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Revealing Potential Bioactive Compounds and Mechanisms of *Lithospermum erythrorhizon* against COVID-19 via Network Pharmacology Study

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Abstract: *Lithospermum erythrorhizon* (LE) is known in Korean traditional medicine for its potent therapeutic effect and antiviral activity. Currently, coronavirus (COVID-19) disease is a developing global pandemic that can cause pneumonia. A precise study of the infection and molecular pathway of COVID-19 is therefore obviously important. The compounds of LE were identified from the Natural Product Activity and Species Source (NPASS) database and screened by SwissADME. The targets interacted with the compounds and were selected using the Similarity Ensemble Approach (SEA) and Swiss Target Prediction (STP) methods. PubChem was used to classify targets linked to COVID-19. The protein–protein interaction (PPI) networks and signaling pathways–targets–bioactive compounds (STB) networks were constructed by RPackage. Lastly, we performed the molecular docking test (MDT) to verify the binding affinity between significant complexes through AutoDock 1.5.6. The Natural Product Activity and Species Source (NPASS) revealed a total of 82 compounds from LE, which interacted with 1262 targets (SEA and STP), and 249 overlapping targets were identified. The 19 final overlapping targets from the 249 targets and 356 COVID-19 targets were ultimately selected. A bubble chart exhibited that inhibition of the MAPK signaling pathway could be a key mechanism of LE on COVID-19. The three key targets (RELA, TNF, and VEGFA) directly related to the MAPK signaling pathway, and methyl 4-prenyloxycinnamate, tormentic acid, and eugenol were related to each target and had the most stable binding affinity. The three bioactive effects on the three key targets might be synergistic effects to alleviate symptoms of COVID-19 infection. Overall, this study shows that LE can play a role in alleviating COVID-19 symptoms, revealing that the three components (bioactive compounds, targets, and mechanism) are the most significant elements of LE against COVID-19. However, the promising mechanism of LE on COVID-19 is only predicted on the basis of mining data; the efficacy of the chemical compounds and the affinity between compounds and the targets in experiment was ignored, which should be further substantiated through clinical trials.

Keywords: COVID-19; *Lithospermum erythrorhizon*; MAPK signaling pathway; methyl 4-prenyloxycinnamate; tormentic acid; eugenol; RELA; TNF; VEGFA

1. Introduction

The outbreak of a new health crisis due to coronavirus disease 2019 (COVID-19) occurred in Wuhan, Hubei Province, China, in December 2019 [1]. On 9 January 2020, the novel coronavirus SARS-CoV-2 was officially acknowledged as the sole contributor to this outbreak [2]. The World Health Organization (WHO) initially called this situation a Public Health Emergency of International Concern on 30 January [3], and then announced it was a global pandemic on 11 March [4].

The novel coronavirus was designated as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2, 2019-nCoV) because of its high genetic similarity (around 80%) to SARS-CoV,
which caused acute respiratory distress syndrome (ARDS) and high mortality between 2002 and 2003 [5]. The SARS-CoV-2 epidemic was considered to have primarily started through a zoonotic transmission linked to a seafood market in Wuhan, China. Subsequently, it was later identified that human to human transmission played a leading role in the ensuing outbreak [6]. At the time of writing, the COVID-19 has infected 213 countries, and the total number of COVID-19 deaths has reached around 6,130,000 [7]. As of 25 March 2022, approximately 478,260,000 cases were reported worldwide, according to Worldometer [7]. The clinical symptoms of COVID-19 are quite varied, ranging from an asymptomatic condition to intense respiratory distress disorder and multiple organ abnormalities [8]. The common symptoms are fever, dry cough, tachypnea, and shortness of breath [9]. Currently, efforts to developing treatments for COVID-19 by repurposing medications are underway and vaccine development continues. However, the attempts have been hampered by limited information on how this coronavirus penetrates human hosts [10]. Alternatively, natural products have played a vital role in providing drug candidates against various diseases, including the emergence of mutant coronaviruses [11]. Folk remedies have led to the discovery of phytochemicals that are invaluable drug resources and have led to drug candidates such as aspirin from *Salix*, Taxol from *Taxus brevifolia*, and artemisinin from *Artemisia annua* [12,13].

The early Korean medical book, *Dongui Bogam*, shows that *Lithospermum erythrorhizon* can be used to treat measles caused by *Measles morbillivirus*. According to an experiment conducted on Beagle dogs, the data suggested that the no observed adverse effect level (NOAEL) of LE extraction was 100 mg/kg/day. Therefore, LE may have a favorable therapeutic effect and is safe to use on virus-related diseases [14]. At present, the bioactive compounds and mechanisms of LE against viruses have not yet been reported. Hence, the exploration of bioactive compounds and mechanisms of LE against the COVID-19 virus should be undertaken to discover more scientific evidence to support its therapeutic application in treating COVID-19. Moreover, we utilized the natural product activity and species source (NPASS) database that we then combined with around 30,000 natural products from diverse traditional and herbal medicines [15]. Moreover, the NPASS is a reliable database with many curated experimental results based on natural compounds [16].

Accordingly, network pharmacology—a multiple analytical mode—can investigate interaction networks such as compounds, genes, protein targets, and diseases [17]. Additionally, network pharmacology can elucidate the mechanism(s) of drug action through networking analysis, which is a role model to shift from “one drug-one target” to “multiple targets” [18]. Therefore, the conception has been extensively utilized to analyze the bioactive compounds and molecular mechanisms of drug candidates against diverse diseases [19]. In this study, network pharmacology was utilized to analyze the bioactive compounds and mechanism(s) of LE against COVID-19. Firstly, compounds from LE were identified using the public database and were confirmed as drug-likeness by the Lipinski rule in SwissADME. Then, targets related to the selected compounds or COVID-19 targets were identified using public databases, and the overlapping targets were selected between compounds and COVID-19 targets. Thirdly, the key bioactive compounds and hub targets of LE against COVID-19 were identified by exploring the interaction of the overlapping targets. Finally, AutoDockTools were used to analyze the binding affinity between promising bioactive compounds and targets. To date, there have been no scientific reports on bioactive compounds and mechanisms of LE against COVID-19. In brief, our study workflow is represented in Figure 1.
2. Materials and Methods

2.1. Selective Compounds’ Construction and Drug-Likeness Evaluation

The compound information of LE was collected by NPASS (http://bidd2.nus.edu.sg/NPASS/, accessed on 21 October 2021), Google Scholar, and SMILES (Simplified Molecular Input Line Entry System). The molecular formula of selective compounds was identified using ChemSpider (https://www.chemspider.com/StructureSearch.aspx) (accessed on 21 October 2021) or PubChem (https://pubchem.ncbi.nlm.nih.gov/) (accessed on 21 October 2021) to confirm compound names or structures. The drug-likeness properties of the identified compounds were confirmed through Lipinski’s rule on SwissADME (http://www.swissadme.ch/) (accessed on 21 October 2021) [20]. The compound structures were drawn in PubChem Sketcher V2.4 (https://pubchem.ncbi.nlm.nih.gov/edit3/index.html) (accessed on 21 October 2021) [21].

