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Araştırma Makalesi / Research Article

Docking Studies of Natural Product Derived Carvacrol Type Aromatic Monoterpenes Against COVID-19 and Comparison with Used Synthetic Drugs: Potential of Carvacryl Acetate Against SARS-CoV-2 (COVID-19)

Doğal Ürün Türevli Karvakrol Tipi Aromatik Monoterpenlerin COVID-19'a Moleküler Modelleme Çalışmaları ve Kullanılmış Sentetik İlaçlarla Karşılaştırılması: Karvakril Asetatın SARS-CoV-2'ye (COVID-19) Karşı Potansiyeli

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ABSTRACT

The COVID-19 pandemic that broken out in 2020 is becoming more worrying for the world. Although there is no 100 % success against COVID-19, certain synthetic drugs are currently used despite various side effects. Therefore, studies on the discovery of new treatment alternatives come to the fore. Studies so far show that natural products are still important resources for the discovery of new therapeutic agents. Plant-derived essential oils are complex volatiles composed of various phytochemicals, mostly containing compounds such as sesquiterpenes, monoterpenes, and phenylpropanoids. In this study, especially thymol and carvacrol compounds specific to the Lamiaceae (Labiate) family and aromatic monoterpenes derived from these compounds were modeled against COVID-19. Results were compared with remdesivir, hydroxychloroquine, and favipiravir used as synthetic drugs. Dock and molecular dynamics simulations analyzed these molecules' potential inhibitor efficiency of the SARS-CoV2 M^{pro}. Lipinski parameters and Docking results were demonstrated that ligands carvacrol (2), carvacryl acetate (11) and cuminaldehyde (12) are potential inhibitors towards

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COVID-19. According to the results, it is seen that medicinal aromatic herbs, which contain these volatile components with the fewer side effects than synthetic drugs, have the potential to be used as supplements in the pharmaceutical industry.

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ÖZ

2020 yılında baş gösteren COVID-19 pandemisi giderek Dünya için daha endişe verici hal almaktadır. COVİD-19'a karşı %100 başarı alınamasa da şuan çeşitli yan etkilerine rağmen belli sentetik ilaçlar kullanılmaktadır. Bu nedenle, yeni tedavi alternatiflerinin keşfi ile ilgili çalışmalar ön plana çıkmaktadır. Şimdiye kadar yapılan çalışmalar doğal ürünlerin, yeni terapötik ajanların keşfi için hala önemli kaynaklar olduğunu göstermektedir. Bitki türevi uçucu yağlar, çeşitli fitokimyasallardan oluşan karmaşık uçucular olup, daha çok monoterpenler, seskiterpenler ve fenilpropanoidler vb. bileşikleri ihtiva eder. Bu çalışmada özellikle Labiate familyasına özgü timol ve karvakrol bileşikleri ile bu bileşiklerin türevi aromatik monoterpenlerin COVİD-19'a karşı moleküller modellemeleri yapılmıştır. Sonuçlar sentetik ilaç olarak kullanılan remdesivir, hydroxychloroquine ve favipiravir ile karşılaştırılmıştır. Dock ve moleküler dinamik simülasyonları, bu moleküllerin SARS-CoV2 M^{pro}'nun potansiyel inhibitör etkinliğini analiz edilmiştir. Lipinski parametreleri ve Docking sonuçları, karvakrol (2), karvakril asetat (11) ve kuminaldehit (12) ligandlarının COVID-19'a karşı potansiyel inhibitörler olduğunu göstermiştir. Sonuçlara göre bu uçucu bileşenleri içeren, sentetik ilaçlara göre yan etkileri çok az olan, tıbbi aromatic bitkilerin ilaç sanayide takviye ürün olarak kullanılma potansiyeli olduğu görülmektedir.

1. INTRODUCTION

Coronaviruses (CoVs) are belong to Coronaviridae family which are a numerous various group of enveloped, and single-stranded RNA viruses [1]. They have been defined in humans and animals like rats, mice, chickens, dogs, cats, rabbits. In addition, they can lead to a diversity of many diseases including enteric, hepatic and respiratory tract diseases [1,2]. There are 4 coronaviruses circulating in humans. These are called HKU1, NL63, 229E and OC43 and induce mild respiratory disease. In the last two decades, two new types of CoVs, called middle east respiratory syndrome CoV (MERS-CoV) and severe acute respiratory syndrome CoV (SARS- CoV), have appeared and caused serious illness in humans [1,2]. During the pandemic, SARS-CoV has infected more than 8000 people worldwide, resulting in a mortality rate of 10%. On the other hand, MERS infected more than 857 official cases and increased the death rate to approximately 35%, with 334 deaths [1,2].

