified charcoal and potential sawn timber.[5] Especially, *Melaleuca cajuputi* essential oil is widely used for medicinal application.[6] Traditionally, cajeput oil is further used in herbal medicinal

[a] *Dr. T. T. A. My, H. T. P. Loan, N. T. T. Hai, Dr. L. T. Hieu, Prof. T. T. Hoa, Dr. N. T. A. Nhung*
Department of Chemistry, University of Sciences, Hue University, Hue City, 530000, Vietnam
E-mail: ntanhung@hueuni.edu.vn
[b] *Dr. B. T. P. Thuy*
Faculty of Fundamental Science, Van Lang University, Ho Chi Minh City 700000, Vietnam
[c] *Prof. D. T. Quang*
Department of Chemistry, University of Education, Hue University, Hue City, 530000, Vietnam
E-mail: dtquang@hueuni.edu.vn
[d] *Dr. N. T. Triet*
Faculty of Traditional Medicine, University of Medicine and Pharmacy at Ho Chi Minh City, 700000, Vietnam
[e] *Dr. T. T. V. Anh, Dr. N. T. X. Dieu*
Faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh City, 700000, Vietnam
[f] *Dr. N. T. Trung*
Laboratory of Computational Chemistry and Modeling, Department of Chemistry, Quy Nhon University, Quy Nhon City, 590000, Vietnam
[g] *Dr. N. V. Hue*
Faculty of Engineering and Food Technology, University of Agriculture and Forestry, Hue University, Hue City, 530000, Vietnam
[h] *Dr. P. V. Tat*
Department of Environmental Engineering, Hoa Sen University, Ho Chi Minh City, 700000, Vietnam
[i] *Dr. V. T. Tung*
University of Sciences, Hue University, Hue City, 530000, Vietnam

Supporting information for this article is available on the WWW under https://doi.org/10.1002/slct.202000822

**Figure 1.** Picture of *Melaleuca cajuputi* leaves.

products to relieve symptoms of coughs, colds, as a laxative, general muscle relaxant and sedative.[7]

Many pharmacological studies have shown that the major constituents of the *Melaleuca cajuputi* essential oil are 1,8-cineol and α-terpineol[4] which have antibacterial, antifungal and antiparasitic activities. Furthermore, there were also reports on the antiviral properties of essential oil from *Melaleuca* species,[7][8] that the major monoterpenes and their derivatives of the essential oil from tea tree (*Melaleuca alternifolia*) including α-terpinene, γ-terpinene, α-pinene, p-cymene, 1,4-terpineol, α-terpineol, thymol, citral, and 1,8-cineol had an antiviral activity against herpes simplex virus type 1 (HSV-1) *in vitro*.[9] The other results showed that tea tree oil and a few of its compounds (e.g. 1,4-terpinenol, terpinolene and α-terpineol) possessed an inhibitory effect on influenza A/PR/8virus subtype H1 N1 replication at non-cytotoxic concentrations.[10] The literature review indicated that cajeput oil might be a promising medicine in preventing the infection of SARS-CoV-2.

The fact is that cajeput essential oil is proved to have antifungal, anti-inflammatory, antioxidant activities.[2],[8][11] According to some folk remedies, people use cajeput oil to help increase resistance, inhibit some bacteria and viruses, freshen air in the room, which are useful for providing postnatal care to women and newborns.[2] In this current SARS-CoV-2 pandemic, there has been a great demand for preventive and curative vaccines as well as measures to prevent and stop the spread of the disease. Based on the traditional usage of using cajeput essential oil in Vietnam, the idea of our research is using the docking simulation to predict the capability of molecular structure in the cajeput oil in inhibiting Angiotensin-converting enzyme 2 (ACE2) protein in human body, causing SARS-CoV-2 to lose its host receptor and destroy its protein (PDB6LU7) at the same time. Noticeably, the ACE2 protein is the host receptor of the SARS-CoV-2 and SARS-CoV,[12],[13][14] therefore, if the ACE2 protein is inhibited, the SARS-CoV-2 could be prevented and treated. Further information of the ACE2 and PDB6LU7 proteins can be found at UniProtKB[15] and Worldwide Protein Data Bank,[16] respectively. In this study, we got the cajeput essential oil derived from Thua Thien Hue, Central Vietnam, and determined the ability to inhibit ACE2 protein in human body and PDB6LU7 protein of SARS-CoV-2. This is important in discovering medicinal herbs and developing treatments to prevent the SARS-CoV-2 pandemic.[16]

## Results and Discussion

### Docking simulation of compounds in *Melaleuca cajuputi* essential oil into ACE2 protein in human body and PBD6LU7 protein in the SARS-CoV-2

So far, to the best of our knowledge, there has been only available result related to coronavirus (SARS-CoV-2) resistance through docking simulation studies of organic compounds in garlic essential oil.[17] We demonstrate successful docking molecules of compounds in the *Melaleuca cajuputi* essential oil into the ACE2 and PDB6LU7 proteins to contribute to the orientation and encourage the use of natural products for prevention of SARS-COV-2 invasion.

The 24 active compounds in the *Melaleuca cajuputi* essential oil were docked into the ACE2 and PDB6LU7 proteins using MOE 2015.10 program. The results indicate that the 10 compounds (identification details in Table 1 and Figure S1 in the Supporting Information Summary) have the strongest interactions on the selected proteins that are the ones with the most powerful inhibitory activity for SAR-CoV-2. The remaining compounds that have weak or almost no interactions with the ACE2 and PDBLU7 proteins are not presented in the main text. Please find more details in Table S1 in the Supporting Information Summary.

Docking simulation results with the interactions between **TA1-TA5**; **TA7**; **TA10**; **TA17-TA19** compounds (accounting for 70.9% in *Melaleuca cajuputi* oil) and 2 proteins (ACE2 and PDB6LU7) are presented in Figures 2 and 4 and Figure S2 (Supporting Information Summary). The results of docking score energy (DS) and root mean square deviation (RMSD) between these 10 compounds in *Melaleuca cajuputi* oil and proteins with various interactions including hydrogen bonds, cation-π bonds, π-π bonds, and ionic interactions as well as the interaction distance between amino acids and the active sites of compounds are shown in Tables 2 and 3 and Table S2 (Supporting Information Summary).

