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MOLECULAR DOCKING STUDY OF LIPOFLAVONOIDS IN THE TREATMENT OF TINNITUS AGAINST THE EFFECTS OF COVID-19

Murat Enöz*
[1]Audiometry Program, Vocational School of Health Services, Istanbul Rumeli University, Turkiye,
murat.enoz@rumeli.edu.tr
(https://orcid.org/0000-0002-2660-1950)

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*Corresponding author

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Abstract

Background/aim:Treatment options are sought for coronavirus disease (COVID-19), which is a global health problem, and the demand for drugs that will eliminate or reduce the effects of SARS-CoV-2 is increasing day by day. The coronavirus disease leaves permanent effects and can even be fatal in patients with weakened immune systems. Considering this important factor, in this study, natural lipoflavonoid nutritional supplement, which is used both to strengthen the immune system and to treat tinnitus, smell and taste disorders, was chosen as the target drug.

Materials and methods: Molecular docking analyzes of lipoflavonoid compounds were performed to understand the molecular interaction mechanisms between SARS-CoV-2, NMDAR and VKORC1 proteins.

Results: In particular, the docking score of thiamine nitrate in NSP16 (-7.97 kcal/mol) and vitamin K epoxide reductase (-7.13 kcal/mol) was found to be high. Riboflavin's insertion score in K epoxide reductase (-8.66 kcal/mol) was also found to be high.

Conclusion: These docking binding scores are indications that these compounds can be used as potential inhibitors. The hypothesis that the common symptoms of COVID-19, olfactory-taste disorder and tinnitus, can be treated in a short time and effectively with lipoflavonoids, and also that the replication of the coronavirus can be stopped, has been theoretically proven.

Keywords: Lipo-Flavonoid compounds, Tinnitus, Bioflavonoids, Covid-19 treatment

Cite

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1. Introduction

Bioflavonoids are a very important class of organic natural products. They are secondary metabolites of plants and are abundant in fruits and vegetables. Given the properties of flavonoids, they are essential for many fields of application. Due to their high pharmacological properties, they are used as medicine or food supplements in the fields of medicine and pharmacy. They have anti-inflammatory, antioxidant, antiviral, anti-mutagenic and anti-cancer therapeutic properties and are amenable to a single component multitarget mechanism. Flavonoids are used to treat various diseases such as cancer, diabetes, heart disease, and also have positive effects against neurological conditions (such as Alzheimer's, Parkinson's and Huntington's diseases and Multiple Sclerosis). Due to their active functional groups (phenyl, methoxy, hydroxy, glucose, etc.), they neutralize free radicals and minimize oxidative damage, a key factor in numerous chronic diseases.

Flavonoids are also used in otolaryngology against dizziness, sudden hearing loss, inner ear nerve damage and tinnitus. It has been reported to be effective in the treatment of tinnitus (tinnitus), objective tinnitus, subjective tinnitus (clicking, pounding sound and pulses in the ear) and Meniere's disease (endolymphatic hydrops of the inner ear). There is limited information in the literature about the mechanism of tinnitus, and it has also been proven that tinnitus is a physiological problem other than a psychological event. Tinnitus can occur through some neurotransmitters and due to damage to the receptors. Repairing these damaged parts plays a key role in tinnitus relief. Glutamate is one of the most important excitatory neurotransmitters in both the peripheral and central nervous

systems. The interaction of vitamins and flavonoids with glutamate receptors can normalize the functions of neuronal activities.

Keeping people in quarantine to protect them from the coronavirus epidemic has increased the psychological pressure on them. Many patients reported increased symptoms such as hallucinations, dizziness, and tinnitus (Situmeang, R. F. V., & Pangestu, A., 2021). A large number of antiviral drugs and vaccine trials for the coronavirus disease, which emerged in December 2019 and spread rapidly all over the world, are being examined both in the computer environment and clinically. Many drugs are in clinical trials to inhibit the coronavirus. In situations where time is limited and the best solution urgently needs to be found, it is very useful to research non-toxic drugs currently in use. It is recommended by medical doctors to consume nutrient-rich fruits and vegetables to keep the immune system strong against coronavirus. Bioflavonoids and vitamins are beneficial metabolites found in abundance in fruits and vegetables. Lipoflavonoid, on the other hand, is a supplement that collects all these components in a single capsule. Consuming bioflavonoids can provide many benefits against both tinnitus and coronavirus (Petridou, A. I., Zagora, E. T., Petridis, P., Korres, G. S., Gazouli, M., Xenelis, I., ... & Kaliora, A. C., 2019).

In this study, the efficacy of bioflavonoids, whose mechanism of action on tinnitus, which is also a symptom of Covid-19 infection, has not been clearly proven in the literature, was investigated for the first time with an in silico analysis, both against tinnitus treatment and against SARS-CoV-2 proteins against coronavirus disease. According to the results of this study, flavonoids can be used in systemic therapy or in nasal wash solutions, nasal sprays, local therapy because of both their antiviral effects and their potential to reduce neural damage. The findings are promising, they can accelerate the recovery processes of patients and will contribute positively to antiviral drug studies.

2. Methods or experimental section

2.1. Lipo-Flavonoid Product

Lipo-Flavonoid is a natural product used in the treatment of tinnitus and Meniere's diseases. It contains eriodictyol glycoside, ascorbic acid, thiamine mononitrate, riboflavin, niacinamide, pyridoxine hydrochloride, cyanocobalamin, calcium pantothenate, choline bitartrate and inositol.

2.2. Preparation of Ligand Molecules

Nicotinic acid (CID938, Niacin), Inositol (CID892), Thiamine nitrate (CID10762), Ascorbic acid (CID54670067, vitamin C), Riboflavin (CID493570, Vitamin B2), Pyridoxine hydrochloride (CID6019), Bitartrate (CID3667129), Choline (CID305))), Pantothenic acid (CID6613), Eriodictyol glycoside (CID382371440), Cyanocobalamin (CID5311498, Vitamin B12), Lopinavir (CID92727), Remdesivir (CID121304016), Remdesivir_nucleoside_monophosphate (CID121310009), x77 (CID121310009), x77 (CID121310009) (CID6323191), N-Acetyl-beta-D-glucosamine (CID24139), S-adenosyl_methionine (CID34756), Cinefungin (CID65482) molecules were downloaded from the PubChem website (ref). They were prepared in Maestro Schrödinger's Epic module. Possible states were produced at pH:7.00. The OPLS3e force field was used.

