Research Article

Identification of Potential COVID-19 Targets and Pathways Derivate from Various Phenolic Compounds from Chives (*Allium schoenoprasum)* by Using Network Pharmacology Approach

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ABSTRACT: *With the uncertainty of COVID-19 disease around the world, the discovery and development of novel treatments for COVID-19 becoming an emerging trend. Network pharmacology has been used for determining the potential targets from several diseases. This research mainly focused on the potential of Allium schoenoprasum against COVID-19 based on a network pharmacology approach. The methods consist of target identification of the compounds, target identification related to COVID-19 disease, compound-target interaction network, protein-protein interaction network and gene ontology and pathway enrichment analysis. Fifthy three main targets obtained from the compound-COVID-19 were identified as main targets from the compounds with MMP9, MPO, TLR4, MMP2, CCNB1, AURKB, PLK1, TOP2A, ALOX5, and CD38 becoming the top 10 core targets. Phenolic compounds in Allium schoenoprasum may act as anti-COVID-19 through several inflammatory and immune response pathways. Based on these results, it seems that phenolic compounds in Allium schoenoprasum might act as anti-COVID-19 via network pharmacology approaches.*

Keywords: *network pharmacology; Allium schoenoprasum; COVID-19*

1. Introduction

The COVID-19 pandemic of respiratory illnes–ses was started in December 2019 by a new coronavirus known as Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). SARS-CoV-2 may have evolved (mutated) in an animal before causing sickness in people. Infections have spread quickly from the outbreak's start to create several epidemics that are occurring simultaneously around the world [1,2]. Around six hundred and thirty-five millions people were verified COVID-19 cases and 6,593,723 COVID-19-related deaths had been documented globally as of the end of October 2022 [3].

It has been determined that the coronavirus is transferred through the air by droplets and virus particles generated when an infected person breathes, talks, laughs, sings, coughs, or sneezes. Small infectious particles can remain in the air and concentrate indoors, especially in areas with high human traffic and inadequate ventilation, while larger droplets may fall to the ground in a matter of seconds [4]. COVID-19 can cause anything from a moderate fever and sore throat to catastrophic lung damage and multiple organ failure, and ultimately death [5]. At this time, there are no effective medicines available that can effectively combat SARS-CoV-2 infection [6] This makes it all the more important to find new medicines to employ against COVID-19, especially from natural resources. *Allium schoenoprasum*, more commonly known as chives, is a member of the lily family (Liliaceae) and is indigenous to both Europe and Asia. Cultivation of this plant dates back at least 4,000 years in China and to the Middle Ages in Europe for use in food preparation and as a medicinal herb. These days, the leaves are commonly utilized in cooking because of their subtle onion flavor [7].

Several pharmacological investigations have reported that *Allium schoenoprasum* might act as an antioxidant, anti-lithogenic, antihypertension, antibacterial, antifungal, anticancer, and etc [8,12]. *Allium schoenoprasum* contains various types of phenolic compounds which makes it one of the promising plants for drug development [13,16]. However, the potential of *Allium schoenoprasum* as anti-Covid-19 has never been reported. In this study, we investigated the potential targets and pathways related to major phenolic compounds from *Allium schoenoprasum* by using a promising computational study via network pharmacological approaches.

2. Methods

2.1. Samples and preparation

Eight major phenolic compounds reported in *Allium schoenoprasum* were retrieved from literature mining such as gallic acid, p-Coumaric acid, ferulic acid, sinapic acid, kaempferol, isorhamnetin, quercetin, and rutin [13-16]. In addition, the chemical structures and SMILE information was collected from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/).

2.2. Drug-likeness, pharmacokinetic, and toxicity prediction

The drug-likeness properties of phenolic compounds from *Allium schoenoprasum* were analyzed based on the Lipinski rule of 5, while the pharmacokinetic properties were based on ADMET prediction. Both drug-likeness and pharmacokinetics were investigated by using pkCSM tool prediction (https://biosig.lab.uq.edu.au/pkcsm/prediction) [17]. Toxicity potential from the compounds was analyzed by using ProTox-II (https://tox-new.charite.de/protox_II/) [18]. All predictions were conducted by inputting the SMILES code from each compound to each database of pkCSM and ProTox-II.

