Cumhuriyet Sci. J., 44(4) (2023) 665-670
DOI: https://doi.org/10.17776/csj.1328641

Cumhuriyet Science Journal

| csj.cumhuriyet.edu.tr | Founded: 2002 ISSN: 2587-2680 e-ISSN: 2587-246X Publisher: Sivas Cumhuriyet University

Investigation of the Effect of Main Components of Wild Thyme on Covid-19 by Computational Methods

Serpil Kaya [1,a], Sultan Erkan [1,b], Duran Karakaş [1,c,*]

[1] *Chemistry Department, Science Faculty, Sivas Cumhuriyet University, Sivas, Türkiye.*
**Corresponding author*

Research Article

History
Received: 17/07/2023
Accepted: 08/11/2023

ABSTRACT

Aromatic plant species of the genus thymus have an important role as they have therapeutic properties such as antirheumatic, antiseptic, antispasmodic, antimicrobial, cardiac, carminative, diuretic and expectorant. It is also known that such plants strengthen the immune system and help cope with infectious diseases such as colds and flu. In this study, the effects of thymol, p-cymene, γ-terpinene, bornyl acetate, borneol, carvacrol, thymol methyl ether, thymol acetate, which are the main components of wild thyme (*thymus serpyllum L.*), on Covid-19 were investigated at the molecular level. Optimizations and molecular docking were done in Docking Server with the MMFF94 method. Major components of wild thyme were docked separately against 6LU7 protein representing the first gene form of Covid-19 and 7KDL protein representing the mutated form. Docking poses and binding energies between target proteins and wild thyme components were calculated. The results were compared with favipiravir, an antiviral drug developed against influenza virus and also used in the treatment of Covid-19. It was found that the thymol molecule, one of the main components of wild thyme, has the highest biological activity against both 6LU7 and 7KDL protein chains of Covid-19.

Keywords: *Thymus serpyllum L.*, Covid-19, Molecular docking.

[a] *sersude@hotmail.com* *https://orcid.org/0000-0003-3360-4735*
[b] *sultanerkan@cumhuriyet.edu.tr* *https://orcid.org/0000-0001-6744-929X*
[c] *dkarakas@cumhuriyet.edu.tr* *https://orcid.org/0000-0002-6770-3726*

Introduction

Studies continue to develop appropriate treatment strategies to reduce the symptoms and deaths caused by Covid-19, which causes a serious pandemic in the world. Drugs that have been tried against other viruses, such as favipiravir, are also being tried against Covid-19. Unfortunately, complete success has not been achieved due to the rapid mutation of the virus. New vaccine and drug development studies continue in this area [1].

As in the past, viruses are still an important cause of morbidity and mortality worldwide. Viruses such as influenza, acquired immune deficiency syndrome (AIDS), Ebola, severe acute respiratory syndrome (SARS) and Covid-19 can still be lethal. It may be a good choice to use plants to discover new antiviral agents and to treat viral diseases.

It is frequently discussed that herbal products can be protective and therapeutic against the Covid-19 virus [2]. Black elderberry (*Sambucus nigra*) is one of the most widely used plants known to have antiviral effects. This herb has been used for medicinal purposes for thousands of years by Native Americans [3] and people of the Mediterranean region [4]. Licorice root *(Glycyrrhiza glabra)* is an antiviral medicinal plant known since ancient times and used in viral respiratory infections such as dry cough and hoarseness [5-7]. Another plant with antiviral activity is echinacea *(Echinacea purpurea)*. Echinacea extract was found to be as effective as the neuraminidase inhibitor oseltamivir in the treatment of influenza by Rauš et al. [8]. Ginger *(Zingiber officinale Roscoe)* has antiviral activity against human respiratory syncytial virus (HRSV). Chang et al found that ginger inhibited HRSV-induced plaque formation in both HEp-2 and A549 cell lines [9]. Rosehip extract *(Cistus incanus)* has been found to exhibit potent antiviral activity in A549 or MDCK cell cultures infected with different influenza strains [10]. Studies were also conducted to find the components responsible for the antiviral activity of garlic *(Allium sativum)*, and thiosulfinates were found as virucidal active components in fresh garlic extract [11]. The antiviral effects of green tea catechins *(Green Tea Catechins)* have been the subject of many studies and have been found to have antiviral activity against many viruses such as HBV, HSV, HIV, HCV, influenza, enterovirus and rotavirus. The mechanisms of their antiviral effects against many viruses have also been described [12]. In the literature, natural compounds of procyanidin A2, procyanidin B1 and cinnamtannin B1 isolated from cinnamon cortex *(Cinnamomi Cortex)* have been found to inhibit SARSCoV infection [13]. It is known that the curcumin molecule in turmeric has antiviral activity and is effective against viruses such as influenza, hepatitis C, HIV, and SARS-CoV [14,15].