2.2. Targets Associated with Selected Compounds or COVID-19

Based on SMILES, targets related to the selected compounds were identified via both SEA (http://sea.bkslab.org/) (accessed on 23 October 2021) [22] and STP (http://www.swisstargetprediction.ch/) (accessed on 24 October 2021) [23] using the “Homo Sapiens” mode. We selected the overlapping targets between SEA and STP databases based on the use of a Venn diagram. Moreover, the COVID-19 targets were adapted from PubChem (https://pubchem.ncbi.nlm.nih.gov/) (accessed on 24 October 2021). The final targets between the selected compounds-related targets and COVID-19 targets were visualized by a Venn diagram plotter.

2.3. The Analysis of the Protein–Protein Interaction (PPI) Networks

The final targets were utilized to construct a protein–protein interaction (PPI) network on STRING (https://string-db.org/) (accessed on 27 October 2021). In the PPI network, the size of circle represents the degree of value. In particular, a target indicated in red color in the most central position was considered the most significant target.

2.4. The Construction of a Bubble Chart

A bubble chart was constructed according to the rich factor, which is defined as the proportion of the number of genes expressed differentially in a signaling pathway [24].

Figure 1. The workflow of this study.
Thus, we identified a hub signaling pathway related to a key target in PPI networks. The bubble chart was plotted in RPackage based on STRING (https://string-db.org/) (accessed on 27 October 2021).

2.5. The Assembly of Signaling Pathways–Targets–Bioactive Compounds (STB) Networks

The STC networks were illustrated the relationships of the three components (signaling pathways–targets–bioactive compounds) and obtained the most critical target in the correlation. In the network, yellow rectangles (nodes) stood for the signaling pathways; blue triangles (nodes) represented targets; and pink circles (nodes) denoted bioactive compounds. The size of the blue triangles marked the number of correlations with the signaling pathways; the size of the pink circles depicted the number of connections with the targets. The merged networks were completed in RPackage.

2.6. The Preparation of the Bioactive Compounds and Targets for Molecular Docking Test (MDT)

The bioactive compounds were associated with the key signaling pathway were extracted .sdf format from PubChem, which were converted into .pdb format using Pymol, and then they were changed into .pdbqt format via AutoDock. The number of three proteins on the MAPK signaling pathway, i.e., TNF (PDB ID: 5YOY), RELA (PDB ID: 2O61), and VEGFA (PDB ID: 3P9W) were obtained using STRING via RCSB PDB (https://www.rcsb.org/) (accessed on 28 October 2021). The targets were converted .pdb format into .pdbqt format via AutoDock (http://autodock.scripps.edu) (accessed on 30 October 2021).

2.7. The MDT on a Key Signaling Pathway

The MDT were performed to verify the affinity between bioactive compounds and targets on a key signaling pathway. The set-up condition consisted of a value of 4 for the energy range and a value of 8 for the exhaustiveness as the default settings in order to identify 10 different poses of bioactive compounds. The center value of each target on a key signaling pathway was RELA (x = 15.616, y = −22.641, z = −18.824), TNF (x = 243.718, y = −425.984, z = 261.631), and VEGFA (x = −12.652, y = 70.481, z = −40.286). The cubic box size of active site was set at x = 40 Å, y = 40 Å, and z = 40 Å. The 2D molecular docking studies were performed in LigPlot⁺ 2.2 [25]. The lower the binding energy (the higher the negative value), the greater the stable binding is between the bioactive and the target.

3. Results

3.1. Potential Bioactive Compounds from LE

We obtained the 82 bioactive compounds in LE through NPASS database and physicochemical properties of these compounds are listed in Table 1. All of them were confirmed by Lipinski’s rule [26] and TPSA (<140 Å²) [27]. Thus, we considered that these compounds might be potential therapeutic agents against COVID-19.

| Compounds                  | PubChem ID | MW  | HBA | HBD | MLogP | Lipinski’s Violations | Bioavailability Score | TPSA  |
|-----------------------------|------------|-----|-----|-----|-------|------------------------|-----------------------|-------|
| 1 (S)-1-Phenylethanol       | 443135     | 122.16 | 1  | 1   | 1.87  | 0                      | 0.55                  | 20.23 |
| 2 3-Methylbutanoic acid     | 10430      | 102.13 | 2  | 1   | 0.89  | 0                      | 0.85                  | 37.30 |
| 3 cis-Caffeic acid          | 1549111    | 180.16 | 4  | 3   | 0.70  | 0                      | 0.55                  | 77.76 |
| 4 Phenylethyl alcohol       | 6054       | 122.16 | 1  | 1   | 1.87  | 0                      | 0.55                  | 20.23 |
Table 1. Cont.