2.3. Preparation of Proteins

The target proteins are Spike S1 subunit, NSP5, NSP12, NSP15 and NSP16, SARS-CoV-2 proteins, NMDA/NR1-4a, NMDA/NR2A, NMDA/NR3A, ACE2 and VKORC1 human proteins. The VKORC1 target protein is not directly related to COVID-19 but is involved in vitamin K metabolism. NMDA/NR1-4a, NMDA/NR2A, NMDA/NR3A target proteins are also not directly related to COVID-19, but are involved in tinnitus metabolism. The following protein database (PDB) IDs were used as the target construct. 7BQY for 3CLpro, 7BV2 for NSP12, 6WXC for NSP15, 6WKQ for NSP16, 6M0J for Spike S1, 6M0J for ACE2 protein, 2A5T for NMDA/1-4a, 2A5T for NMDA/NR2A, 2RC8 for NMDA/NR3A 2RC1 6WV3 for Bu, VKO3 Missing side chains and missing loops in All protein targets used in the study were filled with Maestro's Prime module. Access code 3CLpro: 7BQY has been downloaded and prepared for deployment. The ligand in chain A was defined as the center of the grid box. NSP16:6WKQ with access code was downloaded from the protein database and prepared for insertion. Sinefungin (SFG) was used for the grid box center. Spike S1: 6M0J with access code was downloaded from the protein database and prepared for insertion. ions were removed. NAG was retained. Amino acid residues on the E chain of the Spike model: Tyr449, Asn487, Gly496, Thr500, Gly502 and Tyr505 were entered into the system for grid box production. The ACE protein that we prepared and published in the SARS-COV study was used. The 3D structure with the 2A5T access code was downloaded and prepared for installation. The crystalline A chain was used for the NMDA/1-4a subunit, while the B chain was used for the NMDA/NR2A subunit. NMDA/NR2A's own ligand was stored and identified for grid box center. Access code: NMDA/NR3 with 2RC8 was retrieved from the protein database and prepared for insertion. Dserine was chosen to create the grid box. VKORC1, access code: 6WV3, was retrieved from the protein database and prepared for insertion.

2.4. Molecular Docking Studies

Molecular docking calculations were made using Autodock Vina software. Lipoflavonoid components were provided in 3D from PubChem (https://pubchem.ncbi.nlm.nih.gov/).

The force field parameter MMFF94 was used to minimize the energy and create the lowest energy structure of the lipoflavonoid components. UCSF Chimera 1.16 and Discovery Studio Visualizer v21.1.0.20298 were used to visualize molecular docking results.

3. Results

3.1. Molecular Dockings Results

Molecular docking analyzes of lipoflavonoid compounds were performed to understand the molecular interaction mechanisms between SARS-CoV-2, NMDAR and VKORC1 proteins. In this study, Lipoflavonoid analogues were inserted into the active sites of 3CLpro, NSP12, NSP15, NSP16, Spike S1 subunit, ACE2, NMDA/1-4A, NMDA/NR2A, NMDA/NR3A and VKORC1 using Maestro's Glide Module. Inhibition mechanism of SARS-CoV-2, NMDA and VKORC1 proteins. Molecular docking results are given in Table 1.

Table 1. Docking scores of lipoflavonoid product components at SARS-CoV-2 receptors.It is emphasized that the inhibition energy is higher in those shown in yellow.

	Bitartrate	Choline	Cyanocobalamin	Eriodictyol glycoside	Inositol	Nicotinamide	Pantothenic acid	Pyridoxine HCl	Riboflavin	Thiamine nitrate	Vitamin C
MPro	-4.67	-4.69	-10.38	-8.84	-5.08	-4.44	-5.94	-5.07	-7.66	-6.62	-5.15
Spike S1	-4.36	-4.24	-9.35	-7.76	-4.83	-4.16	-5.28	-4.67	-6.77	-5.75	-5.05
Spike+ACE2	-5.15	-4.70	-10.38	-9.58	-5.64	-4.72	-6.81	-5.30	-7.35	-6.85	-5.32
NSP12	-8.31	-4.67	-30.79	-10.53	-6.74	-4.71	-6.53	-5.69	-8.42	-6.40	-6.70
NSP12+ssRNA	-8.98	-4.73	-14.62	-10.64	-6.16	-5.01	-7.16	-5.68	-9.22	-6.89	-5.73
NSP15	-3.98	-4.38	-8.12	-7.80	-5.05	-4.08	-5.14	-5.04	-5.40	-5.23	-5.24
NSP16	-4.59	-4.37	-10.83	-7.55	-4.49	-4.73	-5.99	-5.11	-6.85	-6.56	-4.95
VKORC1	-4.67	-5.04	-10.94	-10.88	-5.53	-5.11	-6.50	-5.64	-11.47	-7.48	-5.18
NMDAR1-4a	-3.98	-4.22	-9.39	-6.73	-4.22	-4.09	-4.94	-4.44	-5.41	-5.02	-4.42
NMDAR2A	-7.07	-5.50	-9.14	-7.23	-6.51	-5.85	-4.66	-5.67	-5.15	-5.52	-6.67
NMDA/NR3A	-4.35	-3.85	-8.00	-6.57	-4.46	-3.92	-5.17	-4.21	-5.57	-5.31	-4.66

3.2. NMDA Receptors and Tinnitus

The N-methyl-D-aspartate (NMDA) receptor is a type of glutamate receptor and ion channel protein present in neurons. These receptors consist of seven subunits and include an ion donor pathway. These are NR1, NR2A, NR2B, NR2C, NR2D, NR3A and NR3B. The pharmacological and functional features of NMDA receptors depend on the NR1 and NR2 subtypes. Many agonists and antagonists are available for NMDA receptors.

Glutamate is the most important excitatory neurotransmitter in the cochlea and is released from the inner hair structure. After their spread, glutamates bind to N-Methyl D-Aspartate (NMDA) receptors at the terminal ends of

Neurons in the spiral ganglion. Glutamate and glycine become active when bound to the NMDA receptor, allowing positively charged ions to pass through the cell membrane. The ions of the cell's environment (Mg^{2+}) and the earth's (Zn^{2+}) can bind to some places on the NMDA and block the passage of cations such as sodium (Na^{+}), potassium (K^{+}) and emission (Ca^{2+}) through the ion channel. The depolarization of the cell moves the emitted Mg^{2+} and Zn^{2+} ions where they are bound, thus allowing the Na^{+} and Ca^{2+} ions into the cells and the K^{+} ion supplying the cells. By connecting to NMDA receivers, it also operates the ion outputs from the module.

Neuronal damage can occur if NMDA receptors are overstimulated with glutamate. It causes the excito effect of glutamate outside the cell. Similarly, cells entering the cell cause neuronal function cells and cell death. Salicylate, which is the active component of aspirin, is known to inhibit the effect of glutamate and kainic acid as an antioxidant. Free oxygen radicals can cause intracellular shielding and nitric oxide formation to activate the NMDA receptor. At this time, it is seen that aspirin (coraspin), which is among the anti-embolism blood thinners given to COVID-19 patients, can also induce tinnitus.