The aerial parts and volatile components of thyme *(thymus)* are used as herbal tea, condiment and spice [16]. The aerial parts of thyme species have been reported to have activities such as tonic, carminative, digestive, antispasmodic, antimicrobial, antioxidant, antiviral, anti-inflammatory and expectorant [17]. The chemical

composition, antimicrobial, antioxidant and antitumor activities of thyme were investigated by Nikolic et al. [18]. It has been found that there are three species of thymus, t*hymus serpyllum L*, t*hymus algeriensis* and *thymus vulgaris L*, and there are approximately 48 different components in each of these species. The main components in t*hymus serpyllum L* are thymol (38.5%), p-cymene (8.9%), γ-terpinene (7.2%), bornyl acetate (7.0%), borneol (6.0%), carvacrol (4.7%), thymol methyl ether (3.8%) and thymol acetate (2.8%). Other ingredients are low in quantity.

The aim of this study is to examine the biological activities of *thymus serpyllum L* essential components against 6LU7 and 7KDL protein of Covid-19. For this purpose, the main components of *thymus serpyllum L* will be docked against the aforementioned cell lines, docking poses and binding energies will be calculated. The results will be compared with favipiravir, an antiviral drug developed against influenza and also used for the treatment of Covid-19 at the beginning of the coronavirus pandemic, and an activity ranking will be obtained.

Materials and Methods

The molecular structures of thymol, p-cymene, γ-terpinene, bornyl acetate, borneol, carvacrol, thymol methyl ether, thymol acetate, which are the main components of *thymus serpyllum L*, were obtained from the PubChem website, which is an open chemistry database [19]. These molecules were docked against the protein (PDB ID: 6LU7) representing the unmutated form of Covid-19 [20] and the protein representing the first mutated form (PDB ID: 7KDL) [21]. The three-dimensional crystal structure of the 6LU7 and 7KDL proteins was retrieved from the Protein Data Bank. Docking Server was used for docking process [22]. Geometry optimization of essential components of *thymus serpyllum L* on the docking server was performed again with the MMFF94 method [23]. The Gasteiger partial load calculation method was chosen [24]. pH = 7.0 was taken. Grid maps were created using 90×90×90 Å grid points and 0.2 Å spacing. Lamarckian genetic algorithm was used for insertion simulations [22]. The population size was set to 150. The 5 Å quaternion and torsion steps were applied for the studied ligands to search for the appropriate region of the target protein.

Findings and Discussion

Molecular Structures

The molecular structures of the main components of wild thyme *(thymus serpyllum L)* were obtained from the PubChem website, which is an open chemistry database. The three-dimensional molecular structures of these compounds and favipiravir are given in Figure 1.