In late December 2019, an unknown pneumonia outbreak occurred in Wuhan, China. The pathogen that causes COVID-19 was first identified as a novel coronavirus in late January 2020 and was recognized as SARS-CoV-2 (also called as 2019- nCoV) [1]. The WHO classified the COVID-19 outbreak as an "international public health emergency" on January 30, 2020, and defined it as a global epidemic on March 11, 2020, where the first epidemic started, and the spread and severity of the virus [1,2]. Although 100% success has not been achieved against COVID-19, certain synthetic drugs such

as remdesivir, hydroxychloroquine, and favipiravir are currently used despite various side effects. Therefore, studies on the discovery of new treatment alternatives come to the fore.

Studies so far show that natural products are still important resources for the discovery of new therapeutic agents [3-6].

Natural products have always been valuable resources for the pharmaceutical industry. Many drugs derived from or modeled from natural products are greatly beneficial in almost all clinical therapeutic areas [7-10]. The WHO has recommended systematic testing of essential oils and other natural products against Human Immunodeficiency Virus (HIV), due to their general safety and easy availability [11]. The FDA (United States Food and Drug Administration) has stated that essential oils are safe for humans to be used as common food preservatives, flavorings, cosmetics, antiseptics, cleaners, and replenishers [12,13]. Thymol and carvacrol source essential oils have long been used in traditional medicine due to their antimicrobial, anti-inflammatory, antifungal, antiviral, and antioxidant effects [13-20]. Thyme oil, obtained from species such as *Origanum, Satureja, Thymus*, and *Thymbra*, is rich in aromatic monoterpene carvacrol, and its isomeric analog, thymol.

Carvacrol is the major compound of oregano essential oil. It is known that carvacrol has antiviral properties against MNV (nonenveloped murine norovirus) virus, and it has also been shown to be a natural disinfectant for food and surfaces [21-24]. Also, thymol has some biological activities such as antiseptic, antifungal, antiviral, antibacterial, antispasmodic, carminative, etc [25-32].

Recently, the crystal structure of M^{pro} on COVID-19 (6Lu7) has been released. This enzyme shares a similar structure with cysteine protease with an active site lacking the third catalytic residue, it comprises a catalytic dyad, namely Cysteine 145 (C145) and Histidine 41 (H41) [33]. However, some molecular docking studies have been investigated to find a potential inhibitor of M^{pro} activity based on antiviral compounds [33-36]. Until today, there are no specific therapies for COVID-19 disease and research on the treatment of COVID-19 disease is lacking. In this study, we examined the M^{pro} inhibitory potential of thirteen compounds (Thymol, carvacrol and carvacrol derivative aromatic monoterpenes specific to the Lamiaceae family) using a molecular docking approach performed by the Dock6.0 bioinformatic tool. Molecular modeling results of thymol (1), carvacrol (2), 8,9-dehydro-carvacrol (3), methyl carvacrol compounds (4), 4-isopropyl-3-methylphenol (5), chavicol (p-allylphenol) (6), p-cymene (7), o-cymene (8), eugenol (9), anethole (10), carvacryl acetate (11), cuminaldehyde (12) and p-isopropylanisole (13) were compared with remdesivir, hydroxychloroquine and favipiravir used as synthetic drugs. Dock and molecular dynamics (MD) calculations were used to investigate the potential inhibitor effect of these molecules on the target protein.

2. MATERIAL AND METHOD

This study is an illustrative analytical research. In this investigation the intereaction of several permitted compounds used to be studied. Thirteen compounds were proven in opposition to COVID-19 major protease (CoV M^{pro}). N3 ligand was used as a docking goal for evaluation. The constitution of the new CoV M^{pro} stated in entry quantity 6lu7 used to be retrieved from the PDB (Protein Data Bank) database [37]. It conforms to an elaboration between inhibitor N3 and the enzyme. The 6Lu7 model practise contains removing N3 and all water molecules, the new file was previously saved for docking analysis. CoV M^{pro} is formed of three zone: III residues 201-303, II residues 102-184 and I residues 8- 101. The CoV M^{pro}'s enzymes share an enormously preserved substrate-binding pocket, placed in the rift between zones I and II. The cavity benefits as a drug target of our chosen ligands (Figure 1).