2.3. Identification of compound-target network interaction

The target of each compound was obtained via the swiss target prediction database (http://www.swisstargetprediction.ch/?) [19] by using the SMILES code and using *Homo sapiens* as the species. All the predicted targets were downloaded in CSV format and deleted if they

were duplicates. The compound-target network was generated by using Cytoscape v3.8.2 [20] by importing all the integrated targets into the program.

2.4. COVID-19 related disease gene expression

The differential expression (DE) analysis can be employed to investigate genes associated with the disease's condition [21]. Whole blood transcriptomic data from Covid-19 patients were collected from the Gene Expression Omnibus GEO database which can be accessed from the online database (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE171110) with the identifier GSE171110. The data were assigned to Healthy and Infected according to the data sets' information. The data set was prepared using R Studio utilizing the basic function of R and EdgeR package [22]. First, the gene with a count lower than 10 counts per million (CPM) was filtered out. Afterward, the data were normalized using the trimmed mean of the M-values method [23]. Finally, differentially expressed genes were identified using classic edgeR. The results with P-value < 0.01, FDR < 0.05, and absolute value of LFC > 1 were chosen as differentially expressed genes [24,25].

2.5. Protein-protein interaction network

The intersection targets between *Allium schoenoprasum* and COVID-19 targets were uploaded to the online site of STRING version 11.5 (https://string-db.org/) [26]. The protein type was set to "Homo sapiens" and a medium confidence level of 0.4 was selected. The protein interaction network was obtained from the STRING system and the data analysis was imported into Cytoscape v3.9.1, for the identification of the top 10 core targets by using an additional CytoHubba plugin [27].

2.6. Gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis

The enrichment analysis of GO and KEGG was performed according to previously reported [6]. The GO studies were divided into several parameters including biological process, molecular function, and cellular component while the KEGG pathway enrichment was conducted by using obtained data according to the compounds-COVID-19 interaction. The bubble plot was generated by using R software.

3. Results and discussion

3.1. Drug-likeness, pharmacokinetic and toxicity prediction from the compounds

The total of 8 main phenolic compounds in *Allium schoenoprasum* were retrieved from literature mining namely gallic acid, p-Coumaric acid, ferulic acid, sinapic acid, kaempferol, isorhamnetin, quercetin, and rutin. The compound information is shown in Table 1. The drug-likeness was represented by using Lipinski's rule of five, which is a prediction tool used to decide how a compound meets the pharmacological requirements for an oral drug that enters circulation and can have an active effect. As shown in Table 2, all the compounds fulfill the requirements of Lipinski's rule of five except for rutin with 3 violations such as the molecular weight >500, hydrogen bond acceptors >10, and hydrogen bond donors >5. Moreover, the pharmacokinetic properties prediction showed that all compounds except rutin showed high GI absorption. Meanwhile, the Blood Brain Barrier (BBB) ability showed that all compounds are hard to penetrate the BBB. Toxicity prediction in Table 3 showed that all compounds were grouped in classes 3, 4, and 5 with the LD_{50} between 159-5000 mg/kg. it was found that the majority of the compounds used for the network analysis were non-toxic, except for quercetin which might be toxic if swallowed ($50 < LD_{50} \leq 300$ [18].