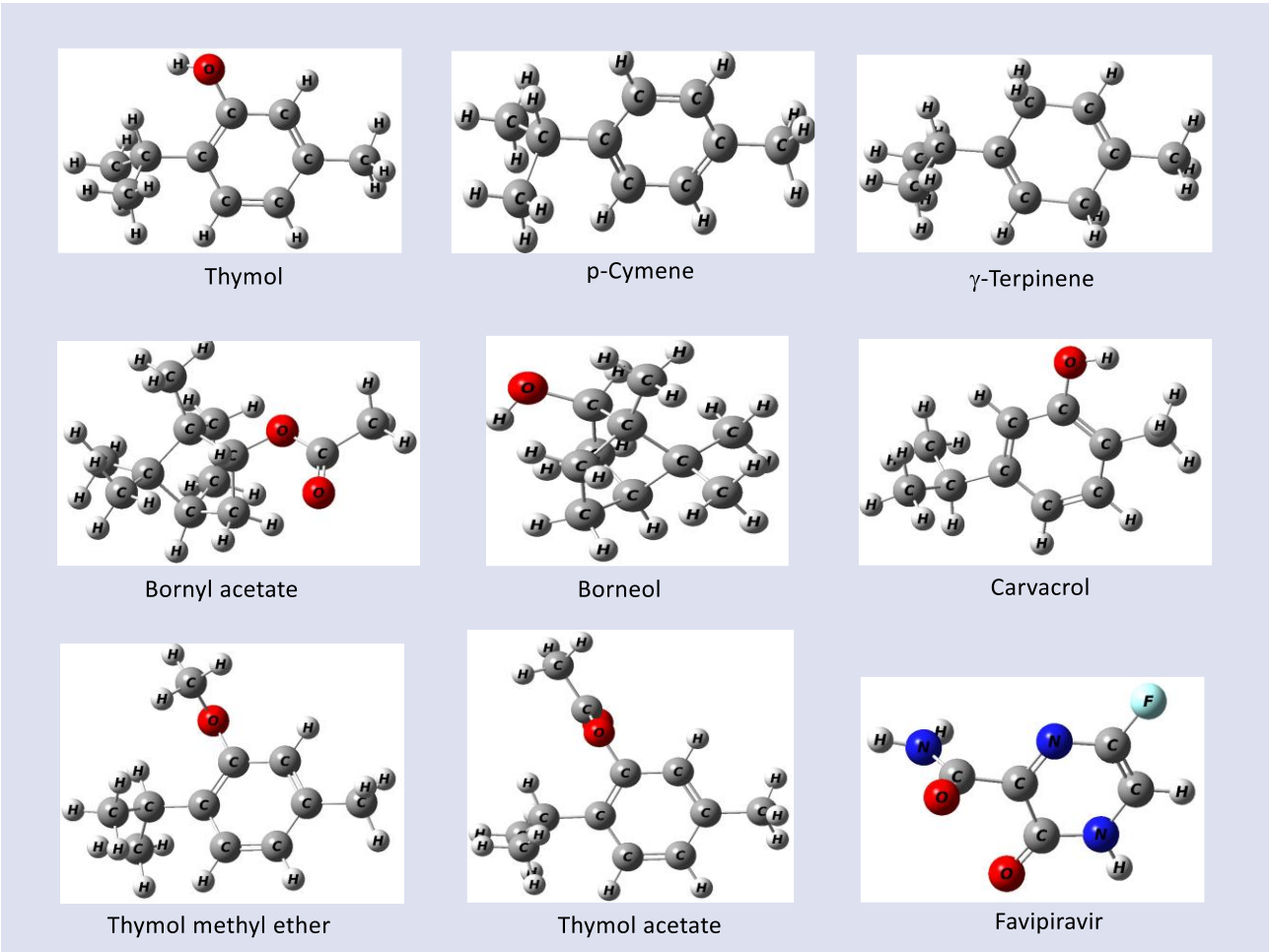

Figure 1. Molecular structures of the main components of *thymus serpyllum L*. and favipiravir

As seen in Figure 1, thymol, borneol and carvacrol have OH^-, bornyl acetate and thymol acetate have CH_3COO^-, thymol methyl ether has CH_3O^- and p-cymene and γ-terpinene have C=C functional groups.

Determination of the Active Component on Covid-19

Drug research and development is an extensive, expensive, and time-consuming process. In drug research and development, new technologies are being developed to shorten the research time and reduce the cost. Molecular docking (MD) is one of the computer aided drug designs (CADD) technologies [25]. Various MD approaches and tools have been developed today [26]. The MD process allows to examine the biological activities of molecules at the molecular level and is one of the popular techniques used in the identification and development of drug candidate molecules. The MD process provides the opportunity to calculate the interactions between the cell lines' minimalized protein structure at the molecular level and the drug candidate molecule. In this way, the binding energies, binding modes and types of secondary chemical interactions between the target protein and the molecule under investigation can be determined [27-30].

In this study, molecular docking process was used to determine the components that are effective on Covid-19 in wild thyme. Molecular docking was done with the Docking Server program. The main components of *thymus serpyllum L.* and favipiravir used as a Covid-19 drug were docked to target proteins with PDB codes 6LU7 and 7KDL. The docking poses between the main components of *thymus serpyllum L.* and the 6LU7 protein are given in Figure 2 and the docking poses between the 7KDL protein are given in Figure 3.