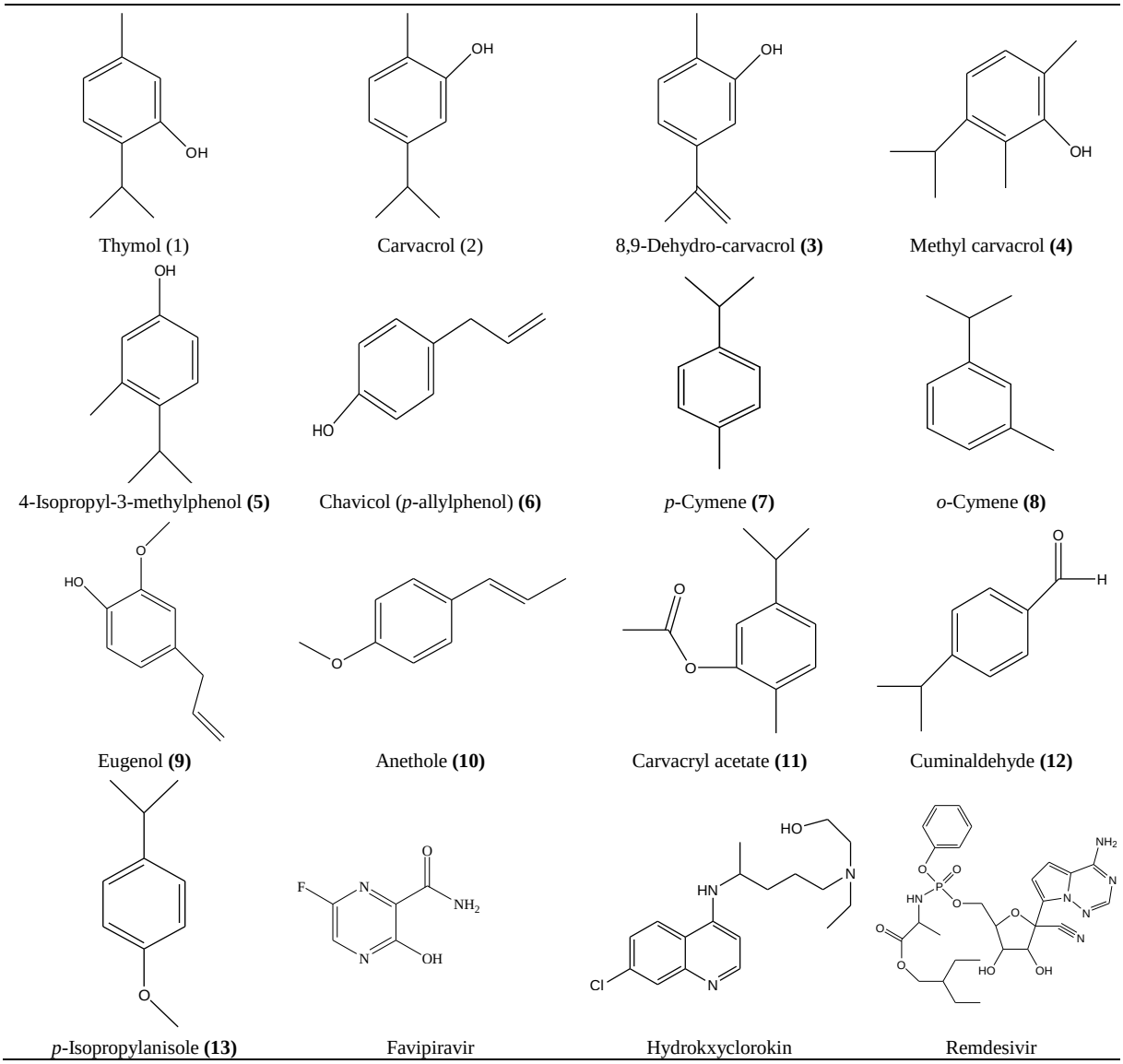


Figure 1. *Studied Compound*

2.1 Molecular Modeling

2.1.1 Molecular Dynamics Simulations

The compounds were used with partial atomic charges derived with the aid of fitting Antechamber acquired by way of electronic constitution simulation module in AMBER and defined the AM1-BCC charges [38-41]. Atoms on 6lu7 were dedicated the PARM99 charges, and all ionisable residues were calibrated default protonation case at neutral pH.