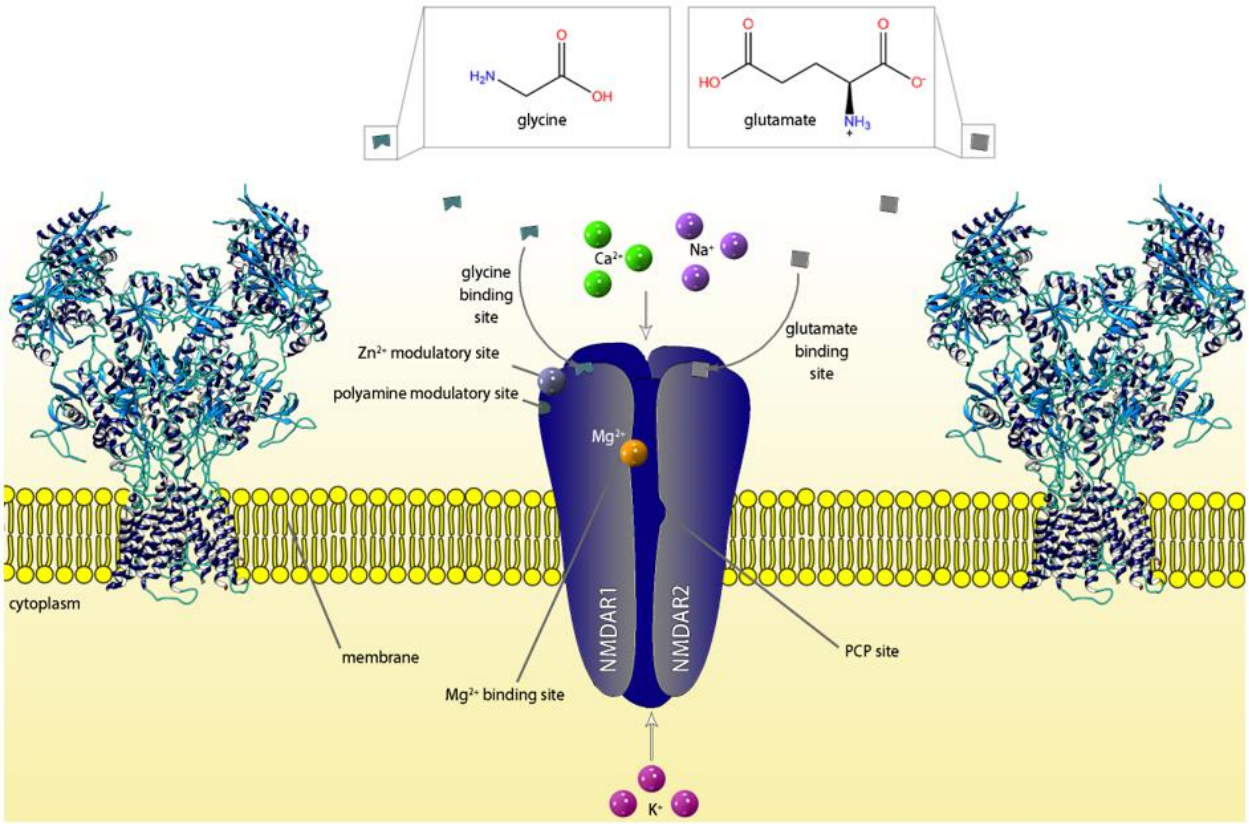

Figure 1. Demonstration of the mechanism of NMDA (N-methyl D-aspartate).

The endogenous glutamate domain agonists used for the NMDA receptor are aspartate, glutamate, homocysteic acid and quinolinic acid. Ibotenic acid is a naturally occurring glutamate domain agonist. Tetrazolylglycine is also a synthetic glutamate site agonist. N-Methyl-D-aspartic acid (NMDA receptor itself) and homoquinolinic acid are national agonists for the glutamate domain. Endogenous glycine domain agonists are alanine, glycine, sarcosine, and serine. Synthetic glycine domain agonists are milasemide, apimostinel, rapastinel, 1-hydroxy-3-amino-2-pyrrolidone, aminocyclopropanecarboxylic acid. Nebostinel is also a synthetic positive allosteric modulator of the glycine domain. Cycloserine is a naturally occurring glutamate site agonist.

Glutamate domain antagonists used for the NMDA receptor are: 5-phosphononorvaline, 2-amino-7-phosphonoheptanoic acid, 2-amino-4-methyl-5-phosphono-3-pentenoic acid, decahydro-6-(phosphonomethyl)-3-isoquinolinecarboxylic acid, midafotel, PEAQX, perzinfotel and selfotel. Kaitocephalin is a naturally occurring glutamate region antagonist. Glycine site antagonists are 4-chloroquinurenin, 7-chloroquinurenic acid and kynurenic

acid. Phenylalanine is an amino acid and a naturally occurring glycine site antagonist. K+, Na+, Cu2+, Zn2+ and Ca2+ are modulators of NMDA receptors.

The prevailing belief is that NMDA receptors are influenced by naturally occurring redox substances, including glutathione, lipoic acid, and the essential nutrient pyrroloquinoline quinone (Hansen, K. B., Yi, F., Gibb, A. J., & Traynelis, S. F., 2018). There are two studies in the literature in which Lipo-Flavonoid components are discussed and investigated for the treatment of tinnitus (Coelho, C., Tyler, R., Ji, H., Rojas-Roncancio, E., Witt, S., Tao, P., ... & Gantz, B. J., 2016). Considering the structure-activity relationship, agonists or antagonists similar to the above-mentioned compounds may be present in Lipo-Flavonoid. Commenting on Lipo-Flavonoid products, based on the interaction of components with proven efficacy on the NMDA receptor, will provide a theoretical idea whether they can be effective in the treatment of tinnitus.

The docking score of glycine to the glycine binding site for NMDA/R1 is -3.99 kcal/mol (ligand efficiency = -0.80). The amine group (-NH3+) on glycine made ion contact with Glu231 and backbone hydrogen donor bond interaction with Glu14. The acetate ion interacts with Glu14 and backbone hydrogen acceptor bond. For NMDA/R2, the docking score of glutamate to the glutamate binding site is -6.40 kcal/mol (ligand efficiency = -0.64). Docking scores of NMDA/R1 glycine binding site agonists are -4.36 for alanine, -8.02 for apimostinel, -4.60 for cycloserine, -4.92 for HA-966, -5.90 for milacamide, -7.75 for nebostinel, -6.12 for phenylalanine, -8.91 for rapastinel. , is -4.55 for sarcosine and -4.40 kcal/mol for serine. The docking scores of glycine binding site antagonists are -6.22 for 7-chlorokynurenic acid, -7.18 for AV-101 and -6.18 kcal/mol for kynurenic acid. Docking scores of NMDA/R2 glutamate binding site antagonists are -5.00 for aspartic acid, -5.54 for homocysteic acid, -5.22 for homoquinolinic acid, -5.31 for ibotenic acid, -5.37 for N-methyl-D-aspartic acid (NMDA), -5.37 for quinolinic acid. -4.99 for tetrazolylglycine and -5.23 kcal/mol for tetrazolylglycine. Docking scores of glutamate binding site antagonists were -5.91 for AP5, -5.76 for AP7, -5.55 for CGP-37849, -6.57 for kaitocephalin, -5.67 for LY-235959, -5.72 for midafotel, -5.99 for PEAQX, -6.13 for perzinfotel, and for selfotel it is -5.48 kcal/mol.