| Compounds                  | Lipinski Rules | Lipinski’s Violations | Bioavailability Score | TPSA  |
|----------------------------|----------------|-----------------------|-----------------------|-------|
|                            | PubChem ID     | MW        | HBA | HBD | MLogP | <500 | <10 | ≤5 | ≤4.15 | ≤1 | >0.1 | <140 Å² |
| Thiophene                  | 8030           | 84.14     | 0   | 0   | 1.12  | 0    | 0   | 0  | 0     | 0  | 0.55 | 28.24   |
| Caryophyllene              | 5281515        | 204.35    | 0   | 0   | 4.63  | 1    | 0   | 0  | 0     | 0  | 0.55 | 0.00    |
| Alkannin                   | 72521          | 288.30    | 5   | 3   | 0.42  | 0    | 0   | 0  | 0     | 0  | 0.55 | 94.83   |
| b-b-Dimethylacylalkannin  | 442720         | 370.40    | 6   | 2   | 1.43  | 0    | 0   | 0  | 0     | 0  | 0.55 | 100.90  |
| Shikonofuran C             | 5321288        | 358.43    | 5   | 2   | 2.36  | 0    | 0   | 0  | 0     | 0  | 0.55 | 79.90   |
| Isovanillin                | 12127          | 152.15    | 3   | 1   | 0.51  | 0    | 0   | 0  | 0     | 0  | 0.55 | 46.53   |
| (R)-2-methylbutanoate      | 6950479        | 102.13    | 2   | 1   | 0.89  | 0    | 0   | 0  | 0     | 0  | 0.85 | 37.30   |
| Ethyl oleate               | 5363269        | 310.51    | 2   | 0   | 5.03  | 1    | 0   | 0  | 0     | 0  | 0.55 | 26.30   |
| Camphor                    | 2537           | 152.23    | 1   | 0   | 2.30  | 0    | 0   | 0  | 0     | 0  | 0.55 | 17.07   |
| (−)-Caryophyllene oxide    | 1742210        | 220.35    | 1   | 0   | 3.67  | 0    | 0   | 0  | 0     | 0  | 0.55 | 3.67    |
| Methyl linolenate          | 5319706        | 292.46    | 2   | 0   | 4.61  | 1    | 0   | 0  | 0     | 0  | 0.55 | 26.30   |
| Totarol                    | 92783          | 286.45    | 1   | 1   | 4.92  | 1    | 0   | 0  | 0     | 0  | 0.55 | 20.23   |
| Oleanolic acid             | 10494          | 456.70    | 3   | 2   | 5.82  | 1    | 0   | 0  | 0     | 0  | 0.85 | 57.53   |
| (S)-2-methylbutanoate      | 6950480        | 101.12    | 2   | 0   | 0.89  | 0    | 0   | 0  | 0     | 0  | 0.85 | 40.13   |
| Hexadecanoic acid methyl ester | 8181           | 270.45    | 2   | 0   | 4.44  | 1    | 0   | 0  | 0     | 0  | 0.55 | 26.30   |
| (−)-Borneol                | 1201518        | 154.25    | 1   | 1   | 2.45  | 0    | 0   | 0  | 0     | 0  | 0.55 | 20.23   |
| Cinnamic aldehyde          | 637511         | 132.16    | 1   | 0   | 2.01  | 0    | 0   | 0  | 0     | 0  | 0.55 | 17.07   |
| Valeric acid               | 7991           | 102.13    | 2   | 1   | 0.89  | 0    | 0   | 0  | 0     | 0  | 0.85 | 37.30   |
| Methyl 4-prenyloxyccinnmate (3S,4S)-4,7,7-| 14414116      | 246.30    | 3   | 0   | 3.48  | 0    | 0   | 0  | 0     | 0  | 0.55 | 35.53   |
| trimethylbicyclo[2.2.1]heptan-3-ol | 12242815      | 154.25    | 1   | 1   | 2.45  | 0    | 0   | 0  | 0     | 0  | 0.55 | 20.23   |
| Paeonol                    | 11092          | 166.17    | 3   | 1   | 0.83  | 0    | 0   | 0  | 0     | 0  | 0.55 | 46.53   |
| β-Selinenene               | 519361         | 204.35    | 0   | 0   | 4.63  | 1    | 0   | 0  | 0     | 0  | 0.55 | 0.00    |
| Docosanol                  | 12620          | 326.60    | 1   | 1   | 5.84  | 1    | 0   | 0  | 0     | 0  | 0.55 | 20.23   |
| Phytoacetalddehyde         | 998            | 120.15    | 1   | 0   | 1.78  | 0    | 0   | 0  | 0     | 0  | 0.55 | 17.07   |
| 11-O-Acetyllakannin        | 137628887      | 330.33    | 6   | 2   | 0.82  | 0    | 0   | 0  | 0     | 0  | 0.55 | 100.90  |
| palmitic acid              | 985            | 256.42    | 2   | 1   | 4.19  | 1    | 0   | 0  | 0     | 0  | 0.85 | 37.30   |
| furfural                   | 7362           | 96.08     | 2   | 0   | −0.56 | 0    | 0   | 0  | 0     | 0  | 0.55 | 30.21   |
| isobutyric acid            | 6590           | 88.11     | 2   | 1   | 0.49  | 0    | 0   | 0  | 0     | 0  | 0.85 | 37.30   |
| isobutyllshikonin          | 479500         | 358.39    | 6   | 2   | 1.28  | 0    | 0   | 0  | 0     | 0  | 0.55 | 100.90  |
| Shikalkin                  | 5208           | 288.30    | 5   | 3   | 0.42  | 0    | 0   | 0  | 0     | 0  | 0.55 | 94.83   |
| shikonin                   | 479503         | 288.30    | 5   | 3   | 0.42  | 0    | 0   | 0  | 0     | 0  | 0.55 | 94.83   |
| Propionylyshikonin         | 153984         | 344.36    | 6   | 2   | 1.06  | 0    | 0   | 0  | 0     | 0  | 0.55 | 100.90  |
| β-hydroxyisovalerylshikonin| 479502         | 388.41    | 7   | 3   | 0.71  | 0    | 0   | 0  | 0     | 0  | 0.55 | 121.13  |
| eugenol                    | 3314           | 164.20    | 2   | 1   | 2.01  | 0    | 0   | 0  | 0     | 0  | 0.55 | 29.46   |
| cis-Anethole               | 1549040        | 148.20    | 1   | 0   | 2.67  | 0    | 0   | 0  | 0     | 0  | 0.55 | 9.23    |
| tormentic Acid             | 73193          | 488.70    | 5   | 4   | 4.14  | 0    | 0   | 0  | 0     | 0  | 0.55 | 97.99   |
| oleic Acid                 | 445639         | 282.46    | 2   | 1   | 4.57  | 1    | 0   | 0  | 0     | 0  | 0.85 | 37.30   |
| No. | Compounds                                                                 | PubChem ID | MW    | HBA | HBD | MLogP | Lipinski’s Violations | Bioavailability Score | TPSA <140 Å² |
|-----|---------------------------------------------------------------------------|------------|-------|-----|-----|-------|-----------------------|-----------------------|---------------|
| 42  | 1-eicosanol                                                               | 12404      | 298.55| 1   | 1   | 5.39  | 1                     | 0.55                  | 20.23         |
| 43  | decanoic acid                                                             | 2969       | 172.26| 2   | 1   | 2.58  | 0                     | 0.85                  | 37.30         |
| 44  | borneol                                                                   | 64685      | 154.25| 1   | 1   | 2.45  | 0                     | 0.55                  | 20.23         |
| 45  | ethyl linolate                                                            | 5282184    | 308.50| 2   | 0   | 4.93  | 1                     | 0.55                  | 26.30         |
| 46  | cetostearyl alcohol                                                       | 62238      | 512.93| 2   | 2   | 7.28  | 2                     | 0.17                  | 40.46         |
| 47  | 2-acetylpyrrole (1R)-2-[(2R,6R)-6-[(2S)-2-hydroxy-2-phenylethyl]-1-methylpiperidin-2-yl]-1-phenylethanol | 14079      | 109.13| 1   | 1   | 0.18  | 0                     | 0.55                  | 32.86         |
| 48  | dimethylacrylsikhonin                                                     | 479499     | 370.40| 6   | 2   | 1.43  | 0                     | 0.55                  | 100.90        |
| 49  | methylbut-2-enoic Acid                                                    | 7127       | 178.23| 2   | 0   | 2.30  | 0                     | 0.55                  | 18.46         |
| 50  | caffeic acid                                                              | 699043     | 180.16| 4   | 3   | 0.70  | 0                     | 0.55                  | 77.76         |
| 51  | β-ionone                                                                  | 638014     | 192.30| 1   | 0   | 2.94  | 0                     | 0.55                  | 17.07         |
| 52  | carene                                                                    | 26049      | 136.23| 0   | 0   | 4.29  | 1                     | 0.55                  | 0.00          |
| 53  | acetylshikhonin                                                           | 479501     | 330.33| 6   | 2   | 0.82  | 0                     | 0.55                  | 100.90        |
| 54  | acetylshikhonin                                                           | 479497     | 372.41| 6   | 2   | 1.51  | 0                     | 0.55                  | 100.90        |
| 55  | methyl tetradecanoate                                                     | 31284      | 242.40| 2   | 0   | 3.94  | 0                     | 0.55                  | 26.30         |
| 56  | 2-pentylfuran                                                             | 98914      | 272.30| 4   | 2   | 1.25  | 0                     | 0.55                  | 74.60         |
| 57  | 2-pentylfuran                                                             | 10089766   | 358.39| 6   | 2   | 1.28  | 0                     | 0.55                  | 100.90        |
| 58  | 2-pentylfuran                                                             | 10931      | 100.12| 2   | 1   | 0.79  | 0                     | 0.85                  | 37.30         |
| 59  | shikonofuran E                                                            | 5321290    | 356.41| 5   | 2   | 2.28  | 0                     | 0.55                  | 79.90         |
| 60  | methyl oleate                                                             | 5364509    | 296.49| 2   | 0   | 4.80  | 1                     | 0.55                  | 26.30         |
| 61  | isovalerylshikhonin                                                       | 479497     | 372.41| 6   | 2   | 1.51  | 0                     | 0.55                  | 100.90        |
| 62  | α-methyl-butylylshikhonin                                                 | 479498     | 372.41| 6   | 2   | 1.51  | 0                     | 0.55                  | 100.90        |
| 63  | isobutylylalkannin                                                        | 137629300  | 358.39| 6   | 2   | 1.28  | 0                     | 0.55                  | 100.90        |
| 64  | Methyl linoleate                                                          | 5284421    | 294.47| 2   | 0   | 4.70  | 1                     | 0.55                  | 26.30         |
| 65  | (−)-camphor                                                              | 444294     | 152.23| 1   | 0   | 2.30  | 0                     | 0.55                  | 17.07         |
| 66  | isovaleryl alkannin                                                       | 5318685    | 372.41| 6   | 2   | 1.51  | 0                     | 0.55                  | 100.90        |
| 67  | pentanoate                                                                 | 19602      | 138.21| 1   | 0   | 1.84  | 0                     | 0.55                  | 13.14         |
| 68  | [(1R)-1-(5,8-dihydroxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-enyl]      | 44438574   | 550.73| 6   | 2   | 3.95  | 1                     | 0.55                  | 100.90        |
| 69  | [(1R)-1-(5,8-dihydroxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-enyl]-6Z,-9Z-octadeca-6,9-dienoate | 9999214    | 370.40| 6   | 2   | 1.43  | 0                     | 0.55                  | 100.90        |
| 70  | [(1R)-1-(5,8-dihydroxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-enyl]-pent-4-enoate | 10475609   | 392.40| 6   | 2   | 1.99  | 0                     | 0.55                  | 100.90        |
| 71  | [(1R)-1-(5,8-dihydroxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-enyl]-benzoate | 145992534  | 476.61| 6   | 2   | 2.15  | 0                     | 0.55                  | 151.50        |
| 72  | [(1R)-1-(5,8-dihydroxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-enyl]-pentanoate | 31289     | 142.24| 1   | 0   | 2.39  | 0                     | 0.55                  | 17.07         |
Table 1. Cont.