3.2. Target identification from compounds and COVID-19

The 8 main candidate compounds in *Allium schoenoprasum* were used for the target prediction. The Swiss Target Prediction database showed

Table 1. Compound information

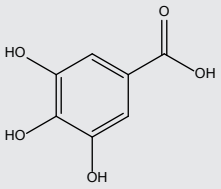
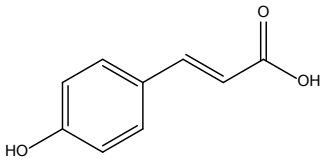
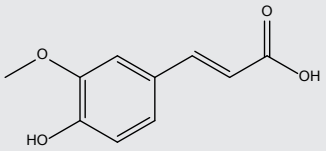
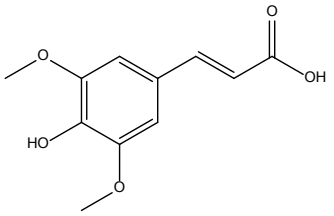
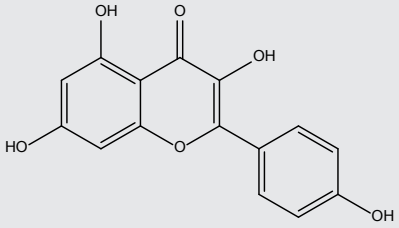
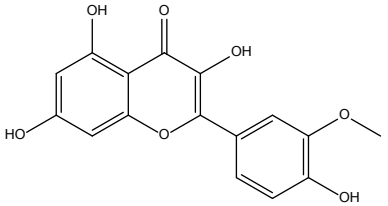
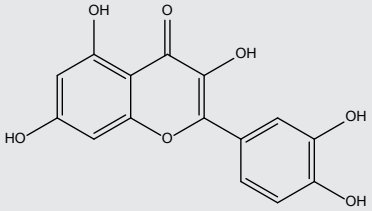
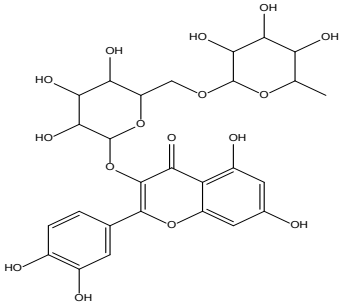
No.	Compounds	Molecular formula	PubChem CID	Structure
1	Gallic acid	$C_7H_6O_5$	370	
2	p-Coumaric acid	$C_9H_8O_3$	637542	
3	Ferulic acid	$C_{10}H_{10}O_4$	445858	
4	Sinapic acid	$C_{11}H_{12}O_5$	637775	
5	Kaempferol	$C_{15}H_{10}O_6$	5280863	
6	Isorhamnetin	$C_{16}H_{12}O_7$	5281654	
7	Quercetin	$C_{15}H_{10}O_7$	5280343	
8	Rutin	$C_{27}H_{30}O_{16}$	5280805	

Table 2. Drug likeness and pharmacokinetic profiles prediction

Compound	MW	Log P	Rotatable bonds	H-Acceptors	H-Donors	Surface Area	%GI absorption	Log BBB	Hepatotoxicity
Gallic acid	170.12	0.50	1	4	4	67.14	43.37	-1.10	No
p-Coumaric acid	164.16	1.49	2	2	2	69.59	93.49	-0.23	No
Ferulic acid	194.19	1.50	3	3	2	81.07	93.69	-0.24	No
Sinapic acid	224.21	1.51	4	4	2	92.54	93.06	-0.25	No
Kaempferol	286.24	2.28	1	6	4	117.31	74.29	-0.94	No
Isorhamnetin	316.27	2.29	2	7	4	128.79	76.01	-1.14	No
Quercetin	302.24	1.99	1	7	5	122.11	77.21	-1.10	No
Rutin	610.52	-1.69	6	16	10	240.90	23.45	-1.90	No

Table 3. Toxicity classes and LD_{50} prediction

Compounds	Toxicity class	LD_{50} (mg/kg)
Gallic acid	4	2000
p-Coumaric acid	5	2850
Ferulic acid	4	1772
Sinapic acid	4	1772
Kaempferol	5	3919
Isorhamnetin	5	5000
Quercetin	3	159
Rutin	5	5000

that a total of 190 potential targets were obtai–ned from the compounds. To summarize the interaction between compounds and target, we generated the network by using Cytoscape v3.8.2 as shown in Figure 1. Moreover, a total of 3429 differentially expressed genes in COVID-19 were collected from the GEO database. As shown in Figure 2, a volcano plot was drawn to show the distribution of the differentially expressed genes. The significant upregulated and downregulated genes are represented with the red dots in the plot representing the significant expression, while the other color represents non-significant expression. We compared the target genes regulated by the active compounds in *Allium schoenoprasum*, and different genes in COVID-19, obtaining 53 common target genes (Figure 3A). Next, we investigated the gene expression from these 53 targets and showed that most target genes were upregulated in COVID-19 patients (Figure 3B).