3.3. Mechanism of SARS-CoV-2 Major Enzymes and Structure-Activity Relationship

3.3.1. Enzymes and Mechanism for SARS-CoV-2

The coronavirus is composed of various components, including the Envelope (E), Membrane (M), Spike (S), and Nucleocapsid (N) proteins, as well as genomic RNA and nonstructural proteins (NSP1-16). Inhibiting these proteins

can impede or reduce the advancement of the coronavirus. While there are potential inhibitors being studied for enzyme inhibition, their effectiveness remains uncertain. Inhibitor N3 (Arafet, K., Serrano-Aparicio, N., Lodola, A., Mulholland, A. J., González, F. V., Świderek, K., & Moliner, V., 2020) for Main protease, Remdesivir nucleoside monophosphate (Jorgensen, S. C., Kebriaei, R., & Dresser, L. D., 2020) for NSP12, Tipiracil [K] for NSP15, Sinefungin [K] for NSP16 and N-Acetyl-beta-D-glucosamine (Gotoh, Y., Yamazaki, T., Ishizuka, Y., & Ise, H., 2021) for Spike+ACE2 are model inhibitors. Despite its small size, favipiravir is an exceptionally potent antiviral due to its ability to form covalent interactions with proteins found in coronaviruses. By taking all these model inhibitors as a reference, new natural inhibitors that are more effective and less toxic can be discovered. The effectiveness of natural compounds on coronavirus proteins has been investigated in the literature, and positive results have been reported especially for vitamins and flavonoids (Alzaabi, M. M., Hamdy, R., Ashmawy, N. S., Hamoda, A. M., Alkhayat, F., Khademi, N. N., Al Joud, S. M. A., El-Keblawy, A. A., & Soliman, S. S. M., 2022). Flavonoids are promising safe therapy against COVID-19 (Jannat, K., Paul, A. K., Bondhon, T. A., Hasan, A., Nawaz, M., Jahan, R., Mahboob, T., Nissapatorn, V., Wilairatana, P., Pereira, M. L., & Rahmatullah, M., 2021). The effect of the components combined under a single drug can sometimes be more advantageous than a single drug. The fact that physicians give immune system-boosting supplements to coronavirus patients is proof of this.

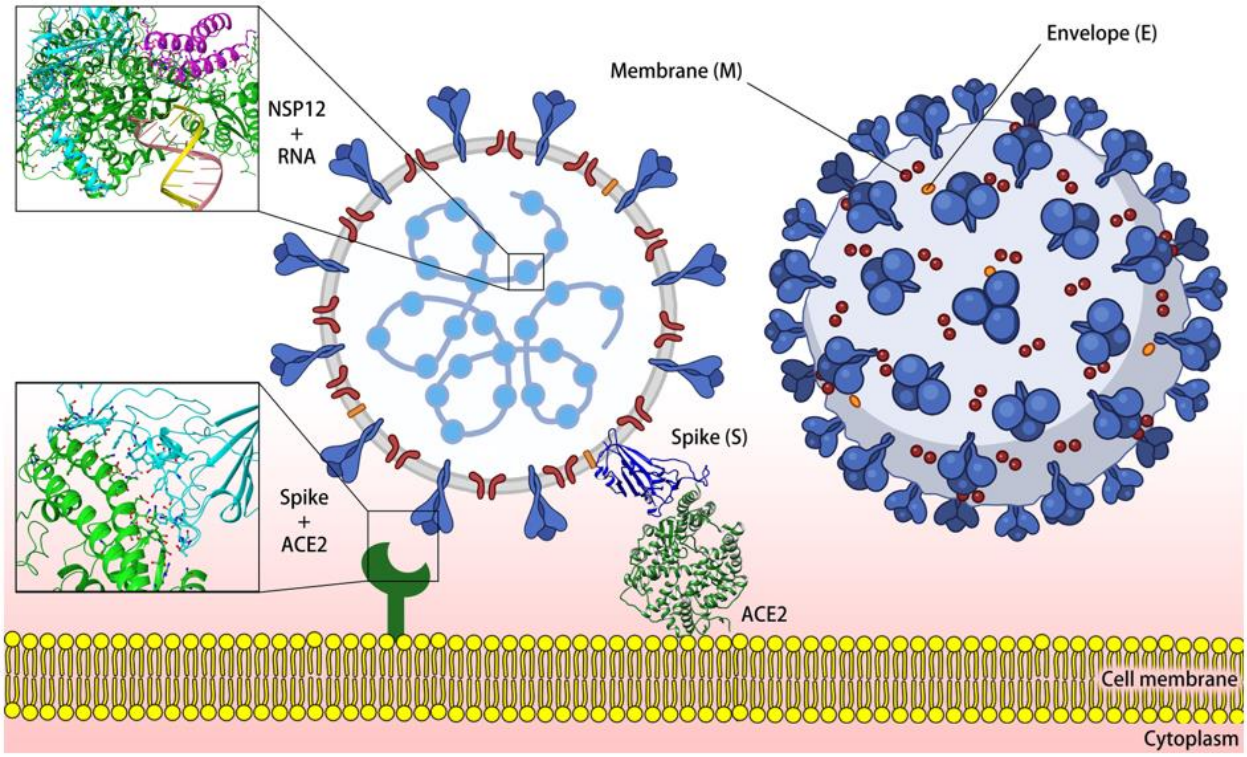

Figure 2. The SARS-CoV-2 and its structure.

The vitamins and flavonoids in the Lipo-Flavonoid product have an immune system strengthening effect and are also used in the treatment of tinnitus. These components are common in coronavirus.

It is thought that it can improve the taste disorder (which is related to smell, taste and sensory communication), which is one of the symptoms, and at the same time, it can prevent the disease from progressing at a severe level by strengthening the immune system.

Docking studies of the model inhibitors favipiravir, hydroxychloroquine, remdesivir, N3, x77, lopinavir and S-adenosyl methionine compounds in the same parameters were performed in the literature using the MOE program (Özdemir, M., Köksoy, B., Ceyhan, D., Sayın, K., Erçağ, E., Bulut, M., & Yalçın, B., 2022). Since favipiravir is a small molecule, its docking score is relatively low, but favipiravir has the ability to interact covalently. Hydroxychloroquine scores high for NSP12 (-11.03 kcal/mol) and NSP12/RNA (-11.12 kcal/mol). Again, the remdesivir compound (contains a phosphorylamine group and is important for phosphorylamine NMDA inhibitors) is a specific inhibitor for NSP12 and NSP12/RNA, and its docking scores are -11.20 and -12.61 kcal/mol, respectively.

Remdesivir is also highly effective against the VKORC1 receptor (-11.85 kcal/mol) and is thought to prevent pulmonary embolism, which is a common COVID-19 symptom and can cause death, by thinning the blood. Compounds N3 and x77 are specific reference compounds for the main protease (3C-like protease) and their scores against MPro were -9.84 and -8.24 kcal/mol, respectively. Lopinavir compound is a specific model inhibitor for endoribonuclease NSP15 (NendoU) and has a score of -7.69 kcal/mol. The model inhibitor for 2′-O-methyltransferases (NSP16), which facilitates viral replication and allows the coronavirus to escape from immune cells, is the S-adenosyl methionine compound and its docking score is -8.13 kcal/mol.