| No. | Compounds            | PubChem ID | MW    | HBA | HBD | MLogP | Lipinski's Violations | Bioavailability Score | TPSA  |
|-----|----------------------|------------|-------|-----|-----|-------|-----------------------|-----------------------|-------|
| 73  | ursolic acid         | 64945      | 456.70| 3   | 2   | 5.82  | 1                     | 0.85                  | 57.53 |
| 74  | methyl decanoate     | 8050       | 186.29| 2   | 0   | 2.87  | 0                     | 0.55                  | 26.30 |
| 75  | hexanal              | 6184       | 100.16| 1   | 0   | 1.39  | 0                     | 0.55                  | 17.07 |
| 76  | 2-methylbutanoic acid| 8314       | 102.13| 2   | 1   | 0.89  | 0                     | 0.85                  | 37.30 |
| 77  | shikonofuran D       | 5321289    | 344.40| 5   | 2   | 2.14  | 0                     | 0.55                  | 79.90 |
| 78  | linoleic acid        | 5280450    | 280.45| 2   | 1   | 4.47  | 1                     | 0.85                  | 37.30 |
| 79  | D-1-phenylethyl      | 637516     | 122.16| 1   | 1   | 1.87  | 0                     | 0.55                  | 20.23 |
| 80  | P-cymene             | 7463       | 134.22| 0   | 0   | 4.47  | 1                     | 0.55                  | 0.00  |
| 81  | phenanthrene         | 995        | 178.23| 0   | 0   | 5.17  | 1                     | 0.55                  | 0.00  |
| 82  | anethole             | 637563     | 148.20| 1   | 0   | 2.67  | 0                     | 0.55                  | 9.23  |

3.2. Targets Associated with the 82 Compounds or COVID-19

As shown in Supplementary Table S1, the total number of 1262 targets (SEA + STP) related to 82 compounds was identified in DisGeNET and OMIM. Then, the overlapping 249 targets between SEA and STP were obtained (Figure 2). The 249 targets (Supplementary Table S2) were analyzed with 356 COVID-19 related targets (Supplementary Table S3). Finally, the Venn diagram (Figure 3) showed that 19 overlapping targets (Supplementary Table S4) were directly associated with response to COVID-19 infection.

Figure 2. The overlapping 249 targets between 550 from SEA and 712 targets from STP.
3.3. The Protein–Protein Interaction (PPI) Networks from 19 Targets

In the PPI networks, the TNF target was considered to be the most significant target with the highest degree of value (15) (Table 2). Moreover, the 19 targets were closely interconnected with each other (19 nodes and 69 edges) (Figure 4). The TNF in the most central position was the most significant target in the PPI networks.