3.3. Protein-protein interaction network

To determine which target is the most potential, we conduct the protein-protein network analysis by using the STRING database and generate the protein cluster by using Cytoscape v3.82. As shown in Figure 4A, a total of 53 nodes and 110 edges as the interaction between each node. The average node degree is 4.15, while the average local clustering coefficient is 0.604. Among all the targets, only 6 targets (Endothelin-converting enzyme 1 (ECE1), Aldo-Keto Reductase Family 1 Member B10 (AKR1B10), Fucosyltransferase 7 (FUT7), Calcium/Calmodulin Dependent Protein Kinase Kinase 2 (CAMKK2), Lysine demethylase

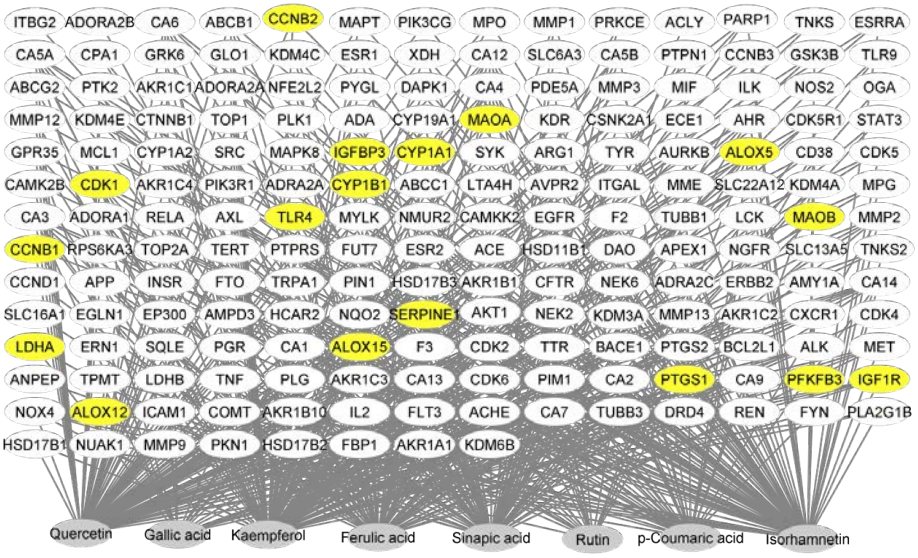


Figure 1. Compound-target interaction network, White oval represents the targets, yellow oval represents gene related to inflammation while the gray oval represents the compounds