Compounds parameters were composed using XLEAP the amber force field (GAFF) [42]. Molecular Dynamics were ordinarily actuated at 300 K and 1 ns. All constructions were extra processed via the xLeAP module of AMBER. The systems in which the energy was reduced did so in two stages: first, the protein and ligand were fixed, only the water molecules were relaxed, and in the second, all atoms were given the go-ahead to move. In the first step, the energy minimizations were carried out in 100 and 2000 steps using the steepest descent and conjugate gradient methods, and in the second step, they were carried out in 100 and 3000 steps using the same methods.

2.1.2 Docking Studies

The Dock 6.5 [43] module enables the execution of all docking technique levels, including the evaluation of binding modes, compound docking, and ligand conformation research. As in this situation, where a rigid receptor approach was used, it is anticipated that the specific receptors taken into consideration will lead to distinct ligand binding modes depend on at the preliminary length of the M^{pro}-binding cavity. As a result, a multistep process was used to park the 13 novel covid-19 inhibitors on the available receptor. Consequently, it will define. A grid of electrical interaction for atoms taking component inside the binding zone was calculated for receptor-binding characteristics. These atoms were acquired from the analysis of every protein–ligand complicated. Default parameters were used in this step. The ligand was then docked by placing it in the pocket and scoring the suggested binding modes using the computed grid.

3. THE RESEARCH FINDINGS

During our studies, we calculated the binding energy of some monoterpenes against 6Lu7 crystal structure using Dock 6.5 [43]. Docking outcomes demostrated that, cuminaldehyde, carvacrol and carvacryl acetate had the best energies of binding 26.90, -27.88 and -33.64 kcal/mol, respectively (Table 1), which is comply with studies. These molecules were compared with three different molecules known as drugs. The first discovered is remdesivir, which used the Dock 6.0 tool to get the best docking results with a low energy of -55.31 kcal/mol. The second was based on the molecular

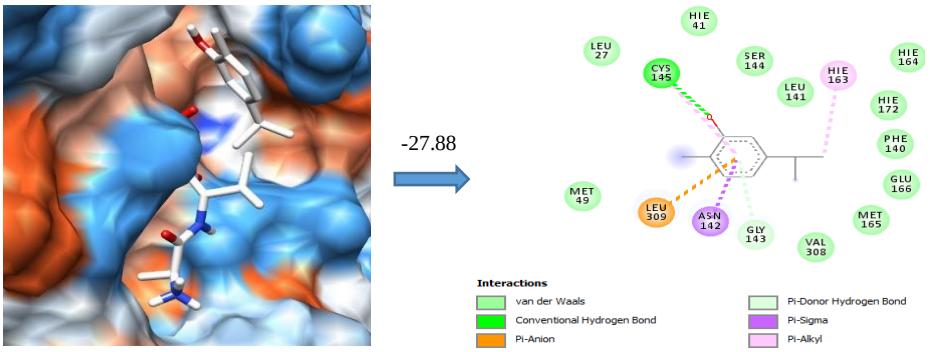
docking of antimalarial drugs against the crystal structure of 6lu7, which showed that hydroxychloroquine bound to Mpro with a score of -41.46, and the final one was favipiravir, which had a score of -29.07 kcal/mol binding energy. Remdesivir, hydroxychloroquine, favipiravir, and 13 other chemicals were found to show that all potential inhibitors were able to settle down the M^{pro} binding cavity when the clusters were evaluated.

Table 1. Molecular docking analysis results for several drugs against 6Lu7 crystal structure. Calculated thermodynamic parameters for complexation of ligands by Docking Method in kcal/mol and Lipinski Log P Parameters.