3.3.2. Structure-Impact Relationship

Bitartrate, choline, cyanocobalamin, eriodictyol glycoside, inositol, nicotinamide, pantothenic acid, pyridoxine HCl, riboflavin, thiamine nitrate, vitamin C have attached to the active sites of SARS-CoV-2 enzymes and interacted with amino acids as a result of molecular docking studies (Figure 3).

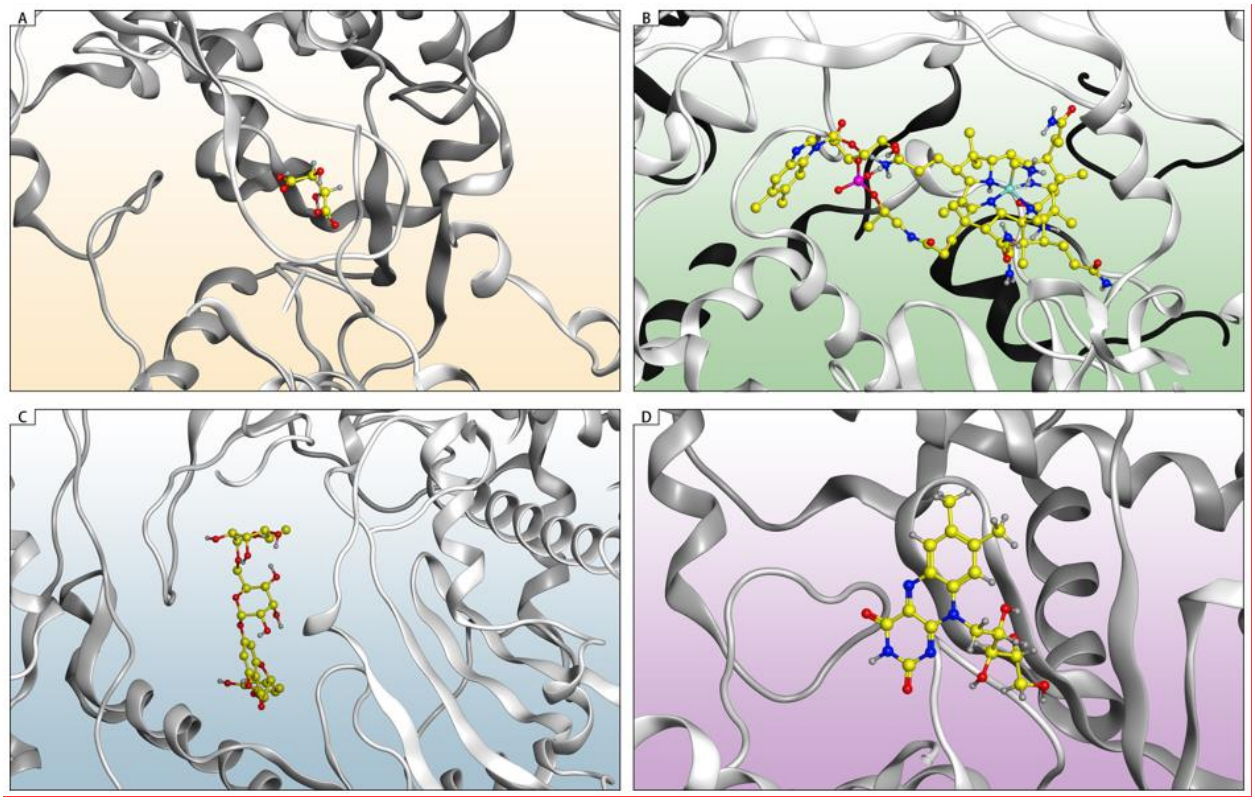

Figure 3. Representation of compounds with the best binding score of SARS-CoV-2 in nonstructural protein 12. A) Bitartrate B) Cyanocobalamin C) Eriodictyol glycoside D) Riboflavin at the active site of NSP12.

The most effective scores for the receptors of the coronavirus generally belong to the compounds cyanocobalamine, eriodictyol glycoside and riboflavin. The compounds cyanocobalamine, eriodictyol glycoside, riboflavin and bitartrate are specific ligands for the NSP12 protein, an RNA polymerase. Cyanocobalamin also has top scores for the main protease (Mpro), Spike, NSP15 and NSP16 proteins, except for NSP12. Although it is a bulky structure, it sits in the active site cavity of the NSP12 receptor and makes non-covalent interactions with amino acids. It has a score of -30.79 kcal/mol against NSP12 without RNA. Sidechain hydrogen donor interactions were made with the hydroxy group Ser682 on the ribose ring on Cyanocobalamin, and with three different amide groups Ser814, Asp618 and Arg55 attached to the central skeleton of Cyanocobalamin. Again, the carbonyl of three different amide groups attached to the central skeleton of Cyanocobalamin made metal/ion contact interaction with the magnesium ion (Mg^{2+}). In the NMDA mechanism, metal interactions are important for regulating ion channel conductivity or for membrane depolarization, and this depolarization is provided by these Mg^{2+} metals interacting with the structure.

The 2 glycoside functional groups on Eriodictyol glycoside add water/blood solubility to the structure and this feature is very important for drug candidates. The sidechain hydrogen donor interacts with the hydroxy group Ser814 on the pyranose ring. One of the hydroxy groups on the benzene ring attached to the flavanone backbone interacted with Asp865 as a sidechain hydrogen donor. The flavanone carbonyl, on the other hand, interacted with Asp833 as a backbone hydrogen acceptor.

Riboflavin's pyrimidine ring in the ptredine skeleton interacts with Arg 836 and arene-H, and a carbonyl group in this ring interacts with Asp833 as a backbone hydrogen acceptor.

Compared to other candidate inhibitors (Cyanocobalamin, eriodictyol glycoside and riboflavin), the bitartrate structure has a lower docking score, but the ligand efficiency is much better. Figure 4 shows the active site of eriodictyol glycoside (A), riboflavin (B) and cyanocobalamin (C) compounds on NSP12 and the single-stranded RNA structure responsible for the replication of the Coronavirus. Hydroxy functional group Gln444 interacted with sidechain hydrogen acceptor. The carbonyl of the carboxylic acid interacted with Ala547, and the acetate group on the other side of the symmetrical structure interacted with Phe442 as a dimeric backbone hydrogen acceptor.

As a result, when the structure-activity relationship is examined, it has been shown by docking studies that inhibitors can slow down or completely stop the work of this enzyme, with strong non-covalent interaction with NSP12.