Table 2. The degree value of targets in PPI networks.

| No. | Target  | Degree of Values |
|-----|---------|------------------|
| 1   | TNF     | 16               |
| 2   | VEGFA   | 15               |
| 3   | CXCL8   | 11               |
| 4   | NFE2L2  | 10               |
| 5   | PPARA   | 10               |
| 6   | HMOX1   | 9                |
| 7   | PPARG   | 9                |
| 8   | ACE     | 8                |
| 9   | RELA    | 8                |
| 10  | HDAC1   | 6                |
| 11  | ANPEP   | 5                |
| 12  | CCR5    | 5                |
| 13  | ERN1    | 5                |
| 14  | GSR     | 5                |
| 15  | TLR9    | 5                |
| 16  | MME     | 4                |
| 17  | S1PR1   | 3                |
| 18  | SIGMAR1 | 3                |
| 19  | ABCG2   | 1                |
Table 2. The degree value of targets in PPI networks.

| No. | Target   | Degree of Values |
|-----|----------|------------------|
| 1   | TNF      | 16               |
| 2   | VEGFA    | 15               |
| 3   | CXCL8    | 11               |
| 4   | NFE2L2   | 10               |
| 5   | PPARA    | 10               |
| 6   | HMOX1    | 9                |
| 7   | PPARG    | 9                |
| 8   | ACE      | 8                |
| 9   | RELA     | 8                |
| 10  | HDAC1    | 6                |
| 11  | ANPEP    | 5                |
| 12  | CCR5     | 5                |
| 13  | ERN1     | 5                |
| 14  | GSR      | 5                |
| 15  | TLR9     | 5                |
| 16  | MME      | 4                |
| 17  | S1PR1    | 3                |
| 18  | SIGMAR1  | 3                |
| 19  | ABCG2    | 1                |

Figure 4. The PPI networks (19 nodes and 69 edges).

3.4. A Bubble Plot and Signaling Pathways–Targets–Bioactive Compounds (STB) Networks

The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis indicated that the 19 targets were associated directly with 18 signaling pathways (False Discovery Rate < 0.05) (Figure 5). The identified 18 signaling pathways were involved in the response to a COVID-19 infection. Detailed information on the 18 signaling pathways is presented in Table 3. Additionally, a bubble plot suggested that the RAS signaling pathway might be a key signaling pathway due to the lowest rich factor (0.008). Moreover, we performed an STB networks analysis to identify the most important target based on the degree of value (Figure 6). Thus, the highest degree of value in STB networks was the RELA target with 17 degrees, which was considered as a notable target against COVID-19 (Table 4). Comprehensively, a signaling pathway that combined both TNF (a key target in PPI networks) and RELA (a key target in STB networks) was the MAPK signaling pathway, which had an antagonistic propensity on the relatively lower rich factor found in the 18 signaling pathways. We observed that the uppermost signaling pathway was not the RAS signaling pathway but the MAPK signaling pathway, which consisted of the two core targets (TNF and RELA).
Table 3. Targets in 18 signaling pathways with enrichment related to COVID-19.

| KEGG ID | Description                                                                 | Target Genes          | False Discovery Rate |
|---------|------------------------------------------------------------------------------|-----------------------|----------------------|
| hsa04933 | AGE-RAGE signaling pathway in diabetic complications                        | RELA, TNF, CXCL8, VEGFA | 0.000067             |
| hsa04920 | Adipocytokine signaling pathway                                              | RELA, TNF, PPARA      | 0.000440             |
| hsa04622 | RIG-I-like receptor signaling pathway                                        | RELA, TNF, CXCL8      | 0.000440             |
| hsa04657 | IL-17 signaling pathway                                                     | RELA, TNF, CXCL8      | 0.000760             |
| hsa04620 | Toll-like receptor signaling pathway                                        | RELA, TNF, CXCL8      | 0.000760             |
| hsa04066 | HIF-1 signaling pathway                                                     | RELA, HMOX1, VEGFA    | 0.000760             |
| hsa04064 | NF-kappa B signaling pathway                                                | RELA, TNF, CXCL8      | 0.000760             |
| hsa04071 | Sphingolipid signaling pathway                                             | RELA, TNF, S1PR1      | 0.000950             |
| hsa04621 | NOD-like receptor signaling pathway                                         | RELA, TNF, CXCL8      | 0.000950             |
| hsa04062 | Chemokine signaling pathway                                                | RELA, CXCL8, CCR5     | 0.002100             |
| hsa05120 | Epithelial cell signaling in Helicobacter pylori infection                   | RELA, CXCL8           | 0.005700             |
| hsa03320 | PPAR signaling pathway                                                     | PPARA, PPARG          | 0.006400             |
| hsa04010 | MAPK signaling pathway                                                     | RELA, TNF, VEGFA      | 0.007200             |
| hsa04660 | T cell receptor signaling pathway                                           | RELA, TNF             | 0.010200             |
| hsa04668 | TNF signaling pathway                                                       | RELA, TNF             | 0.011800             |
| hsa04926 | Relaxin signaling pathway                                                  | RELA, VEGFA           | 0.016500             |
| hsa04024 | cAMP signaling pathway                                                      | RELA, PPARA           | 0.031200             |
| hsa04014 | Ras signaling pathway                                                      | RELA, VEGFA           | 0.039900             |

Table 4. The degree value of targets in the STB networks.

| No. | Target | Degree of Values |
|-----|--------|------------------|
| 1   | RELA   | 17               |
| 2   | TNF    | 11               |
| 3   | CXCL8  | 8                |
| 4   | VEGFA  | 5                |
| 5   | PPARA  | 3                |
| 6   | PPARG  | 2                |
| 7   | CCR5   | 2                |
| 8   | HMOX1  | 1                |
| 9   | S1PR1  | 1                |
| 10  | NFE2L2 | 0                |
| 11  | ACE    | 0                |
| 12  | HDAC1  | 0                |
| 13  | ANPEP  | 0                |
| 14  | ERN1   | 0                |
| 15  | GSR    | 0                |
| 16  | TLR9   | 0                |
| 17  | MME    | 0                |
| 18  | SIGMAR1| 0                |
| 19  | ABCG2  | 0                |
Figure 5. A bubble plot of 18 signaling pathways related to the response to LE bioactive compounds against COVID-19.