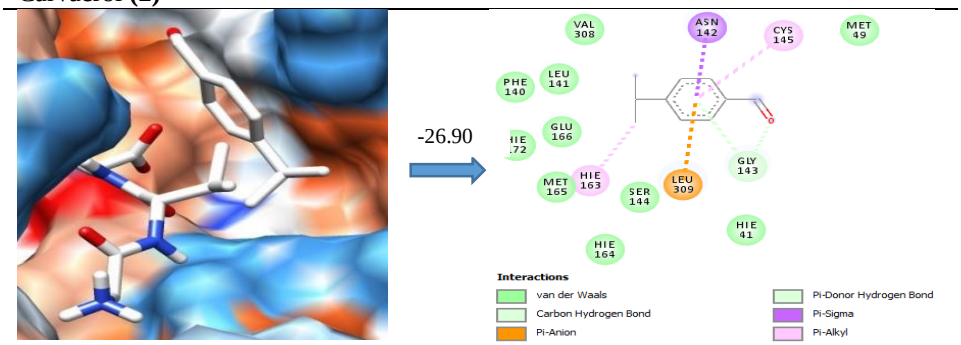
Comp. No	van der Waals Energy	Electroatic Energy	Internal Energy	Dock Score Energy	Log P
1	-24.83	-0.043	8.13	-24.87	3.34
2	-26.60	-1.28	9.49	-27.88	3.81
3	-22.49	-0.27	4.55	-22.75	3.03
4	-18.46	-3.07	9.38	-21.53	3.51
5	-18.04	-4.05	4.75	-22.09	2.87
6	-22.28	-0.44	1.74	-22.72	2.28
7	-21.08	-1.18	7.25	-22.26	3.90
8	-21.67	-0.95	8.83	-22.62	3.87
9	-24.31	0.86	5.43	-25.17	2.10
10	-23.99	-0.23	8.78	24.22	3.10
11	-32.28	-1.36	8.031	-33.64	3.38
12	-25.70	-1.20	7.64	-26.90	3.24
13	-26.25	-0.65	8.68	-26.56	3.61
Favipiravir	-23.43	-5.64	1.67	-29.07	-0.98
Hydrokxyclorokin	-39.59	-2.36	8.89	-41.46	3.73
Remdesivir	-48.47	-6.83	13.31	-55.31	3.60

Molecules with high activity established effective H- bond with E166 and N142 residues, in other respects, some compounds were found to form multiple H-bonds with E166, Gly143, and L141, residues, respectively. In addition, when the results were examined, it showed that the pi anion and van der waals interactions in this study were also significant [44].

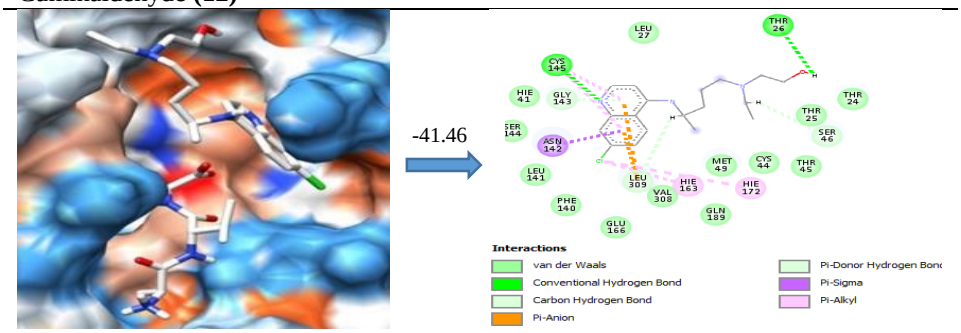
The log P analysis values of these twelve medications were within an acceptable range, according to drug similarity and pharmacokinetic features (Table 1). Carvacrol (2), carvacryl acetate (11) and cuminaldehyde (12) substances were shown by dock score findings and Lipinski [45] criteria to be potential inhibitors of The active amino acids Thr190, Gln189, Asp187, Arg188, Val186, Pro168, His172, Leu167, Met165, Glu166, His163, Phe140, Cys145, Asn142, His41, Met49, Pro39, Leu27, and in protein are present in the pocket that was selected for virtual screening, according to COVID-19 (Figure 2).