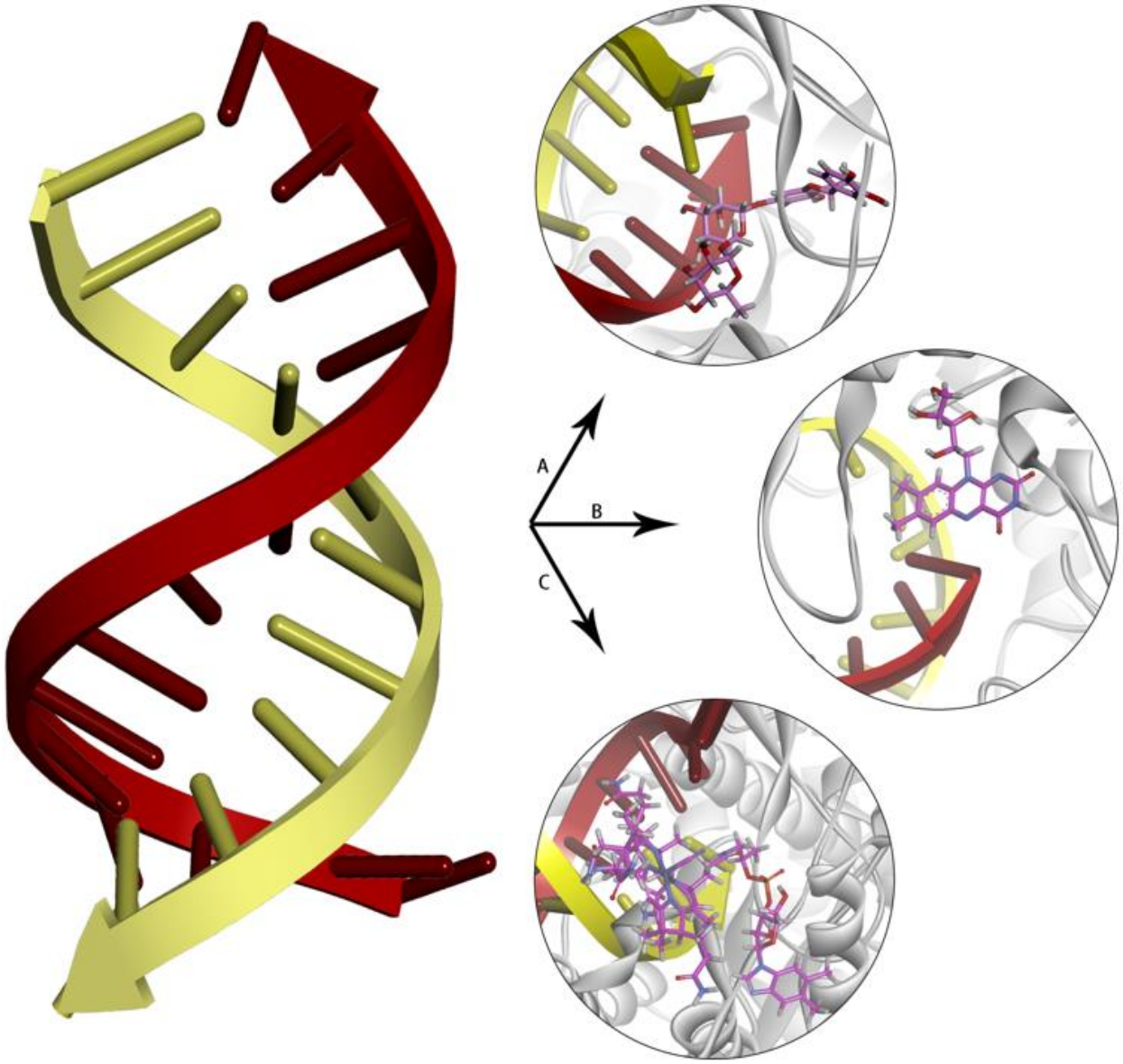

Figure 4. The single-stranded RNA structure responsible for the replication of the coronavirus and the active site of eriodictyol glycoside (A), riboflavin (B) and cyanocobalamin (C) compounds on NSP12, RNA-replication polymerase.

SARS-CoV-2 is a single-stranded RNA virus. Coronavirus RNA transcription and replication cause the virus to reproduce continuously in living organisms. Inhibiting the coronavirus RNA or RNA polymerase will inhibit the replication of the virus and reduce its effectiveness. Cyanocobalamin, one of the specific inhibitors for NSP12, gave sidechain hydrogen donor interactions with two uracil (B18 and B20) and adenine (B19) nucleobases. The amine in

the amide group and the hydroxy functional groups in the ribose ring tend to interact with RNA nucleobases (Uracil and Adenine). In addition, it was determined that magnesium metal interacts with amide carbonyls attached to the cyanocobalamin skeleton, just like in NSP12 modeling without RNA.

The hydroxy functional groups of the two pyranose rings on the Eriodictyol glycoside inhibitor are side-linked to uracil (2 with 3 hydroxy groups B18 on the same structure and 1 on the adjacent ring) and adenine (with 2 hydroxy groups on the same ring, B19). chain hydrogen donor interactions.

It interacted with the benzotetrahydropyrazine part of the riboflavin compound, uracil (B20). The pteridine group interacted with the carbonyl uracil (C10) and the sidechain hydrogen acceptor. In addition, the polar side OH groups in riboflavin have sidechain acceptor interactions with Asp760 and side-chain donor Asn 691. Bitartrate, on the other hand, does not interact with any RNA nucleobase.

3.4. VKORC1 Mechanism

The VKORC1 gene encodes Vitamin K epOxide Reductase Complex subunit 1. This protein complex is responsible for reducing the Vitamin K epoxide to the active Vitamin K form, which is important for effective coagulation. Effective inhibitors of VKORC1 are given to heart patients as blood thinners (coumadin or warfarin). It has been reported in the literature that coronavirus causes pulmonary or brain embolism (Sampson, C., & Ukah, O., 2023). Enoxaparin sodium anticoagulant therapy is also applied during the treatment of COVID-19 disease. Since flavonoids and coumarins have high anticoagulant properties, it is thought that Lipo-flavonoid nutritional supplement can prevent blood clot and eliminate vital risks.