It is found that Terpineol (**TA2**), Guaiol (**TA5**), and Linalool (**TA19**) are the best 3 compounds to have the binding ability toward the ACE2 and PDB6LU7 proteins with lots of convergence points and the inhibitory intensity of these compounds on ACE2 and PDB6LU7 proteins is almost similar (Table 2 and Figure 2).

Specifically, the **TA2** has the strongest inhibitory effects on the ACE2 protein in human body and PDB6LU7 protein in the SARS-CoV-2 (Figure 2) with the docking simulation results as follows: *i)* Docking of **TA2-ACE2** has DS of $-11.0$ kcal.mol$^{-1}$ and RMSD of 1.97 Å, while the site-site bonding interaction exhibiting the length of the binding of compound with amino acid between –OH of **TA2** and amino acids Asn 103 is 2.25 Å and 2.08 Å for Gln 101; *ii)* Docking of **TA2-SARS-CoV-2** has DS = -10.9 kcal.mol$^{-1}$ and RMSD = 1.16 Å, and site-site bonding interaction between –OH and amino acid His 163 is 2.38 Å. It can be realized that terpineol (**TA2**) is a monoterpene alcohol easily found in several essential oils such as petitgrain oil, cajuput oil, and pine oil. **TA2** has a pleasant scent similar to lilacs and it is a common ingredient in perfumes, cosmetics and fragrances.[18] The monoterpene compounds including α-terpinene, γ-terpinene, α-pinene, p-cymene, terpinen-4-ol, α-terpineol, thymol, citral and 1,8-cineol in the *Melaleuca alternifolia* essential oil were reported against HSV-1 *in vitro*.[9] Besides, α-terpineol attracts great attention from scientists because it has a wide range of biological applications such as an effective inhibition of pathogenic viruses that helps protect our health, treatment of insect bites or wounds, relieving uncomfortable itching and reducing swelling rapidly, and curing respiratory infections. Moreover, terpineol in peppermint essential oil was proved as inhibitory agent for liver microsomes with $IC_{50}$ value of 14.8 μM.[19] The use of α-terpineol in

**Table 1.** Identification of some bioactive compounds in *Melaleuca cajuputi* essential oil.

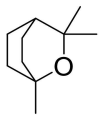
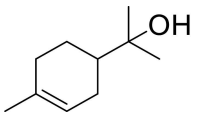
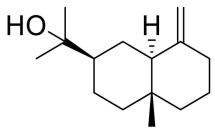
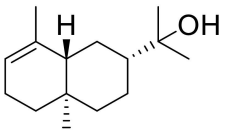
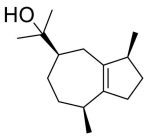
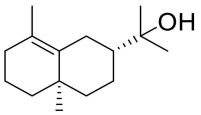
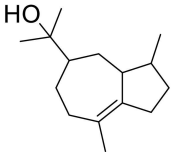
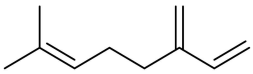
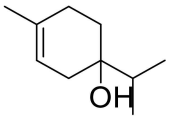
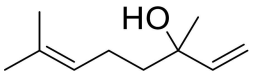
| No. | Compound | Formula | Structure | Symbol | Percentage (%) |
|---|---|---|---|---|---|
| 1 | Cineol | $C_{10}H_{18}O$ |  | **TA1** | 31.6 |
| 2 | Terpineol | $C_{10}H_{18}O$ |  | **TA2** | 10.7 |
| 3 | β-Selinenol | $C_{15}H_{26}O$ |  | **TA3** | 6.8 |
| 4 | α-Eudesmol | $C_{15}H_{26}O$ |  | **TA4** | 6.7 |
| 5 | Guaiol | $C_{15}H_{26}O$ |  | **TA5** | 6.5 |
| 6 | γ-Eudesmol | $C_{15}H_{26}O$ |  | **TA7** | 4.3 |
| 7 | Bulnesol | $C_{15}H_{26}O$ |  | **TA10** | 1.9 |
| 8 | β-Myrcene | $C_{10}H_{16}$ |  | **TA17** | 0.9 |
| 9 | Terpinen-4-ol | $C_{10}H_{18}O$ |  | **TA18** | 0.9 |
| 10 | Linalool | $C_{10}H_{18}O$ |  | **TA19** | 0.6 |

**Table 2.** Docking simulation results with docking score energy (DS) and root mean square deviation (RMSD) between the most active compounds (**TA2**, **TA5**, **TA19**) in the *Melaleuca cajuputi* oil and the ACE2 and PBD6LU7 proteins.

| Compound | Symbol compound-protein | DS (kcal·mol$^{-1}$) | RMSD (Å) | Interaction with amino acid |
|---|---|---|---|---|
| Terpineol (TA2) | TA2-ACE2 | -11.0 | 1.97 | Asn 103 (2.25 Å), Gln 101 (2.08 Å), His 195, Gln 102, Gln 81, Ala 193, Tyr 196 |
| | TA2-SARS-CoV-2 | -10.9 | 1.16 | His 163 (2.38 Å), Leu 41, Met 165, Phe 140, Glu 166, His 164, Asn 142, Ser 144, Cys 145, Gly 143, His 41 |
| Guaiol (TA5) | TA5-ACE2 | -11.1 | 1.49 | Asn 103 (2.22 Å), Gln 101 (1.80 Å), Leu 85, Án 194, His 195, Tyr 196, Gln 102 |
| | TA5-SARS-CoV-2 | -10.9 | 0.84 | His 163 (2.51 Å), Glu 166, His 164, Met 165, Met 40, Gln 189, His 41, Leu 141, Ser144, Asn 142, Phe 140, Cys 145, His 122 |
| Linalool (TA19) | TA19-ACE2 | -10.9 | 1.77 | Asn 210 ( 1.96 Å), Gln 98, Lys 562, Asp 206, Ala 396, Trp 566, Glu 208, Glu 564, Pro 565, Leu 95, Val 209, Leu 91, Lys 94 |
| | TA19-SARS-CoV-2 | -11.1 | 1.18 | Gly 143 ( 2.89 Å); Cys 145 (2.82 Å), Ser 144, Asn 142, Leu 141, Leu 27, Phe 140, His 41, Glu 166, His 163, Et 165, His 164 |