Carvacrol **(2)**



Cuminaldehyde **(12)**



Hydroxychloroquine

Figure 2. *Docking poses of different drugs against protease M^{pro} is shown as colourfull surface background, inhibitors are in elements colors and 2D schematic interaction diagram*

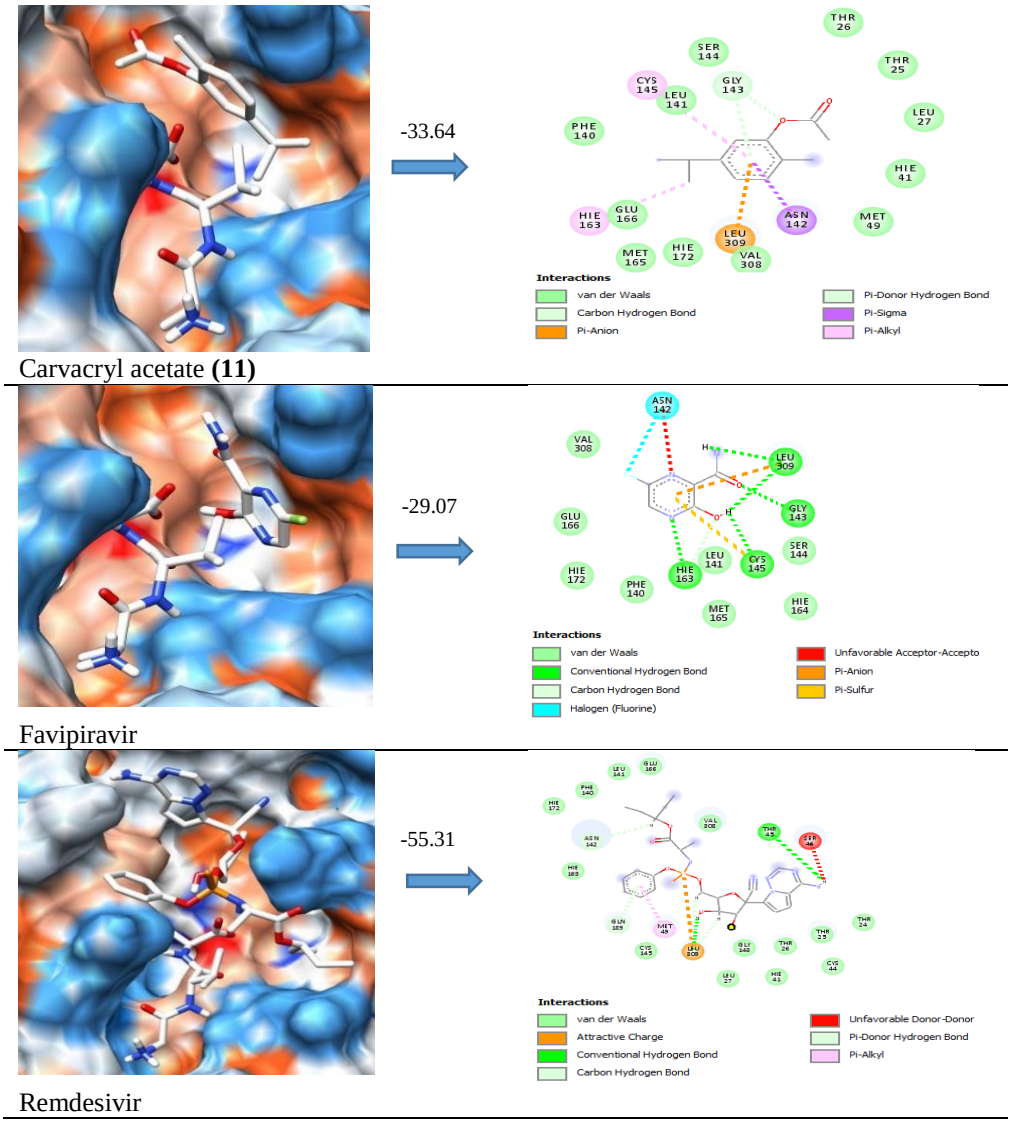


Figure 2 Continued. *Docking poses of different drugs against protease M^{pro} is shown as colourfull surface background, inhibitors are in elements colors and 2D schematic interaction diagram (Continued)*

3.1 Structure-Activity Relationship

Thymol (1) and carvacrol (2) are aromatic monoterpene structures that are structurally identical to each other. They are isomeric structures in which only the hydroxyl group is replaced.

Despite these similar structures, the docking score values of thymol and carvacrol compounds are -24.87 and -27.88 kcal/mol. The hydroxyl group in thymol compound is adjacent the isopropyl group. The hydroxyl group and methyl group are more closely spaced in carvacrol. Since the steric barrier of isopropyl in the thymol compound for the hydroxyl is higher, both hydrogen bond interactions and electrostatic contacts will be less than carvacrol while the compounds in question interact with the active region of the protein. The difference in docking score values of the two

compounds can be explained by the steric barrier caused by the isopropyl group. When the structure of these two compounds is compared to remdesivir and hydroxychloroquine as drugs against COVID-19, it is seen that both vanderWaals and electrostatic interactions are less, especially since the number of polar groups and hydrogen bonding is low.