Figure 6. The STB networks. Yellow triangle: signaling pathway; blue sky triangle: target; pink circle: LE bioactive compound.
3.5. The Molecular Docking Test on MAPK Signaling Pathway against COVID-19

The potential bioactive compounds were docked against three targets (RELA, TNF, and VEGFA) to measure the binding energy. The MDT of the affinity between R1–R5 and RELA target (PDB ID: 2O61) in the “Homo sapiens” setting was analyzed. The binding energy of R1–RELA, R2–RELA, R3–RELA, R4–RELA, and R5–RELA demonstrated at $-7.1$, $-6.2$, $-5.7$, $-5.5$, and $-5.4$ kcal/mol, respectively (Table 5). Methyl 4-prenyloxyccinnamate (R1) had the strongest affinity for RELA (PDB ID: 2O61) (Figure 7A). The binding energy between T1–T12 and TNF target (PDB ID: 5YOY) in the “Homo sapiens” setting was revealed. The presented binding energy of T1–TNF, T2–TNF, T3–TNF, T4–TNF, T5–TNF, T6–TNF, T7–TNF, T8–TNF, T9–TNF, T10–TNF, T11–TNF, and T12–TNF were exposed to $-7.3$, $-7.1$, $-6.6$, $-6.4$, $-6.3$, $-6.3$, $-6.2$, $-6.1$, $-5.6$ and $-5.0$ kcal/mol, respectively (Table 6). Tormentic acid (T1) manifested the strongest affinity for VEGFA (PDB ID: 3P9W) (Figure 7B). The binding energy between V1–V6 and VEGFA target in the “Homo sapiens” setting was uncovered. The presented binding energies of V1–VEGFA, V2–VEGFA, V3–VEGFA, V4–VEGFA, V5–VEGFA, and V6–VEGFA were $-6.1$, $-5.2$, $-4.7$, $-4.6$, $-4.2$, and $-3.7$ kcal/mol, respectively (Table 7). Eugenol (V1) exhibited the strongest affinity for VEGFA (Figure 7C). Collectively, methyl 4-prenyloxyccinnamate, tormentic acid, and eugenol of LE on COVID-19 were promising bioactive compounds to dampen MAPK signaling pathway.

Table 5. Binding energy of potential active compounds on RELA (PDB ID: 2O61).

| Protein (PDB ID: 2O61) | Ligand | PubChem ID | Symbol | Grid Box | Hydrogen Bond Interactions | Hydrophobic Interactions |
|------------------------|--------|------------|--------|----------|--------------------------|------------------------|
| RELA                   | Methyl 4-prenyloxyccinnamate | 14414116 | R1 | $\text{Binding Energy (kcal/mol)} = -7.1$ | $\text{Center: x = 15.616, Dimension: size_x = 40}$ | $\text{Amino Acid: Arg33}$ | $\text{Amino Acid: Gln247, Lys218, Arg187}$ |
| RELA                   | Paeonol | 11092 | R2 | $\text{Binding Energy (kcal/mol)} = -6.2$ | $\text{Center: x = 15.616, Dimension: size_x = 40}$ | $\text{Arg246}$ | $\text{Lys272, Lys241}$ |
| RELA                   | Isovanillin | 12127 | R3 | $\text{Binding Energy (kcal/mol)} = -5.7$ | $\text{Center: x = 15.616, Dimension: size_x = 40}$ | $\text{N/A}$ | $\text{Arg33, Arg187, Lys218}$ |
| RELA                   | Anethole | 637563 | R4 | $\text{Binding Energy (kcal/mol)} = -5.5$ | $\text{Center: x = 15.616, Dimension: size_x = 40}$ | $\text{Arg305}$ | $\text{Val248, Lys218, Arg246, Gln247, Phe307}$ |
| RELA                   | Cinnamic aldehyde | 637511 | R5 | $\text{Binding Energy (kcal/mol)} = -5.4$ | $\text{Center: x = 15.616, Dimension: size_x = 40}$ | $\text{N/A}$ | $\text{Pro189, Asp185, Cys120, His88, Tyr36, Leu154, Val121, Asn155, Ala188}$ |
4. Discussion

The compounds–targets network indicated that LE compounds might be significant ligands that could be used to alleviate COVID-19 symptoms. Of these, RELA has a more significant effect than any other kinds of targets. Furthermore, based on the pathway enrichment, the MAPK signaling pathway is the uppermost mechanism of LE against

Figure 7. (A) The MDT of Methyl 4-prenyloxyccinnmate (PubChem ID: 14414116) on RELA (PDB ID: 2O61). (B) The MDT of Tormentic acid (PubChem ID: 73193) on TNF (PDB ID: 5YOY). (C) The MDT of Eugenol (PubChem ID: 3314) on VEGFA (PDB ID: 3P9W).
Table 6. Binding energy of potential active compounds on TNF (PDB ID: 5YOY).

| Protein (PDB ID: 5YOY) | Ligand | PubChem ID | Symbol | Binding Energy (kcal/mol) | Grid Box | Hydrogen Bond Interactions | Hydrophobic Interactions |
|------------------------|--------|------------|--------|---------------------------|----------|---------------------------|-------------------------|
| TNF                    | Tormentic acid | 73193 | T1 | −7.3 | x = 243.718, size_x = 40 | Arg31 | Arg32, Ala33, Leu29, Asn19, Gln21, Thr89, Val91, Lys90, Arg32, Ser147 |
|                        | [(1R)-1-(5,8-dihydroxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-enyl]pent-4-enoate | 9999214 | T2 | −7.1 | x = 243.718, size_x = 40 | Asn30 | Lys128, Arg31, Ala84, Leu29, Arg82, Tyr87, Gln27, Trp28, Asn46, Asp45, Leu43, Gly127 |
| Dimethylacrylshikonin  | 479499 | T3 | −6.6 | x = 243.718, size_x = 40 | Trp94, Phe144 | Gln21, Ala145, Gly105, Lys65, Asp143, Pro20 |
| Isovalerylshikonin     | 479497 | T4 | −6.5 | x = 243.718, size_x = 40 | Asn93, Phe144 | Pro20, Gln21, Gly105, Lys65, Asp143, Ala145, Thr94 |
| Isobutylalkannin       | 137629300 | T5 | −6.4 | x = 243.718, size_x = 40 | Thr69, Tyr60, Ser85 | Lys65, Gly66, Phe68, Arg67, Gly66, Lys58 |
| α-Methylbutylshikonin  | 479498 | T6 | −6.3 | x = 243.718, size_x = 40 | Ala33, Ala145 | Val17, Arg32, Ala18, Pro20, Gln21, Gly146, Arg31, Val91, Ser147 |
| Isobutylalkannin       | 137629300 | T7 | −6.3 | x = 243.718, size_x = 40 | Thr69, Tyr60, Ser85 | Lys65, Gly66, Phe68, Arg67, Lys58 |
| Protein                  | Ligand                                                                 | PubChem ID | Symbol | Binding Energy (kcal/mol) | Center | Dimension | Grid Box | Hydrogen Bond Interactions | Hydrophobic Interactions |
|-------------------------|-------------------------------------------------------------------------|------------|--------|--------------------------|--------|-----------|----------|---------------------------|-------------------------|
| β-β-Dimethylacrylalkannin | 442720                                                                  | T8         | −6.3   | x = 243.718              | size_x = 40 | Ser56, Asp54, Tyr53, Gln67, Lys65 | His73, Leu75, Pro113 |
| Buthylshikonin          | 10089766                                                                | T9         | −6.2   | x = 243.718              | size_x = 40 | Thr79, Ser95, Gln149 | Lys90, Asn92, Ser81 |
| [(1R)-1-(5,8-dihydroxy-1,4-dioxonaphthalen-2-yl)-4-methylpent-3-enyl]pentanoate | 145992534                                         | T10        | −6.1   | x = 243.718              | size_x = 40 | Thr69, Tyr60 | Gly66, Lys65, Lys58 |
| Ethyl oleate            | 5363269                                                                 | T11        | −5.6   | x = 243.718              | size_x = 40 | N/A | Glu127, Arg82, Ala35, Leu36, Gln125 |
| Ethyl linoleate         | 5282184                                                                 | T12        | −5.0   | x = 243.718              | size_x = 40 | N/A | Gly101, Tyr53, Asn57, Ser56, His73, Leu75, Pro113, Ala111, Ser52, Ala33, Gln67 |