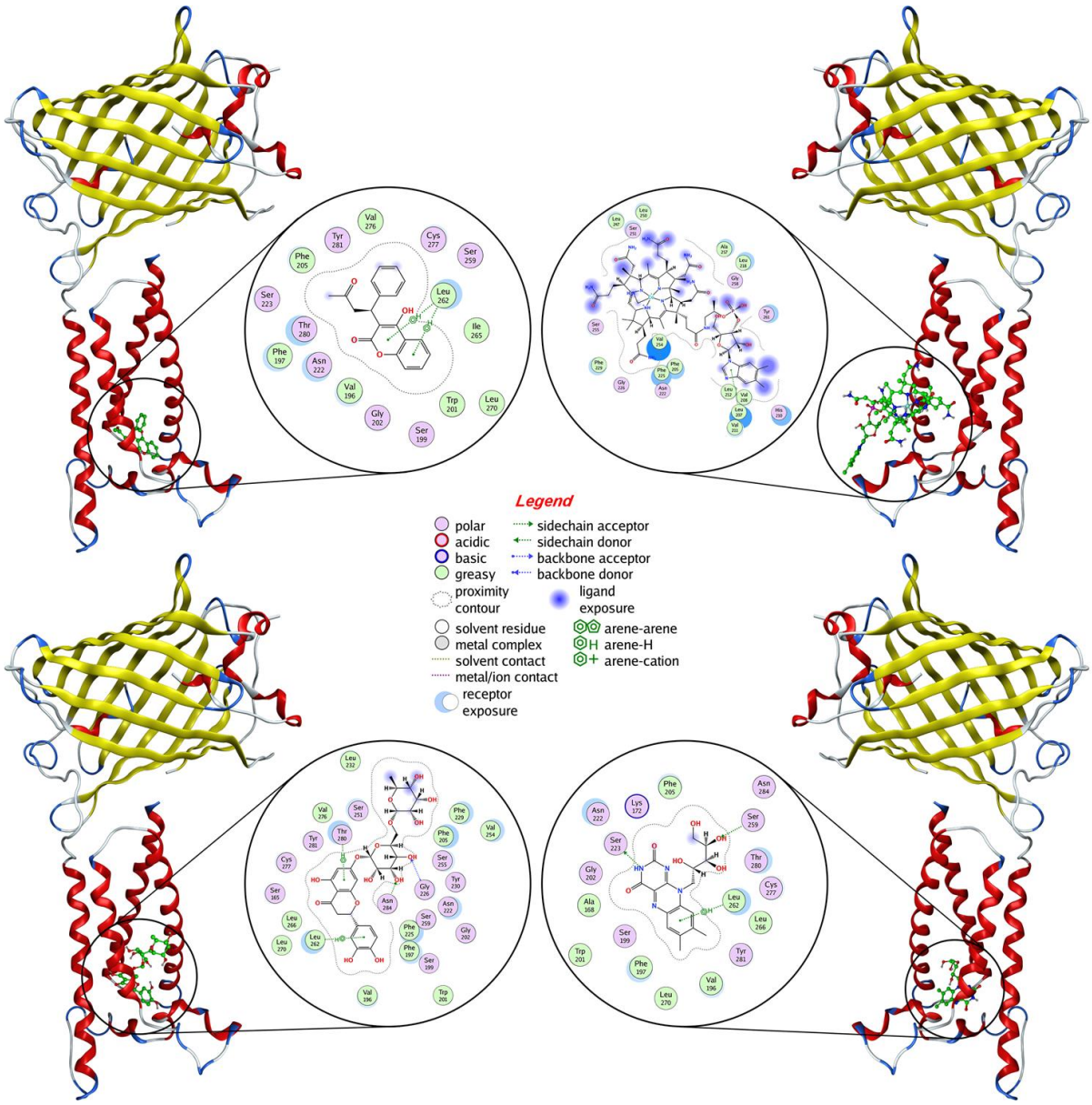

Figure 5. Positions and molecular interactions of lipoflavonoid components on the VKORC1 enzyme.

In the docking study on VKORC1, the warfarin anticoagulant had binding affinity of -8.11 kcal/mol (ligand efficiency: -0.35) and enoxaparin anticoagulant -11.94 kcal/mol (ligand efficiency could not be calculated due to the polymer form). Cyanocobalamin has a docking score of -10.69 kcal/mol (ligand efficiency -0.12), eriodictyol glycoside -11.47 kcal/mol (ligand efficiency -0.27) and riboflavin -8.87 kcal/mol (ligand efficiency -0.39). The

position of the model inhibitor and candidate inhibitors in the active site and their interaction with the VKORC1 receptor are given in Figure 5.

4. Discussion

In the scientific study published by Ogunyemi et al. , the inhibitory effect of alkaloids and lipo-flavonoids on SARS-CoV-2 RNA-dependent RNA polymerase was emphasized. In addition, in our study, we determined that lipo-flavonoid compounds may have blood thinning effects due to their inhibitory effect on the Vitamin K epOxide Reductase Complex and may also have antiviral activity with their inhibitory effect on the Main Protease enzyme (Ogunyemi, O. M., Gyebi, G. A., Elfiky, A. A., Afolabi, S. O., Ogunro, O. B., Adegunloye, A. P., & Ibrahim, I. M., 2020). Therefore, not only for the purpose of reducing COVID-19 viral replication; It may be beneficial in reducing secondary thromboembolic diseases that may cause increased mortality and morbidity of the disease.

Ngwa, W., Kumar, R., Thompson, D., Lyerly, W., Moore, R., Reid, T. E., Lowe, H., & Toyang, N. (2020). emphasized that lipo-flavonoid components have binding affinities especially to the ACE-2 metallopeptidase domain and therefore may have possible prophylactic roles in the treatment of COVID-19 (Ngwa, W., Kumar, R., Thompson, D., Lyerly, W., Moore, R., Reid, T. E., Lowe, H., & Toyang, N., 2020).. In our study, we determined that ACE-2 receptor binding was not as strong as binding affinity to NSP12, Main Protease and others. In our literature review, we did not find any other clinical study on the binding of lipo-flavonoids to NSP12 and its possible benefits in the treatment of tinnitus.

In contrast to other viruses, the distinct symptom of anosmia (loss of sense of smell) without accompanying rhinitis and rhinorrhea in SARS-CoV-2 may be attributed to the virus specifically binding to ACE-2 receptors on olfactory neurons without triggering an inflammatory response. Anosmia has been observed as a primary symptom of COVID-19, and considering the virus's ability to invade the nervous system, individuals experiencing this symptom should undergo thorough evaluation, particularly when other symptoms are absent (Xydakis, M. S., Dehgani-Mobaraki, P., Holbrook, E. H., Geisthoff, U. W., Bauer, C., Hautefort, C., Herman, P., Manley, G. T., Lyon, D. M., & Hopkins, C., 2020). Accurate and timely diagnosis is also very difficult and it is not easy to distinguish between coronavirus and influenza respiratory syndromes when diagnosed without RT-PCR diagnostic test (Chan, J. F., Yuan, S., Kok, K. H.,

To, K. K., Chu, H., Yang, J., Xing, F., Liu, J., Yip, C. C., Poon, R. W., Tsoi, H. W., Lo, S. K., Chan, K. H., Poon, V. K., Chan, W. M., Ip, J. D., Cai, J. P., Cheng, V. C., Chen, H., Hui, C. K., … Yuen, K. Y., 2020). In patients infected with COVID-19, who have only olfactory disorders and no other symptoms, it may be beneficial to add flavonides to treatment protocols because of their possible antiviral efficacy, although ACE-2 receptor binding is not very high.

Studies for the development of intranasal COVID-19 vaccines have recently been initiated in order to create an intranasal mucosal immunity or barrier effect, and it is thought that the use of intranasal antiviral barrier or products that may have antiviral effects may be effective in both the treatment and reduction of the contagiousness of the COVID-19 virus. Results of an in vitro study performed at Utah State University Northwestern University in the USA showed that xylitol and grapefruit seed extract (GSE) can potentially prevent SARS-CoV-2 infection. It has been emphasized that these products can be used as nasal sprays to control the spread of coronavirus (Cannon, M. L., Westover, J. B., Bleher, R., Sanchez-Gonzalez, M. A., & Ferrer, G. A., 2020).