Carvacryl acetate (11) is obtained by acetylation of the hydroxy group in the carvacrol (2). The carbonyl group in the acetoxy group in the carvacryl acetate makes both π- π and hydrogen bonds withe the active site of the protein. Also, since it is connected through 3 bonds from the ring, there is a little steric barrier in these interactions. The bonding of an oxygen to the carbonyl group through 2 bonds also contributes to the increase of electrostatic interactions. The interactions based on mass are greater in carvacrol than in carvacryl acetate because there are more oxygen and methyl groups in the latter. Besides, the carvacryl acetate compound interacts with 17 amino acids of the protein, while carvacrol interacts with 16 amino acids. For these reasons, carvacryl acetate (docking score: -33.64 kcal/mol) binds to the active center of the protein better than carvacrol and shows a higher effect.

According to theoretical calculations, carvacrol acetate appears to be more effective than favipiravir (docking score: -29.07 kcal/mol) compound used as a drug against COVID-19.

4. CONCLUSION

The present study was carried out for the discovery of novel inhibitor molecules against three enzymes M^{pro}. Consequently, some aromatic monoterpenes (Thymol (1), carvacrol (2), 8,9-dehydro-carvacrol (3), methyl carvacrol (4), 4-isopropyl-3-methylphenol (5), chavicol (p-allylphenol) (6), p-cymene (7), o-cymene (8), eugenol (9), anethole (10), carvacryl acetate (11), cuminaldehyde (12) and p-isopropylanisole (13)) were analyzed by molecular docking techniques. The results of the 13 ligands were compared with reference molecules (remdesivir, hydroxychloroquine and favipiravir) of protein, which demonstrates that these compounds can bind more efficiently and act as inhibitors. Thus, we conclude that these compounds can be utilized as potential antiviral candidates. These novel molecules could be utilized for further innovation and development of antiviral compounds against Coronavirus.

This study showed that these drug-like compounds are screened by deep learning method subjected to molecular docking and these compounds showed better energy against M^{pro} receptor and can be used against coronavirus. Various studies are going on nowadays to treat coronavirus an existing can play a big role to treat this disease. So, this study can be used to find some novel compounds against coronavirus disease.

Thyme oil, obtained from species such as *Origanum, Satureja, Thymus*, and *Thymbra,* is rich in aromatic monoterpene 5-isopropyl-2-methylphenol (carvacrol), and its isomeric analog, 2-

isopropyl-5-methylphenol (thymol). Carvacrol, which has an aromatic monoterpene structure, has been defined as a natural food preservative due to its very high broad spectrum antimicrobial activity [21-23]. Additionally, it is mentioned in the literature that it can be used to increase the shelf life of packaged products due to its antioxidant and antimicrobial activity [24]. It is included in the literature that especially carvacrol-derived essential oils have antiviral effects on different viruses such as yellow fever virus, HSV, IFV, HIV, and avian influenza, etc. [27,28].

Moreover, studies about the carvacrol compound to be effective against COVID-19 are mentioned in the literature [46]. For these reasons, it can be said that the use of species belonging to the genus such as *Origanum, Satureja, Thymus*, and *Thymbra*, which contain carvacryl acetate and carvacrol without side effects, will be beneficial against the COVID-19 epidemic that threatens our world and humanity.

CONFLICTS OF INTEREST

The authors confirm that this article content has no conflict of interest.

AUTHORS' CONTRIBUTIONS

Şafak ÖZHAN KOCAKAYA and Abdulselam ERTAŞ: Conceptualization, methodology, validation, writing-review and editing, development or design of methodology, supervision, data interpretation. İsmail YENER, Enes ARICA, and Demet DİNÇEL: Writing-original draft preparation, data collection, data curation, visualization, data interpretation, development or design of methodology, data presentation.

DECLARATION OF ETHICAL CODE

In this study, the authors undertake that they comply with all the rules within the scope of the "Higher Education Institutions Scientific Research and Publication Ethics Directive" and that they do not take any of the actions under the heading "Actions Contrary to Scientific Research and Publication Ethics" of the relevant directive.

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