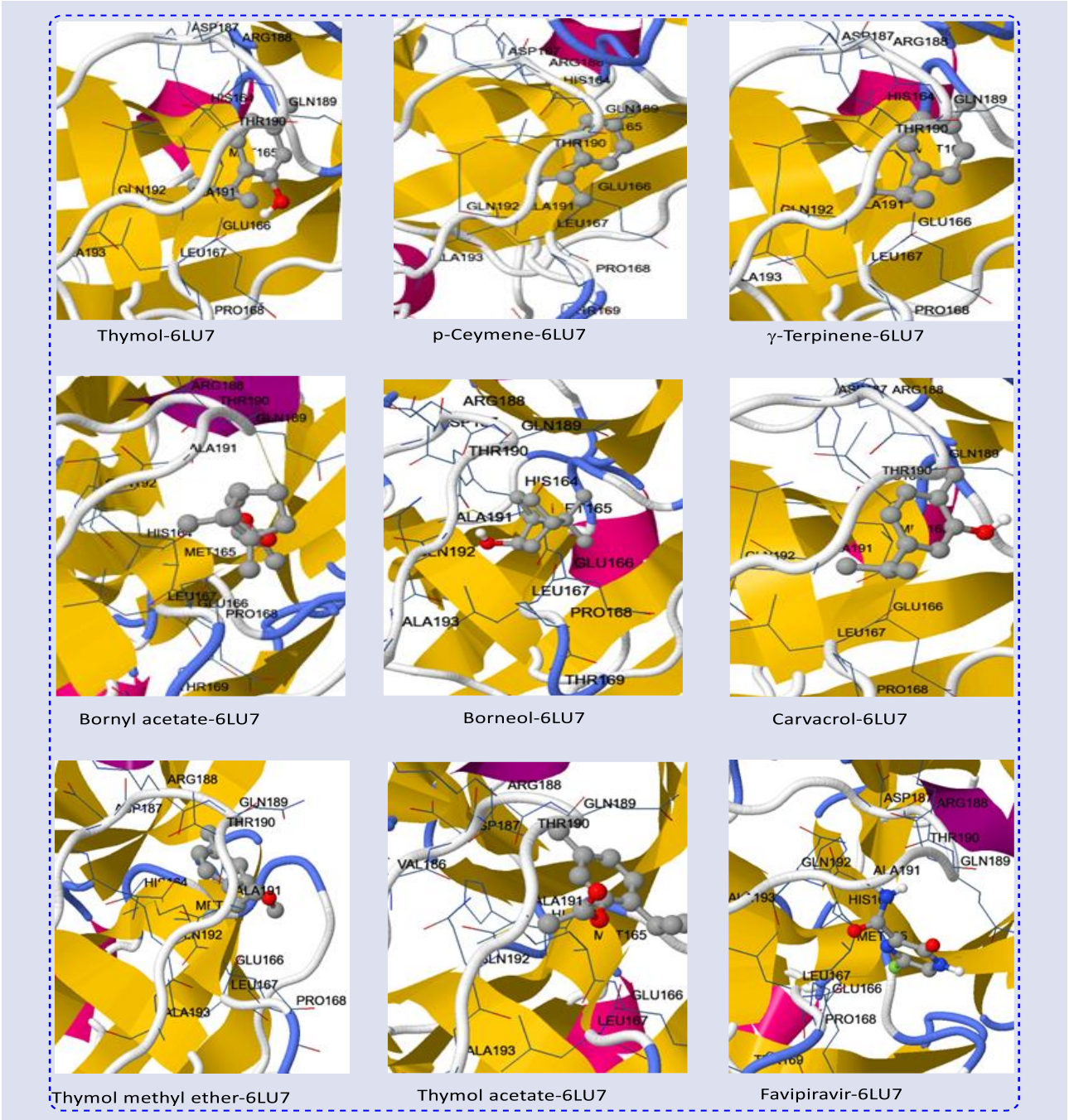

Figure 2 Docking poses between the main components of *thymus serpyllum L.* and the 6LU7 target protein