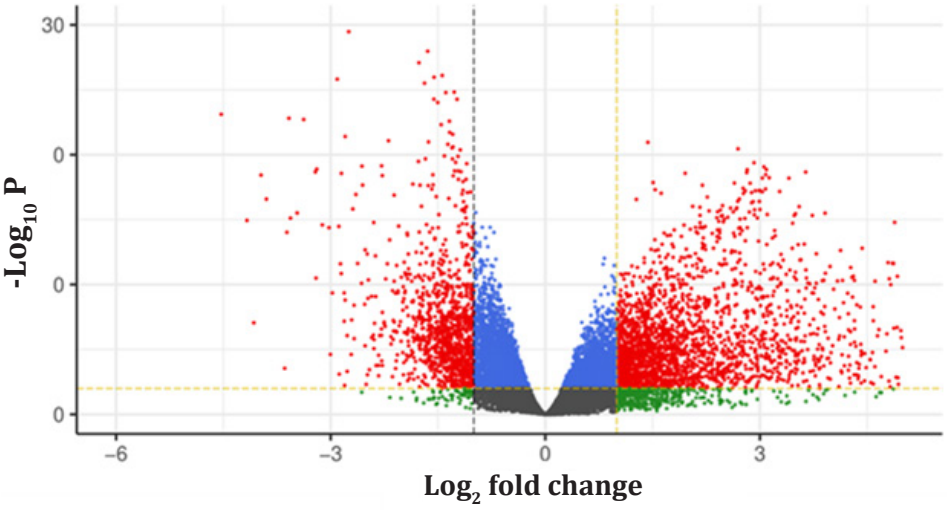


Figure 2. Differentially expressed genes in COVID-19 patients from differentially expressed genes (3429 genes). The significantly expressed genes with log fold change lower than -1 or higher than 1 are plotted in red. The blue plot shows genes that do not satisfy the minimum log fold change values. Other plots below the horizontal black dashed line are non-significantly different expressed genes, with a p-value higher than 0.01

6B (KDM6B), and Human Quinone Reductase 2 (NQO2)) that were not formed the cluster with another target. The protein-protein interaction network formed 1 main protein cluster (Figure 4B) and 3 small protein clusters (Figure 4C). Among all the targets, Matrix metallopeptidase 9 (MMP9), Myeloperoxidase (MPO), Toll-like receptor 4 (TLR4), Matrix metallopeptidase 2 (MMP2), Cyclin B1 (CCNB1), Aurora kinase B (AURKB), Polo-like kinase 1 (PLK1), DNA topoisomerase IIα (TOP2A), Arachidonate 5-Lipoxygenase (ALOX5), and Cluster of differentiation 38 (CD38) were the most important target from the compounds in *Allium schoenoprasum* which are associated with the COVID-19. Of the top 10 main targets, mostly all the targets were related to inflammatory responses. As the most important target, both MMP-9 and MMP-2 during inflammatory responses play a significant role in breaking down the basement membrane surrounding blood vessels

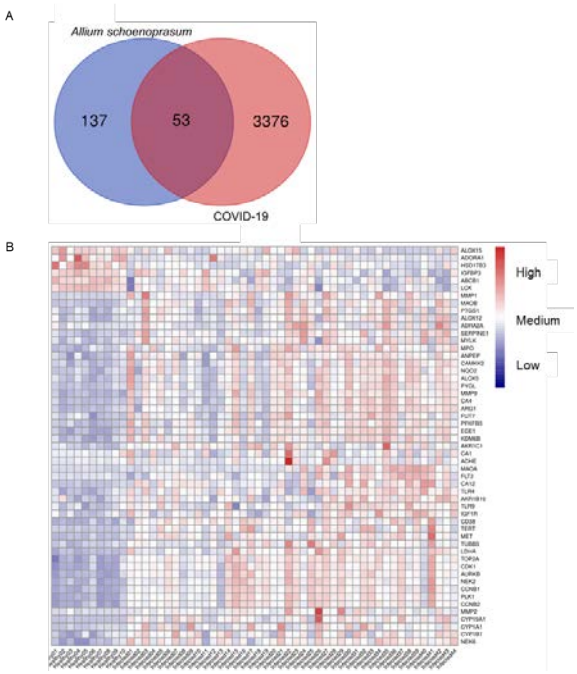


Figure 3. (A)Venn diagram represents the intercept targets between compounds from *Allium schoenoprasum* and COVID-19. (B) Heat map of gene expression. The samples with high gene expression across samples are represented in red. The blue color shows that the gene expression within the sample is relatively low compared to other samples. In addition, the white color indicating the gene within the sample has an average expression level compared to other samples