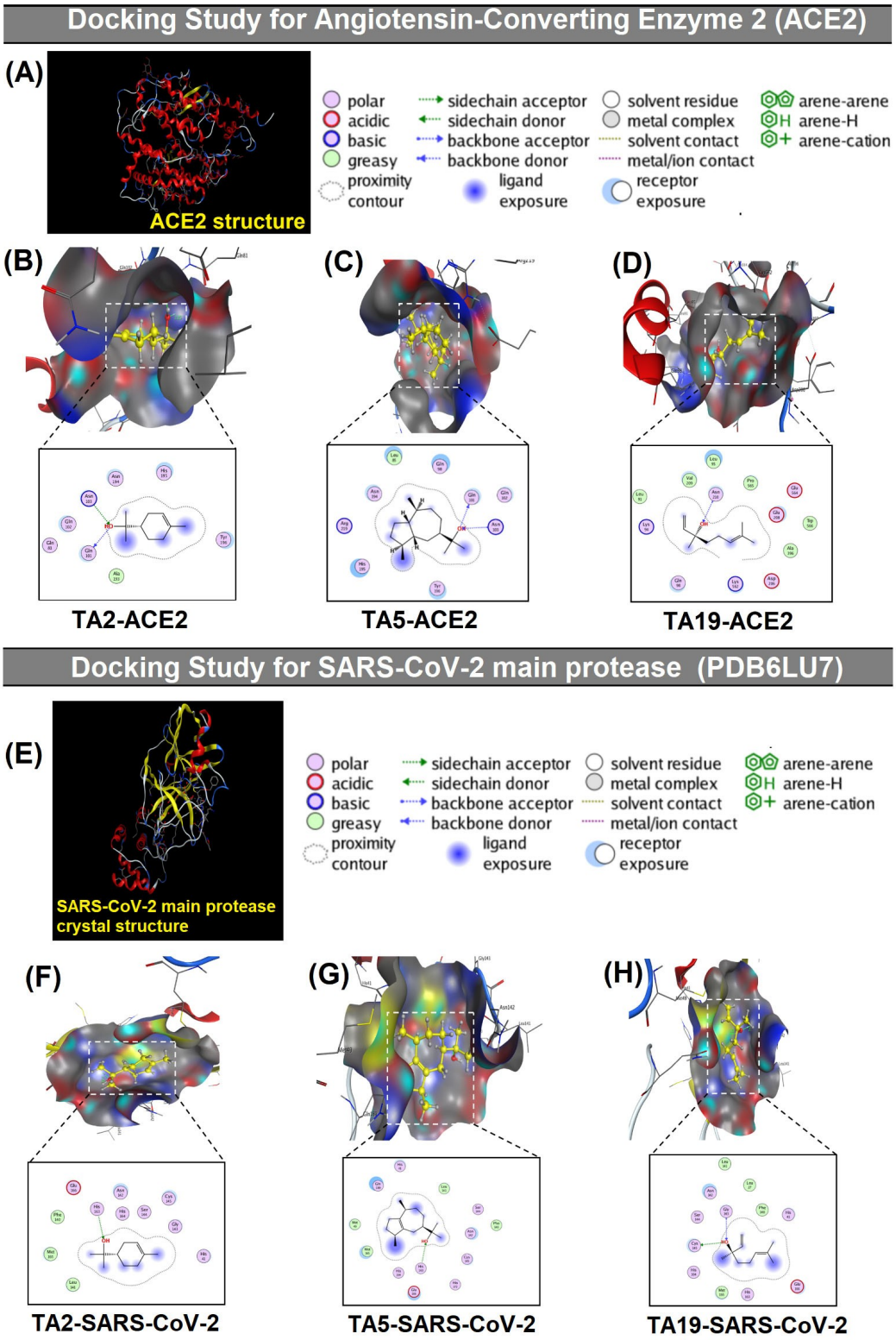



**Figure 2.** Terpineol (**TA2**), Guaiol (**TA5**), and Linalool (**TA19**) docked with ACE2 and PDB6LU7 proteins in the range of **TA2** ≈ **TA5** ≈ **TA19**; (A) Native human angiotensin converting enzyme 2 (ACE2) crystal structure. Docking simulation with the interaction between compounds (B) **TA2**, (C) **TA5**, (D) **TA19** and ACE2 protein in human body; (E) Crystal structure of the SARS-CoV-2 main protease in complex (PDB6LU7). Docking simulation with the interaction between compounds (F) **TA2**, (G) **TA5**, (H) **TA19** and PDB6LU7 protein of SARS-CoV-2.