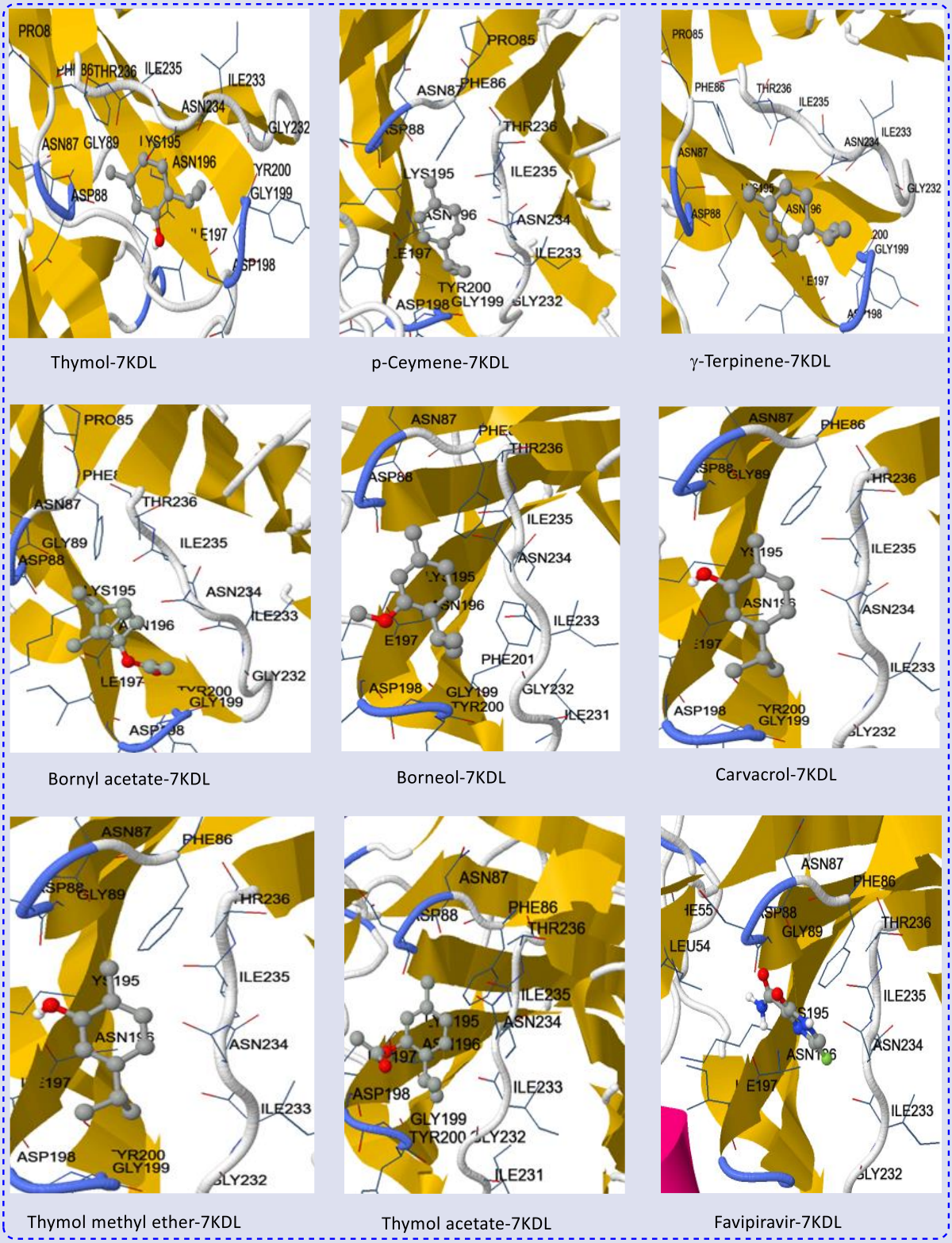

Figure 3 Docking poses between major components of *thymus serpyllum L*. and 7 KDL target proteins

The regions where the main components of *thymus serpyllum L.* enter in 6LU7 and 7KDL coded proteins and the amino acids found in these regions are shown in Figure 2 and Figure 3. For example, as seen from the thymol-6LU7 docking pose in Figure 2, the thymol ligand entered the MET165, GLU166, LEU167, GLN189, THR190 and GLN192 amino acid regions of the 6LU7 protein, and various secondary chemical interactions occurred between these amino acids and the thymol ligand. In this study, the energies of secondary chemical interactions between the main components of *thymus serpyllum L.* and 6LU7 and 7KDL proteins were calculated and given in Table 1.

Table 1 Binding energies between major components of *thymus serpyllum L.* and proteins 6LU7 and 7KDL

Molecules	6LU7	7KDL
Thymol	-4.61	-4.61
p-Cymene	-4.37	-3.84
γ- Terpinene	-4.23	-3.70
Bornyl acetate	-4.40	-4.28
Borneol	-4.42	-4.03
Carvacrol	-4.45	-3.96
Thymol methyl ether	-4.26	-3.85
Thymol acetate	-4.44	-4.07
Favipiravir	-4.39	-4.27
Energies are in kcal/mol		