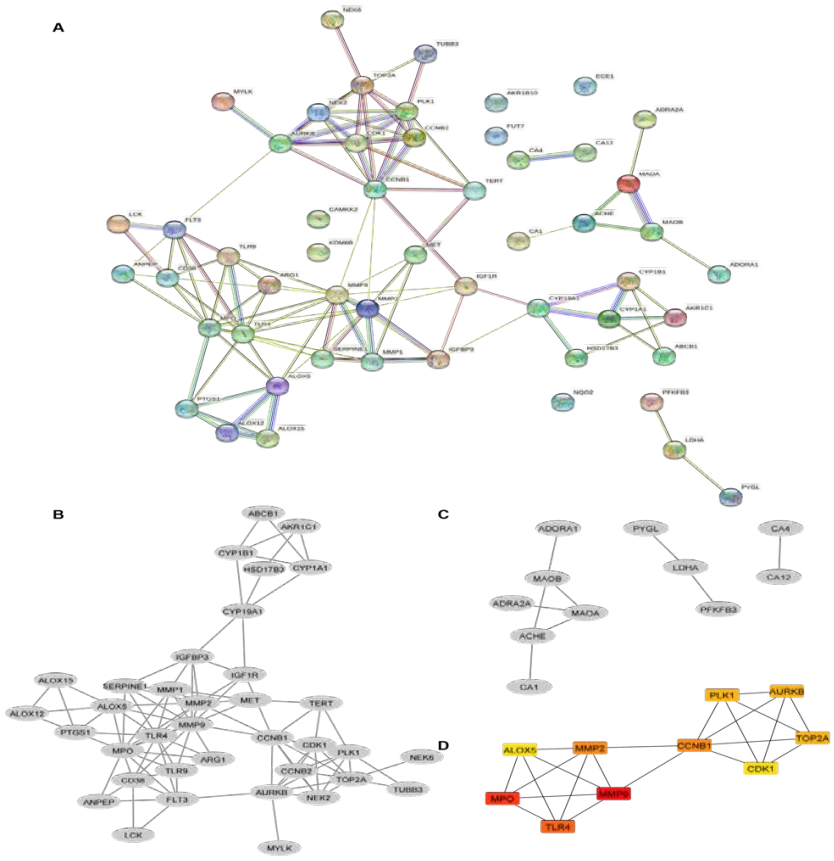


Figure 4. Protein-protein interaction network. (A) PPI obtained from STRING database. (B) Main cluster of PPI. (C) Small cluster of PPI. (D) Top 10 most important target ranked by number of degree (Red/high to yellow/low)