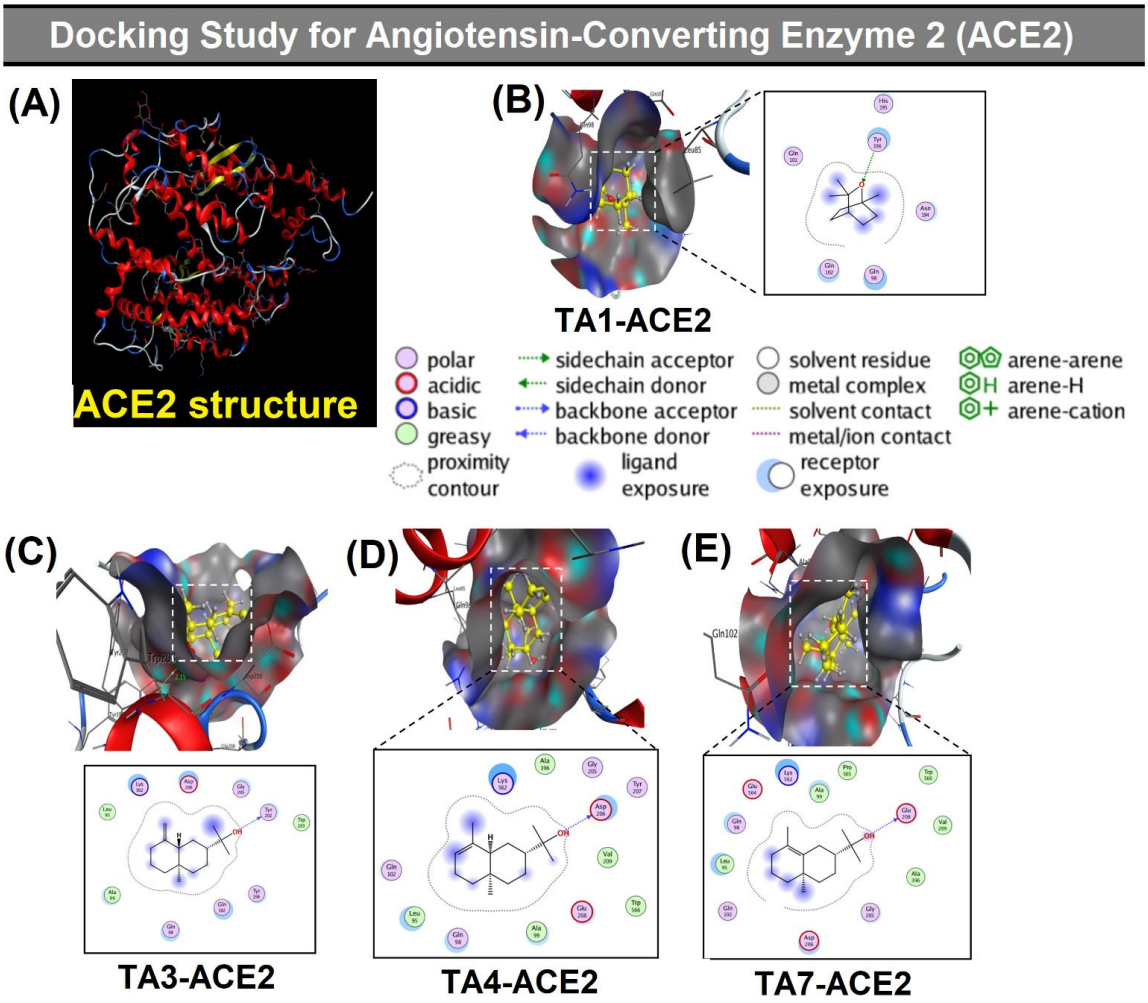



**Figure 3.** Native human angiotensin converting enzyme 2 (ACE2) protein docked with Cineol (**TA1**), β-Selinenol (**TA3**), α-Eudesmol (**TA4**), and γ-Eudesmol (**TA7**) in the range of **TA1** > **TA3** > **TA4** > **TA7**; (A) ACE2 crystal structure. Docking simulation with the interaction between compounds (B) **TA1**, (C) **TA3**, (D) **TA4**, (E) **TA7** and ACE2 protein in human body.

**Table 3.** Docking simulation results with docking score energy (DS) and root mean square deviation (RMSD) between the most active compounds (**TA1**, **TA3**, **TA4**, **TA7**) in the *Melaleuca cajuputi* essential oil and the proteins (ACE2 and PBD6LU7).

| Compound | Symbol compound-protein | DS (kcal·mol$^{-1}$) | RMSD (Å) | Interaction with amino acid |
|---|---|---|---|---|
| Cineol (**TA1**) | **TA1-ACE2** | -10.8 | 1.28 | Tyr 196 (2.32 Å), Gln 102, Gln 98, Asn 194, His 195, Gln 101 |
| | **TA1-SARS-CoV-2** | -10.9 | 1.00 | Glu 143 (2.31 Å), Asn 142, Leu 142, Thr 26, His 41, Met 49, His 164, Cys 145 |
| β-Selinenol (**TA3**) | **TA3-ACE2** | -10.1 | 1.80 | Tyr 202 (2.15 Å), Gly 205, Asp 206, Lys 562, Leu 95, Ala 99, Gln 98, Gln 102, Tyr 196, Trp 203 |
| | **TA3-SARS-CoV-2** | -10.8 | 2.89 | Leu 141 (1.97 Å), His 163, His 164, Glu 166, Gln 189, His 172, His 41, Met 49, Cys 145, Met 165, Gly 142, Ser 144, Phe 140, Asn 142 |
| α-Eudesmol (**TA4**) | **TA4-ACE2** | -9.9 | 1.96 | Asp 208 ( 2.83 Å), Trp 565, Ala 95, Val 289, Gln 102, Trp 203, Ala 396, Pro 565, Lys 562, Tyr 202. |
| | **TA4-SARS-CoV-2** | -9.4 | 2.18 | His 163 ( 2.23 Å), Phe 140, Glu 166, His 172, His 164, Ser 144, Asn 142, Cys 145, Met 165, Gly 143, Gln 189, His 41, Met 49, Leu 141, Phe 140. |
| γ-Eudesmol (**TA7**) | **TA7-ACE2** | -9.9 | 1.21 | Glu 208 (2.37 Å), Trp 566, Pro 565, Lys 562, Glu 564, Gln 98, Leu 95, Gln 102, Asp 206, Gly 205, Ala 396, Val 209. |
| | **TA7-SARS-CoV-2** | -9.4 | 1.15 | Leu 141 (1.90 Å), His 164, Glu 166, His 163, Met 165, Ser 144, Phe 140, Asn 142, Gln 189, Cys 145. |

medical and pharmaceutical industry plays an important role in therapeutic applications. It can be used for making up topical or inhaled medications.[18][19] Therefore, we recognize that the results will support future research for the use of α-terpineol in the SARS-CoV-2 prevention.