Vofo, G., Brodie, R., & Gross, M. (2020) emphasized the development and utilization of angiotensin Converting Enzyme-2 (ACE-2) receptor agonists or angiotensin receptor blockers (ARBs) as nasal lavage to reduce the viral load in individuals who have tested positive for the virus. They also suggested using this approach as a preventive measure, particularly for high-risk patients. These medications are easily accessible, and testing this concept involves determining the appropriate dosage of angiotensin receptor blockers or ACE inhibitors (diluted in water) for nasal lavage, followed by efficacy trials. It is important to monitor for potential side effects such as low blood pressure or changes in heart rate. Medicated nasal lavage can be administered more easily and quickly distribute within the nasal mucosa. (Vofo, G., Brodie, R., & Gross, M., 2020).

Quercetin, one of the most commonly used and discussed flavonoid compounds, is a widely distributed flavonoid in nature and has been reported to inhibit the oxidation of other molecules, so it has been classified as an antioxidant in vitro. Flavonoids occur naturally in fruits, vegetables, and beverages such as tea and wine. Quercetin is the major flavonoid belonging to the class called flavonols. Quercetin is found in apples, tea, onions, nuts, strawberries, cauliflower, cabbage, and many other foods. Quercetin's anti-inflammatory, antioxidant, analgesic and inflammatory properties have been reported. Severe inflammation is one of the main life-threatening conditions in COVID-19 patients. Due to the anti-inflammatory and antioxidant effects of Quercetin, it is recommended in various herbal

supplements. Quercetin's inhibitory effects on IL-17 may be particularly important in the treatment of COVID-19 (Saeedi-Boroujeni, A. & Mahmoudian-Sani, MR., 2021).

Quercetin can also act as an inhibitor of SARS-CoV-2 by binding to the active sites of SARS-CoV-2 major proteases 3CL and ACE2. Inhibition of inflammatory, cell apoptosis-related signaling pathways may be critical mechanisms by which Quercetin protects kidney and other organs from SARS-CoV-2 damage. (Gu, Y. Y., Zhang, M., Cen, H., Wu, Y. F., Lu, Z., Lu, F., Liu, X. S., & Lan, H. Y., 2021) In addition, the antiviral effects of lipo-flavonoids on COVID-19 may increase their importance in the treatment of the COVID-19 epidemic.

There is a high rate of infection from viral pneumonia caused by SARS-CoV-2, associated inflammation, and acute respiratory distress syndrome (ARDS). Serious pneumonia, secondary infections and cardiovascular events are the main causes of death (Li, Q., Guan, X., Wu, P., Wang, X., Zhou, L., Tong, Y., Ren, R., Leung, K. S. M., Lau, E. H. Y., Wong, J. Y., Xing, X., Xiang, N., Wu, Y., Li, C., Chen, Q., Li, D., Liu, T., Zhao, J., Liu, M., Tu, W., … Feng, Z., 2020). Among the drugs used in the treatment of COVID-19, remdesivir, which has the ability to inhibit RNA-dependent RNA polymerase (RdRp), can be used (Huang, J., Song, W., Huang, H., & Sun, Q., 2020). In our virtual docking study, we determined that lipoflavonoids may also have antiviral activity with their inhibitory effect on RNA-dependent RNA polymerase and additionally with their inhibitory effect on Main Protease enzyme.

Istifli, E. S., Netz, P. A., Sarikurkcu, C., & Tepe, B. (2022) In the study where they evaluated the interactions of 23 different phytochemicals belonging to different flavonoid subgroups with the RBD of the 2019-nCoV, TMPRSS2, CatB and CatL spike glycoprotein, they found that anthocyanidins, isoflavones and flavanones had stronger interactions with target proteins. Although epicatechin gallate cannot cross the blood-brain barrier; emphasized that since it has no toxic effect on cells, it can be considered as a candidate molecule in drug development processes against 2019-nCoV .

Gorla, U. S., Rao, K., Kulandaivelu, U. S., Alavala, R. R., & Panda, S. P. (2021) identified that biochanin A and silymarin bioflavonoids as potent inhibitors of ACE-2 targets, emphasizing the need for further studies to confirm their therapeutic potential in COVID-19.

Deng, J. G., Hou, X. T., Zhang, T. J., Bai, G., Hao, E. W., Chu, J. J. H., Wattanathorn, J., Sirisa-Ard, P., Soo Ee, C., Low, J., & Liu, C. X. (2020) emphasized the antiviral effects of Baicalin, a flavonoid compound used in various tea, lozenges and herbal formulations due to its anti-inflammatory and antioxidant properties, on SARS-CoV-2 in East Asian Countries, European Countries and many different regions.

While developing COVID-19 treatment protocols, lipo-flavonoids are used in patients with suppressed immune system due to various drug use or diseases, cardiovascular diseases or risk group for thromboembolic diseases, smell disorder, taste disorder or central nervous system involvement. It may be beneficial to use its components in systemic or intranasal local treatment. It is appropriate to conduct adequate and comprehensive scientific clinical studies on the subject.

5. Conclusion

In this study, theoretical calculations of Lipo-Flavonoid natural nutritional supplement components, their effects against tinnitus disease, their contribution to the inhibition of coronavirus proteins, and the contribution of flavone derivatives to the coagulation process since blood thinning (thinning) properties are known. Lipo using molecular insertion studies against NMDA receptors NMDA/R1, NMDA/R2 and NMDA/R3, MPro, NSP12 with RNA, NSP15, NSP16 and Spike, which are important receptors of SARS-CoV-2, and ACE2 and VKORC1 receptors responsible for coagulation. Flavonoid components were studied and their binding scores to receptors were compared with model inhibitors and structure-activity relationship was established. It was found to be very effective against lipo-flavonoid components cyanocobalamin, eriodictyol glycoside, riboflavin and bitartrate NSP12 and NSP12 with RNA. Since cyanocobalamin has a bulky structure and has functional groups acting on it, it generally has binding scores above -10.00 kcal/mol. It has been proven by ligand/amino acid interactions that Lipo-Flavonoid compounds can prevent extracellular glutamate accumulation by providing depolarization or ion flow and preventing the closure of the NMDA

pathway due to their potential to interact with ions or metals. Since flavonoid compounds can be obtained from natural herbal sources and their side effects are low, it may be appropriate to recommend them not only in medical treatment but also in nutritional supplements. Lipo-Flavonoid compounds, which can also be used in the treatment of tinnitus, can treat symptoms such as immune system failure, taste and smell disorder caused by coronavirus, and moreover inhibit coronavirus. We think that not only systemic but also nasal topical use of lipo-flavonoids in the future may be beneficial in the transmission of COVID-19 virus, reducing the viral load in the nasal air cavity and reducing possible neural damage.

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CONFLICT OF INTEREST

The authors must declare that they have not any conflict of interest.

AUTHOR STATEMENT

The authors have to declare that if there is no any ethical approval, consent to participate, consent for publication, availability of data and material, and code availability etc.

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