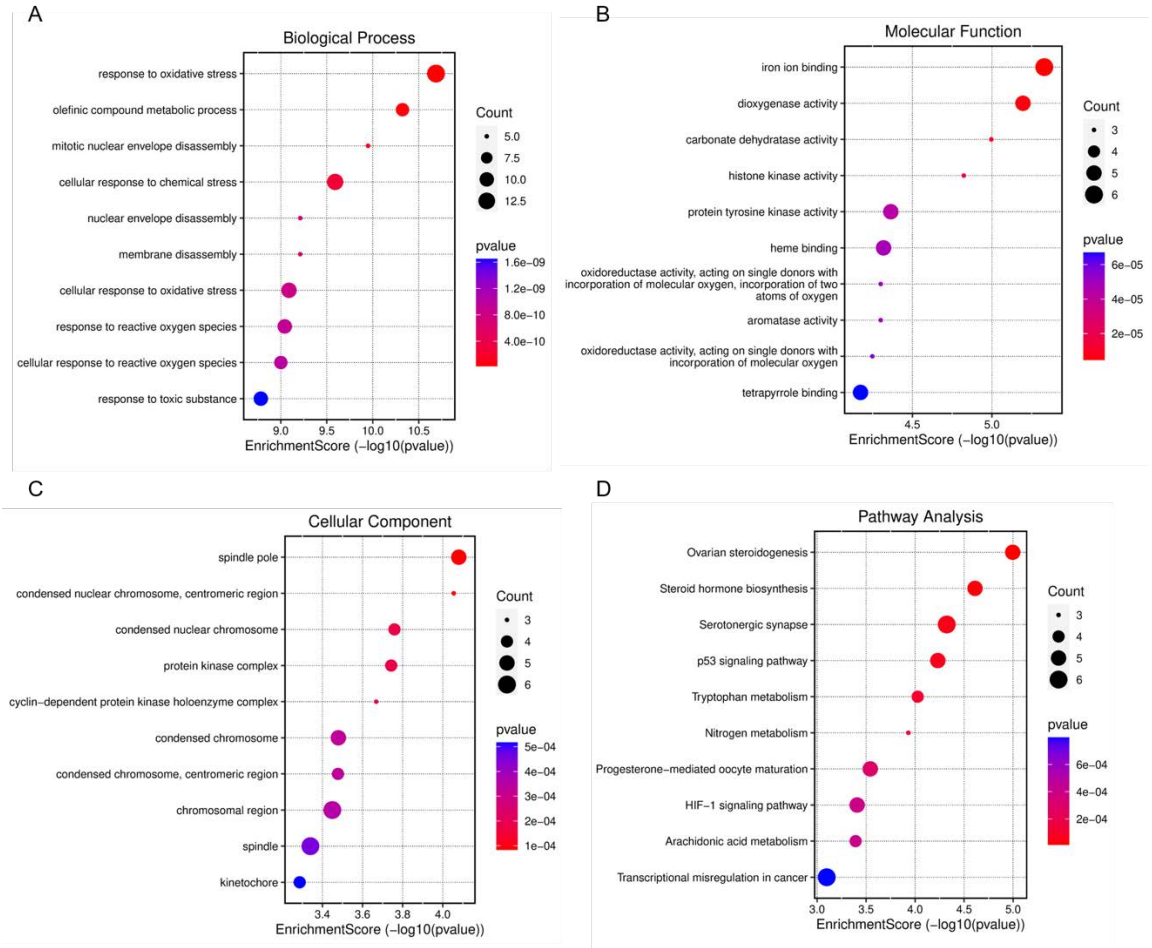


Figure 5. GO and KEGG enrichment analysis. (A) Biological process (B) Molecular function (C) Cellular component (D) KEGG pathway. The size of the dots represents the number of gene count

as well as the parenchymal extracellular matrix thereby facilitating leukocyte infiltration [28]. MPO which is a leukocyte-derived enzyme that produces the reactive oxygen species might contribute to tissue damage during inflammation [29]. The hyperactivation of TLR4 might facilitate the production of proinflammatory cytokines which mainly contributes to the severity of COVID-19 [30]. Meanwhile, ALOX5 regulates the production of the inflammatory marker of COX-2 and plays a role in the severity of COVID-19, especially in hyperglycemic patients [31]. Over–expression of CD38 was associated with the hyperactivation of the immune system resulting in immune exhaustion and uncontrolled release of inflammatory cytokines [32]. It is suggested that MMP9, MPO, TLR4, MMP2, CCNB1, AURKB, PLK1, TOP2A, ALOX5, and CD38 may be ten key targets for the anti-COVID-19 activity of selected compounds from *Allium schoenoprasum*.

3.4. Gene ontology and KEGG enrichment analysis

To further explore possible mechanisms of the 53 candidate targets for the treatment of COVID-19, R software with $p<0.05$ was used for generating the bubble plot for GO enrichment analysis with the candidate target and KEGG pathway analysis. Figure 5A-5C showed the parameter of biological process, molecular function, and cellular component, respectively. The biological process revealed that the response to oxidative stress is the main regulator in interacting with COVID-19. Molecular function showed that several types of binding and protein kinase activity might be related with the COVID-19 and compounds relation. Cellular component showed that the targets were mainly distributed in the several types of spindle and protein kinase complex. Figure 5D showed that