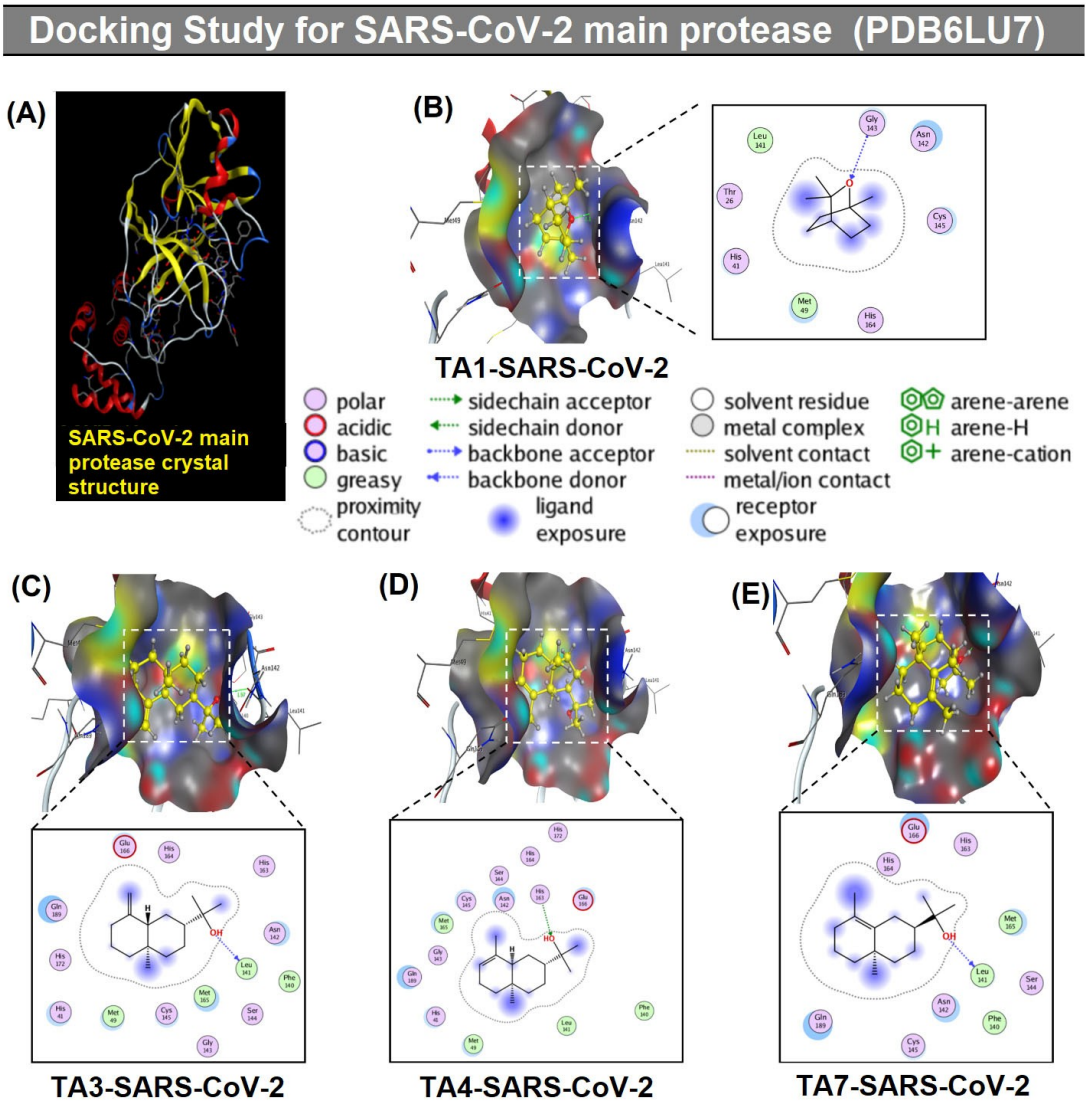



**Figure 4.** PDB6LU7 protein of the SARS-CoV-2 docked with Cineol (**TA1**), β-Selinenol (**TA3**), α-Eudesmol (**TA4**), and γ-Eudesmol (**TA7**) in the range of **TA1** > **TA3** > **TA4** > **TA7**; (A) Crystal structure of the SARS-CoV-2 main protease in complex (PDB6LU7). Docking simulation with the interaction between compounds (B) **TA1**, (C) **TA3**, (D) **TA4**, (E) **TA7** and PDB6LU7 protein of SARS-CoV-2.

The inhibitory effects of Guliol (**TA5**) on the ACE2 and PDB6LU7 proteins are not significantly different compared to the **TA2** based on the docking results as follows: *iii)* Docking of **TA5-ACE2** has DS of −11.1 kcal.mol$^{-1}$ and RMSD of 1.49 Å, and the site-site bonding interaction exhibiting the length of the binding of ligand with amino acid between –OH of **TA2** and Asn 103 is 2.22 Å and 1.80 Å for Gln 101; *iv)* Docking of **TA5-SARS-CoV-2** has DS of −10.9 kcal.mol$^{-1}$ and RMSD of 0.84 Å, and site-site bonding interaction between –OH and amino acid His 163 is 2.51 Å. The fact is that **TA5** is a sesquiterpenoid alcohol, and its molecule containing –OH group has strong interactions with amino acids Asn 103 and His 163 of ACE2 and PDB6LU7 proteins, respectively. Recent study has indicated that **TA5** with strong antibacterial activity is also present in guaiacum and cypress pine oils .[20] This docking simulation gives a clear evidence for this activity of **TA5**.

Lonalool (**TA19**) is capable of binding to ACE2 and PDB6LU7 proteins and has quite good interactions with amino acid Asn 210 of ACE2 protein, and Gly 143 as well as Cys 145 of PDB6LU7 protein (Table 2 and Figure 2): *v)* Docking of **TA19-ACE2** has DS = -10.9 kcal.mol$^{-1}$, RMSD = 1.77 Å, and site-site bonding interaction between –OH and amino acid Asn 210 is 1.96 Å. *vi)* Docking of **TA19-SARS-CoV-2** has DS of −11.1 kcal.mol$^{-1}$, and RMSD of 1.18 Å, and site-site bonding interactions between –OH and amino acid Gly 143 and Cys 145 are 2.89 Å and 2.82 Å, respectively. **TA19** is a monoterpenoid

having very flexible –OH group at C3 position, which is the active center of the **TA19** that interacts with proteins. This compound can be found in oranges, tangerines, mangoes, other herbs such as basil, coriander, and lavender. It helps reduce stress, inhibit cancer cells, and fight inflammation.[21] Moreover, **TA19** is also a major ingredient in some essential oils and used as aroma to produce perfumes, pharmaceuticals, and cosmetics.[21]