y = \[-425.984\]  
z = \[261.631\]  
size_y = 40  
size_z = 40
Table 7. Binding energy of potential active compounds on VEGFA (PDB ID: 3P9W).

| Protein                  | Ligand            | PubChem ID | Symbol | Binding Energy (kcal/mol) | Grid Box | Hydrogen Bond Interactions | Hydrophobic Interactions |
|--------------------------|-------------------|------------|--------|--------------------------|----------|---------------------------|-------------------------|
| VEGFA (PDB ID: 3P9W)    | Eugenol           | 3314       | V1     | −6.1                     | x = −12.652 x = 40 | Glu65, Ile83, Pro85, Glu64 | Ile46, Asn62, Ile46 |
|                         |                   |            |        | y = 70.481               | y = 40    |                           |                         |
|                         |                   |            |        | z = −40.286              | z = 40    |                           |                         |
|                         | Ethyl linoleate   | 5282184    | V2     | −5.2                     | x = −12.652 x = 40 | N/A | Ile46, Pro85, His86 |
|                         |                   |            |        | y = 70.481               | y = 40    |                           |                         |
|                         |                   |            |        | z = −40.286              | z = 40    |                           |                         |
|                         | Methyl tetradecanoate | 31284  | V3     | −4.7                     | x = −12.652 x = 40 | Glu64 | Ile46, Pro85, Asp63 |
|                         |                   |            |        | y = 70.481               | y = 40    |                           |                         |
|                         |                   |            |        | z = −40.286              | z = 40    |                           |                         |
|                         | Ethyl oleate      | 5363269    | V4     | −4.6                     | x = −12.652 x = 40 | N/A | Ile83, Pro85, Glu64 |
|                         |                   |            |        | y = 70.481               | y = 40    |                           |                         |
|                         |                   |            |        | z = −40.286              | z = 40    |                           |                         |
|                         | Hexadecanoic acid methyl ester | 8181  | V5     | −4.2                     | x = −12.652 x = 40 | N/A | Ile46, Ile83, Glu64 |
|                         |                   |            |        | y = 70.481               | y = 40    |                           |                         |
|                         |                   |            |        | z = −40.286              | z = 40    |                           |                         |
|                         | Methyl decanoate  | 8050       | V6     | −3.7                     | x = −12.652 x = 40 | N/A | Ile46, Ser50, Phe36 |
|                         |                   |            |        | y = 70.481               | y = 40    |                           |                         |
|                         |                   |            |        | z = −40.286              | z = 40    |                           |                         |

4. Discussion

The compounds–targets network indicated that LE compounds might be significant ligands that could be used to alleviate COVID-19 symptoms. Of these, RELA has a more significant effect than any other kinds of targets. Furthermore, based on the pathway enrichment, the MAPK signaling pathway is the uppermost mechanism of LE against COVID-19. Thus, three targets (RELA, TNF, and VEGFA) linked to the MAPK signaling pathway might be promising targets for use against COVID-19. Accordingly, the docking score on the three targets suggested that three bioactive compounds (methyl 4-prenylcinnamate, tormentic acid, and eugenol) were considered as the most notable compounds of LE against COVID-19. Meanwhile, the results of the KEGG pathway enrichment analysis showed that nine targets might play important roles against COVID-19. The relationships of the 18 signaling pathways with anti-virus were discussed as follows.
• AGE-RAGE signaling pathway in diabetic complications: Activation of the binding of AGE to its receptor RAGE can stimulate cytokine production, can cause tissue damage, and the suppression of AGE-RAGE can effectively reduce inflammation [28].

• Adipocytokine signaling pathway: Most adipocytokines are pro-inflammatory factors and they are closely linked to chronic inflammation [29,30].

• RIG-I-like receptor (RLR) signaling pathway: The RIG-I-like receptors (RLRs), RIG-I, MDA5, and LGP2, play an important role in pathogen recognition of RNA virus infections that instigate and regulate antiviral immunity [31].

• IL-17 signaling pathway: IL-17 can play vital roles in responding to pathogenicity in diverse tissues, as well as being important for inflammation balance and tissue cohesion during viral attacks [32].

• Toll-like receptor (TLR) signaling pathway: Nucleic acids originating from bacteria and viruses can be recognized by the intracellular Toll-like receptor (TLR) and they are also sensitive to self-nucleic acids in disease conditions such as autoimmunity [33].

• HIF-1 signaling pathway: The dysfunction of HIF-1α develops influenza A virus (IAV) replication by triggering autophagy in alveolar epithelial cells [27].

• NF-κb (Nuclear Factor kappa-light-chain-enhancer of activated B cells) signaling pathway: Viruses have evolved to exploit NF-κb-driven cellular functions, and the understanding of molecular mechanisms might be a new strategy against viral diseases [34].

• Sphingolipid signaling pathway: Sphingolipid metabolites, such as ceramide and sphingosine-1-phosphate, are signaling messengers that tune a wide range of cellular processes and are essential for immunity, inflammation, and inflammatory disorders [35].

• NOD-like receptor (NLR) signaling pathway: NLRs have been linked to human diseases, including infections, inflammatory disorders, and even chronic inflammation [36].

• Chemokine signaling pathway: In COVID-19 patients, inhibiting the secretion of cytokines and chemokines dulled the cytokine storm that represented the severity of the disease and was a negative side effect [37].