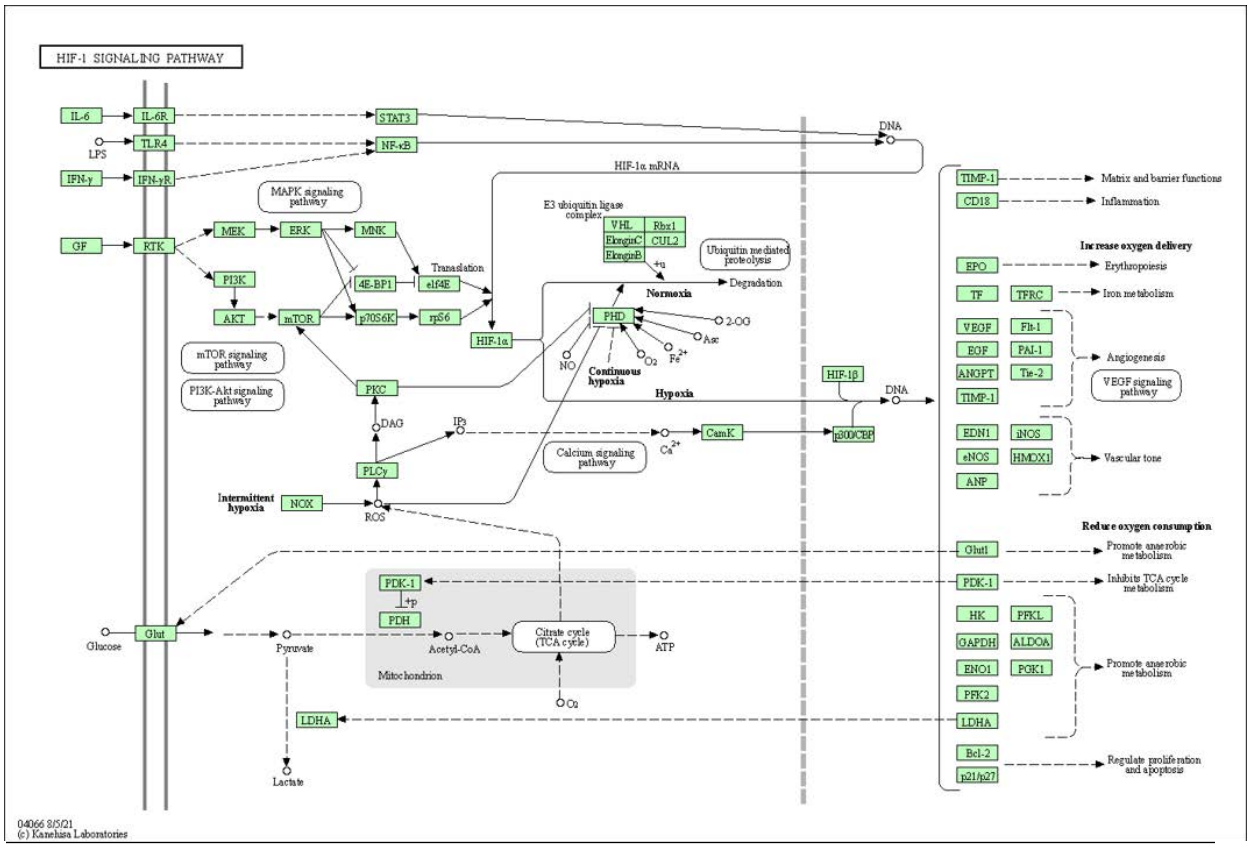


Figure 6. HIF-1 signaling as one potential target from *Allium schoenoprasum*

several majority pathways of serotonergic, p53, tryptophan, arachidonic acid, and HIF-1 signaling might contribute to the inflammatory and immune response. Figure 6 showed one potential pathway which could become a target from the selected flavonoids from the *Allium schoenoprasum*. Apart from inflammation and immune regulation pathway, several pathways related to ovarian steroidogenesis, steroid bio–synthesis, progesterone, and transcriptional mis–regulation might be involved.

4. Conclusion

This study showed that 8 major phenolic components in *Allium schoenoprasum* had potential anti-COVID-19 activity, involving 53 target genes related to COVID-19. MMP9, MPO, TLR4, MMP2, CCNB1, AURKB, PLK1, TOP2A, ALOX5, and CD38 might be the core target in treatment of COVID-19. The obtained results revealed that the 8 major phenolic components in *Allium schoenoprasum* compounds may exert multiple mechanisms in regulating inflammatory and immune response, which indicates the potential of *Allium schoenoprasum* against COVID-19.

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