Figures 3 and 4, and Table 3 present the docking results of ACE2 and PDB6LU7 proteins by Cineol (**TA1**), β-Selinenol (**TA3**), α-Eudesmol (**TA4**), and γ-Eudesmol (**TA7**). The anti-SARS-CoV-2 and anti-ACE2 activities of the cajuput essential oil composition into the PDB6LU7 and ACE2 proteins are shown in the following order: **TA1** > **TA3** > **TA4** > **TA7**. Although TA1 is the highest content in the cajuput essential oil (31.6%) in cajeput essential oil, its interactions with the ACE2 and PDB6LU7 proteins are weaker compared to **TA2**, **TA5**, and **TA19** (Figure 4) as follows: *vii)* **TA1-ACE2** has DS = -10.8 kcal.mol$^{-1}$ and RMSD of 1.28 Å, the site-site bonding interaction between –O– with amino acid Tyr 196 of 2.32 Å. *viii)* **TA1-SARS-CoV-2** exhibits the DS value of −10.9 kcal.mol$^{-1}$ and RMSD = 1.00 Å, and the site-site bonding interaction between –O– with amino acid Glu 143 is 2.31 Å. **TA1** is present in *Cinnamomum camphora*, *Laurus nobilis*, *Artemisia vulgaris*, *Ocimum basilicum*, *Salvia rosmarinus*. **TA1** is commonly used in dentistry or used as cough remedy at an acceptable amount.[22] Some studies have shown that **TA1** has good antimicrobial, antiviral, anti-inflammatory and antioxidant properties.[22][23] **TA3** exhibits the interactions with ACE2 and PDB6LU7 proteins in which the docking results are displayed as follows: *ix)* **TA3-ACE2** has DS value of −10.1 kcal.mol$^{-1}$ and RMSD = 1.80 Å. The site-site bond length between –OH and amino acid Tyr 202 is 2.15 Å. *x)* **TA3-SARS-CoV-2** has DS = -10.8 kcal.mol$^{-1}$, RMSD of 2.89 Å, and the bonding interaction between –OH ligand with amino acid Leu 141 is 1.97 Å. The **TA3** compound can be found in fischeriana essential oil, devrra tortuosa oil, which have been recently studied on antioxidant activity and antifungal activity.[24][25]

Docking simulation of the interactions between **TA4** and the ACE2 and PDB6LU7 proteins are presented as **TA4-ACE2** and **TA4-PDB6LU7. TA4-ACE2** has DS = -9.9 kcal.mol$^{-1}$, RMSD = 1.96 Å and the bonding length interaction between –OH with amino acid Asp 208 is 2.83 Å while **TA4-SARS-CoV-2** consists of DS = -9.4 kcal.mol$^{-1}$, RMSD gives 2.18 Å, and site-site distance between –OH and amino acid His 163 of 2.23 Å. **TA4** is an eudesmane sesquiterpenoid which exhibits a double bond between C3 and C4 in compound; the flexible -OH group at C11 has strong interactions with amino acids Asp 208 of ACE2 and His 163 of PDB6LU7. The fact is that **TA4** can also be found in *Asteraceae* oil, *Centaurea appendicigera* oil, and *Centaurea helenioides* oil and has great antibacterial and anti-oxidant activities.[26] The docking simulation results of **TA7** and the two proteins indicate a similar trend but rather weak interaction compared to **TA4. TA7-ACE2** has DS = -9.9 kcal.mol$^{-1}$ and RMSD = 1.21 Å, the site-site bonding between –OH of compound with Glu 208 of 2.37 Å. **TA7-SARS-CoV-2** has the similar DS value of −9.4 kcal.mol$^{-1}$ and RMSD = 1.15 Å, and the bond distance of –OH and amino acid Leu 141 is 1.90 Å.

The above results can affirm that the strongest interactions of the most active compounds of cajeput essential oil with the ACE2 and PDB6LU7 proteins are described in this order: **TA2** ≈ **TA5** ≈ **TA19** > **TA1** > **TA3** > **TA4** > **TA7**.

The compounds **TA10**, **TA17**, and **TA18** indicate the weak inhibitory effects on the ACE2 and PDB6LU7 proteins (Table S2 and Figure S1 in the Supporting Information Summary). The docking results of **TA10-ACE2** exhibit DS = -9.7 kcal.mol$^{-1}$, RMSD = 0.95 Å and the site-site distance between –OH and Glu 208 is 1.77 Å. **TA10-SARS-CoV-2** has DS = -9.2 kcal.mol$^{-1}$, RMSD = 2.75 Å and site-site distance between –OH with Gln 189 is 2.90 Å. DS and RMSD of **TA18-ACE2** are bit larger than those of **TA18-SARS-COV-2**. Noticeably, **TA17** has the weakest inhibitory effects on the **ACE2** and **PDB6LU7** proteins in the 10 compounds of the cajeput essential oil. **TA17 -ACE2** has DS of −8.3 kcal.mol$^{-1}$ and RMSD of 1.30 Å, and site-site bonding between carbon of compound and aroma of amino acid Phe 40 is 3.03 Å. **TA17-SARS-CoV-2** gives DS = -7.3 kcal.mol$^{-1}$, RMSD = 2.12 Å, and the site-site bonding interaction between carbon of ligand and aroma of amino acid His 41 is 2.72 Å. Based on the docking simulation results analyzed above, the interaction order of the compounds in the cajeput essential oil is listed as follows: **TA2** ≈ **TA5** ≈ **TA19** > **TA1** > **TA3** > **TA4** > **TA7** > **TA10** > **TA18** > **TA17.**

Interestingly, the co-interaction of the above 10 compounds (**10TA**) with ACE2 and PDB6LU7 proteins was also investigated and shown in Figure 5. There were 12 and 13 interactions between **10TA** in cajuput essential oil with the ACE2 and PDB6LU7 proteins, respectively. The results exhibit the main interactions of –OH and –O– groups in **10TA** compounds **TA1-TA5, TA7, TA10, TA17-TA19** with amino acids including His 163, His 41, Leu 141, Gly 143, Glu 143, Gln 189 of PDB6LU7 protein, and average DS value is −9.9 kcal.mol$^{-1}$. The main interactions of **10TA** with amino acids in ACE2 are Asn 103, Asn 210, Tyr 196, Tyr 202, Glu 208, Glu 209, Asp 208, Phe 40 of ACE2 protein, and its average DS value is −10.1 kcal.mol$^{-1}$.

The study demonstrates the strong inhibitory effects of active sites of the compounds in cajeput essential oil on the ACE2 and PDB6LU7 proteins. Therefore, they are crucial for the use of the cajeput essential oil in preventing viruses that cause flu, pneumonia in general and the SARS-CoV-2 in specific.