• PPAR (Peroxisome Proliferator-Activated Receptor) signaling pathway: The regulation of PPAR-α (Peroxisome Proliferator-Activated Receptor-alpha) with agonists enhanced herpesvirus replication and reactive oxygen species (ROS) production [38].

• MAPK (Mitogen-Activated Protein Kinase) signaling pathway: it was reported that the virus’s existence in hosts could activate the MAPK signaling pathway; some viral specific proteins can maintain the persistent activation of the MAPK signaling pathway [39].

• T cell receptor (TCR) signaling pathway: The TCR recognizes pathogens on major histocompatibility complex molecules with the cooperation of CD4 (Cluster of Differentiation 4) or CD8 (Cluster of Differentiation 8) co-receptors and produces cytokines [40].

• TNF (Tumor necrosis factor-alpha) signaling pathway: TNF-α boosters influenza A virus-induced production of antiviral cytokines by activating RIG-I (Retinoic acid-inducible gene I) gene expression [41].

• Relaxin signaling pathway: Relaxin receptor abnormality enhances vascular inflammation and damages external remodeling in arteriovenous fistulas [42].

• cAMP (Cyclic Adenosine MonoPhosphate) signaling pathway: cAMP stimulates interleukin-10 production as the anti-inflammatory cytokine [43].

• RAS (Renin-Angiotensin System) signaling pathway: The use of RAS antagonists might increase the risk of developing a SARS-CoV-2 infection. However, it is not sufficient evidence for discontinuing RAS blockers in patients with hypertension [44].

Based on the pathway enrichment analysis, RELA was considered as a hub target in LE against COVID-19. The RELA was directly enriched in 17 out of 18 signaling pathways by
the MAPK signaling pathway, indicating that the MAPK signaling pathway might be a hub signaling pathway in LE against COVID-19. The other two targets (TNF and VEGFA) that are directly related to the MAPK signaling pathway might be important targets for creating synergistic effects against COVID-19. A report demonstrated that inhibition of RELA of the NF-κB component reduce cytokine production and thus could alleviate inflammation severity [45]. Most recently, it has been reported that anti-TNF treatment for COVID-19 patients with rheumatoid arthritis diseases showed preventive effects against the high levels of cytokines involved in the immune response of infection, and the therapeutic application of anti-TNFs can lessen the incidence of severe inflammation of COVID-19 [46]. Notably, a report indicated that vascular endothelial growth factor A (VEGFA) antagonized by angiotensin-converting enzyme 2 (ACE2) that is upregulated by COVID-19 infection because COVID-19 inhibits the expression of ACE2. Consequently, VEGFA increases vascular permeability and lessens endothelial damage [47].

Endothelial cell inflammation is a serious symptom of COVID-19 infection, and its uncontrollable cytokine production in tissues and cells causes a severe immune reaction, which is defined as a “cytokine storm” that results in aggravating pneumonia. Moreover, inhibition of the other two targets (TNF and VEGFA) related to the MAPK signaling pathway contribute to anti-proinflammation and anti-vascular permeability against COVID-19. Therefore, the key mechanism of LE against COVID-19 might be the ability to block inflammation and vascular permeability in tissues and/or cells by inactivating the MAPK signaling pathway (Figure 8). However, this research still has some limitations. The incompleteness of the natural products dataset might create a fallacy and COVID-19 data is updated continually as a new version. During the analysis, some results might cause an error unexpectedly, if we are only focused on computational methods.

Thus, we need to validate the pharmacological mechanism via in vitro or in vivo tests. Thereby, the usefulness of computational approach might be represented. As a matter of fact, network pharmacology is a holistic perspective to search for multiple factors against specific diseases, which might provide significant clues about the relationships between components such as signaling pathways, targets, and compounds.

**Figure 8.** The potential mechanism effectors of LE against COVID-19.
5. Conclusions

The bioactive compounds and mechanism(s) of LE against COVID-19 were first explored using network pharmacology. The findings of this study suggest that the final bioactive compounds were methyl 4-prenylcinnamate, tormentic acid, and eugenol and their targets were RELA, TNF, and VEGFA, respectively. The mechanism(s) of LE against COVID-19 might inhibit cell inflammation and permeability against COVID-19 by inactivating the MAPK signaling pathway. This research provides a scientific indication to support LE’s therapeutic effects on COVID-19, and thus, the proper application of these three bioactive compounds against COVID-19 might lead to promising synergistic effects such as anti-inflammation and anti-permeability in order to alleviate COVID-19 symptoms. In parallel, this study needs to be further verified through in vitro or in vivo models.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/cimb44050123/s1, Table S1: SEA (Similarity Ensemble Approach): 550 targets; STP (SwissTargetPrediction): 712 targets. Table S2: The overlapping targets between SEA and STP: 249 targets. Table S3: COVID 19-related targets: 356 targets. Table S4: The final 19 overlapping targets related to COVID-19.

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**Abbreviations**

- ACE2: Angiotensin Converting Enzyme 2
- AGE-RAGE: Advanced Glycation Endproduct–Receptor for Advanced Glycation Endproduct
- cAMP: Cyclic Adenosine MonoPhosphate
- CD4: Cluster of Differentiation 4
- CD8: Cluster of Differentiation 8
- COVID-19: Coronavirus disease 2019
- HIF-1: Hypoxia-inducible factor 1
- HIF-1α: Hypoxia-inducible factor 1α
- IL-17: Interleukin 17
- KEGG: Kyoto Encyclopedia of Genes and Genomes
- LE: Lithospermum erythrorhizon
- LGP2: Laboratory of Genetics and Physiology 2
- MAPK: Mitogen-Activated Protein Kinase
- MDA5: Melanoma Differentiation-Associated protein 5
- NF-kb: Nuclear Factor kappa-light-chain-enhancer of activated B cells
NLRs  NOD-like receptors

NPASS  Natural Product Activity and Species Source

PPAR  Peroxisome Proliferator-Activated Receptor

PPAR-α  Peroxisome Proliferator Activated Receptor Alpha

RAS  Renin-Angiotensin System

RELA  v-rel avian reticuloendotheliosis viral oncogene homolog

RIG-I  Retinoic acid-inducible gene I

RLRs  RIG-I-like receptors

RNA  Ribonucleic acid

ROS  Reactive Oxygen Species

SARS-CoV-2  Severe Acute Respiratory Syndrome Coronavirus 2

SEA  Similarity Ensemble Approach

SMILES  Simplified Molecular Input Line Entry System

STP  Swiss Target Prediction

TCR  T Cell Receptor

TLR  Toll-like Receptor

TNF  Tumor Necrosis Factor

TNF-α  Tumor Necrosis Factor Alpha

VEGFA  Vascular Endothelial Growth Factor A

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