## Conclusion

*Melaleuca cajuputi* essential oil is a valuable natural pharmaceutical source that can inhibit the ACE2 protein - the host receptor of PDB6LU7 protein in the SARS-CoV-2 as well as attack the PDB6LU7 protein at the same time. Docking simulation results indicated the active bonding sites of 10 (**TA1-TA5**, **TA7**, **TA10**, **TA17 – TA19**) out of 24 compounds of the *Melaleuca cajuputi* essential oil are capable of inhibiting ACE2 and resisting PDB6LU7 protein in SARS-CoV-2. The three substances Terpineol (**TA2**), Guaiol (**TA5**), and Linalool (**TA19**) have the strongest inhibitory effects on ACE2 and PDB6LU7

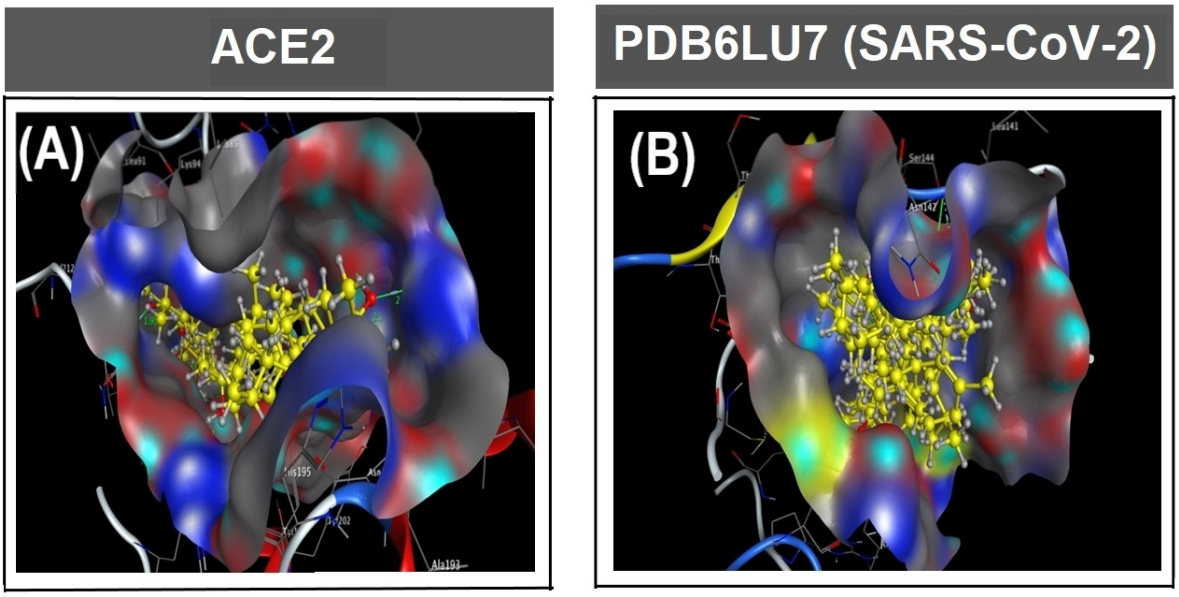



**Figure 5.** 3D-docking simulation of the interactions between the 10 most active compounds (**10TA**): **TA1, TA2, TA3, TA4, TA5, TA7, TA10, TA17, TA18, TA19** and (A) ACE2 protein including 12 interactions and (B) PDB6LU7 protein of the SARS-CoV-2 including 13 interactions.

proteins. The order of the active compounds inhibiting the ACE2 and PDB6LU7 proteins is: **TA2** ≈ **TA5** ≈ **TA19** > **TA1** > **TA3** > **TA4** > **TA7** > **TA10** > **TA18** > **TA17**. Interestingly, the docking simulation results also indicate the synergistic interactions of 10 substances in the *Melaleuca cajuputi* essential oil exhibit the significant inhibition into the ACE2 and PDB6LU7 proteins. This prevents protein maturation of the virus and the spread of infection. This study brings out a recommendation to use cajeput essential oil in preventing infections and limiting the spread of SARS-CoV-2.

## Materials and methodology

**Docking simulation**: The docking method requires information of the protein structures and the ligand structures. When the docking is conducted, all of these structures are used to simulate the interactions between the ligands and the proteins and then the bonding and docking score energy results are evaluated. The result is displayed as the ligands configurations and the docking score (DS), root mean square deviation (RMSD), types of interactions, and respective distances between the ligands and proteins. The molecular docking modeling has 3 steps.[,[27],[28],[29][30]

### *1) Protein and ligands preparation*

Structures of ACE2 and PDB6LU7 proteins are available at UniProtKB[14] and Worldwide Protein Data Bank,[15] respectively. Water molecules are deleted in the protein structure with the Sequence Editor of the MOE 2015.10; Quickprep tool is then used to repair the protein structure and 3D protonation: The protein active zone is determined based on the ligand position within a radius of 4.5 Å and the presence of important amino acids, then the protein structures are saved in *.pdb format. The ligands, which are the 24 compounds in the *Melaleuca cajuputi* essential oil, were optimized via: Conj Grad for minina energy; Termination for energy change = 0.0001 kcal.mol$^{-1}$; Max interactions = 1000; Modify charge: gasteiger – Huckel. Next, molecular dynamics on the MOE 2015.10 system are performed and the molecule structures in *.sdf format are saved.

### *2) Molecules docking into proteins - Docking investigation*

The docking simulation parameters displayed via the number of poses to keep for further analysis of interaction is 10; the maximum number of solutions per iteration is 1000; maximum number of solutions per fragmentation is 200.

### *3) Docking results analysis*

Docking score energy (DS) demonstrates the binding between ligands and the site – site distance of ligands and proteins. It is also necessary to analyze the interactions formed between ligands and important amino acids in the site – site distances. Hydrogen bonds, ion bonds, arene – arene (π - π), cation-arenes (cations - π), and van der Waals interactions are detected by contacts with hydrophilic, hydrophobic, and solvent interaction. Evaluation of docking score energy (DS) exhibits the analysis of interaction between the compounds of the essential oil and targeted proteins, and performance of interaction on 2D, 3D plane using MOE 2015.10 software. Various interactions such as hydrogen bonds, cation-π, π-π bonds, and ionic interactions, interactions distance between amino acids and the active sites of compounds are plotted. Besides, van der Waals interactions are detected by contact

with hydrophilic and hydrophobic surfaces between the compounds and the bonding point. Finally, the ligand-protein interactions are determined.

### Supporting Information Summary

Supporting Information Summary is supplied for Experiment Section, docking simulation results of other compounds in *Melaleuca cajuputi* essential oil that are not shown in the main text of this paper.

## Acknowledgements

*This research is supported by the Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 02/2020/ĐX.*

## Conflict of Interest

The authors declare no conflict of interest.

**Keywords:** ACE2 · Melaleuca cajuputi oil · Biological activity · PDB6LU7 · Computational Chemistry · SARS-CoV-2

[1] J. Chen, *Microbes Infect.* **2020**. *22*, 69–71.
[2] N. D. Cuong, T. T. Xuyen, O. Motl, K. Stránský, J. Presslova, Z. Jedlickova, V. Serý, *J. Essent. Oil Res.* **1994**, *6*, 63–67.
[3] Y. Yoon, Y. Sornnuwat, J. H. Kim, K. H. Liu, T. Kitirattrakarn, C. Anantachoke. Essential leaf oils from Melaleuca cajuputi. In *III WOCMAP Congress on Medicinal Aromatic Plants-Volume 6: Traditional Medicine Nutraceuticals* **2003**, p. 65–72.
[4] J. C. Doran, J. W. Turnbull, *Australian trees and shrubs: species for land rehabilitation and farm planting*, ACIAR Monograph, Australian Centre for International Agricultural Research, Canberra, Australia, **1997**, 24.
[5] A. D. Amis, R. Prakash, T. H. Vuong, D. T. Huynh, in *Proceedings IEEE INFOCOM 2000. Conference on Computer Communications. Nineteenth Annual Joint Conference of the IEEE Computer and Communications Societies (Cat. No. 00CH37064)*, IEEE, **2000**, *1*, p. 32–41.
[6] L. C. A. Barbosa, C. J. Silva, R. R. Teixeira, R. M. S. A. Meira, A. L. Pinheiro, *Agric. Conspec. Sci.* **2013**, *78*, 11–23.
[7] L. P. A. Oyen, N. X. Dung, *Plant resources of South-East Asial: Essential Oil Plants. Backhuys Publ. Leiden, the Netherlands* **1999**, *19*.
[8] S. Tariq, S. Wani, W. Rasool, M. A. Bhat, A. Prabhakar, A. H. Shalla, M. A. Rather, *Microb. Pathog.* **2019**, 103580.
[9] A. Astani, J. Reichling, P. Schnitzler, *Phytother. Res.* **2010**, *24*, 673–679.
[10] A. Garozzo, R. Timpanaro, B. Bisignano, P. Furneri, G. Bisignano, A. Castro, *Lett. Appl. Microbiol.* **2009**, *49*, 806–808.
[11] J. Zhang, H. Wu, D. Jiang, Y. Yang, W. Tang, K. Xu, *Nat. Prod. Res.* **2019**, *33*, 2545–2548.
[12] A. Roberts, D. Deming, C. D. Paddock, A. Cheng, B. Yount, L. Vogel, B. D. Herman, T. Sheahan, M. Heise, G. L. Genrich, *PLOS Pathog.* **2007**, *3*.
[13] L. E. Gralinski, V. D. Menachery, *Viruses* **2020**, *12*, 135.
[14] D. Paraskevis, E. G. Kostaki, G. Magiorkinis, G. Panayiotakopoulos, G. Sourvinos, S. Tsiodras, *Infect. Genet. Evol.* **2020**, 104212.
[15] A. D. Amis, R. Prakash, in *Proceedings 3rd IEEE Symposium on Application-Specific Systems and Software Engineering Technology*, IEEE, **2000**, pp. 25–32.
[16] Z. Guo, L. Cupples, A. Kurz, S. Auerbach, L. Volicer, H. Chui, R. Green, A. Sadovnick, R. Duara, C. DeCarli, *Neurology* **2000**, *54*, 1316–1323. J. Sharifi-Rad, B. Salehi, E. M. Varoni, F. Sharopov, Z. Yousaf, S. A. Ayatollahi, F. Kobarfard, M. Sharifi-Rad, M. H. Afdjei, M. Sharifi-Rad, *Phytother. Res.* **2017**, *31*, 1475–1494.
[17] B. T. P. Thuy, T. T. A. My, N. T. T. Hai, L. T. Hieu, T. T. Hoa, H. T. P. Loan, N. T. Triet, T. T. V. Anh, P. T. Quy, P. V. Tat, N. V. Hue, D. T. Quang, N. T. Trung, V. T. Tung, L. K. Huynh, N. T. A. Nhung, *ACSOmega* **2020**, *5*, 8312–8320.
[18] C. Khaleel, N. Tabanca, G. Buchbauer, *Open Chem.* **2018**, *16*, 349–361.
[19] A. De-Oliveira, A. Fidalgo-Neto, F. J. R. Paumgartten, *Toxicology* **1999**, *135*, 33–41.
[20] P. Eppenberger, F. Galassi, F. Rühli, *Br. J. Clin. Pharmacol.* **2017**, *83*, 2118.
[21] A. T. Peana, P. S. D'Aquila, F. Panin, G. Serra, P. Pippia, M. D. L. Moretti, *Phytomedicine* **2002**, *9*, 721–726.
[22] J. Safaei-Ghomi, A. A. Ahd, *Pharmacogn. Mag.* **2010**, *6*, 172.
[23] S. Mulyaningsih, F. Sporer, J. Reichling, M. Wink, *Pharm. Biol.* **2011**, *49*, 893–899.
[24] S.-P. Fang, X. Xing, P.-X. Lai, J.-J. Huang, *Chem. Nat. Compd.* **2018**, *54*, 591–592.
[25] A. Guetat, A. Boulila, M. Boussaid, *Nat. Prod. Res.* **2019**, *33*, 2708–2713.
[26] M. Malek, B. Safa, N. Nabil, A. A. Nelly, *Pharm. Chem. J.* **2016**, *3*, 91–98.
[27] O. Tarasova, V. Poroikov, A. Veselovsky, *Molecules* **2018**, *23*, 1233.
[28] T. M. C. Babu, S. S. Rajesh, B. V. Bhaskar, S. Devi, A. Rammohan, T. Sivaraman, W. Rajendra, *RSC Adv.* **2017**, *7*, 18277–18292.
[29] T.-D. Ngo, T.-D. Tran, M.-T. Le, K.-M. Thai, *Mol. Divers.* **2016**, *20*, 945–961.
[30] K.-M. Thai, D.-P. Le, T.-D. Tran, M.-T. Le, *J. Theor. Biol.* **2015**, *385*, 31–39.

Submitted: February 28, 2020
Accepted: May 15, 2020