As seen in Table 1, the binding energy between favipiravir used as a Covid-19 drug and 6LU7 representing the unmutated form of Covid-19 was calculated as -4.39 kcal/mol. It can be said that ligands with lower absolute binding energies than these values have lower inhibition efficiency against 6LU7 cell line than favipiravir. When Table 1 is examined, the binding energies of p-cymene, γ-terpinene and thymol methyl ether ligands are lower than those of favipiravir. Therefore, it can be said that the main components of *thymus serpyllum L.* p-cymene, γ-terpinene and thymol methyl ether are not active against the 6LU7 cell line. As seen in Figure 1, the molecular structures of p-cymene and γ-terpinene are composed of carbon and hydrogen atoms and contain only the C=C functional group. On the other hand, although thymol methyl ether contains CH_3O functional group, its binding energy is low. Therefore, it can be said that thymol methyl ether cannot be an active ingredient against 6LU7. The order of biological activity against 6LU7 according to the binding energies of the ligands is as follows.

γ-Terpinene < Thymol methyl ether < p-Cymene < Favipiravir < Bornyl acetate < Borneol < Thymol acetate < Carvacrol < Thymol

According to this order, it can be said that the molecules on the left of favipiravir are inactive against the 6LU7 cell line, while the molecules on the right are active. Considering the binding energies of 6LU7 and components, the component with the highest biological activity is thymol.

A similar interpretation can be made according to the binding energies between the main components of *thymus serpyllum L.* and the 7KDL cell line. The order of biological activity between the main components of *thymus serpyllum L.* and the 7KDL cell line according to their binding energies is as follows.

γ-Terpinene < p-Cymene < Thymol methyl ether < Carvacrol < Borneol < Thymol acetate < Favipiravir < Bornyl acetate < Thymol

According to this order, it can be said that only bornyl acetate and thymol are active against the 7KDL cell line, and other ligands are inactive.

As a result, the inhibition efficiency of bornyl acetate, borneol, thymol acetate, carvacrol and thymol against the 6LU7 target protein of Covid-19, and of bornyl acetate and thymol against the 7KDL protein is higher than favipiravir. It is seen that the biological activity of the thymol molecule, which is the highest amount in wild thyme, is high against the examined cell lines. According to the results obtained, it is thought that the thymol molecule and its derivatives may be a molecule that should be considered in drug development against Covid-19.

Conclusions

In this study, the antiviral effects of thymol, p-cymene, γ-terpinene, bornyl acetate, borneol, carvacrol, thymol methyl ether, thymol acetate, which are the main components of wild thyme, on Covid-19 were investigated. Molecules were docked to the 6LU7 and 7KDL target protein of Covid-19. Docking poses and binding energies between target proteins and molecules were calculated. The results were compared with favipiravir, an antiviral drug developed against influenza virus and also used in the treatment of Covid-19. It was predicted that the thymol molecule, one of the main components of wild thyme, has antiviral activity against Covid-19 and should be considered in drug development.

Conflicts of interest

There are no conflicts of interest among the authors of this study.

References

[1] Chintagunta A. D., Kumar M., Sampath Kumar N. S., Jeevan Kumar S. P., Differential diagnosis and possible therapeutics for coronavirus disease 2019, *In Diagnostic Strategies for COVID-19 and other Coronaviruses,* (2020) 51-71.

[2] Islam M. T., Sarkar C., El-Kersh D. M., Jamaddar S., Uddin S. J., Shilpi J. A., Mubarak M. S., Natural products and their derivatives against coronavirus: A review of the non-clinical and pre-clinical data, *Phytotherapy Research,* 34(10) (2020) 2471-2492.

[3] Ulbricht C., Basch E., Cheung L., Goldberg H., Hammerness P., Isaac R., Wortley J., An evidence-based systematic review of elderberry and elderflower (Sambucus nigra) by the Natural Standard Research Collaboration, *Journal of dietary supplements,* 11(1) (2014) 80-120.

[4] Valles J., Bonet M. A., Agelet A., Ethnobotany of Sambucus nigra L. in catalonia (Iberian Peninsula): The integral exploitation of a natural resource in mountain regions, *Economic botany,* 58(3) (2004) 456-469.

[5] Armanini D., Fiore C., Mattarello M. J., Bielenberg J., Palermo M., History of the endocrine effects of licorice, *Experimental and clinical endocrinology & diabetes,* 110(06) (2002) 257-261.

[6] Fiore C., Eisenhut M., Krausse R., Ragazzi E., Pellati D., Armanini D., Bielenberg J., Antiviral effects of Glycyrrhiza species, *Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives,* 22(2) (2008) 141-148.

[7] Fiore C., Eisenhut M., Ragazzi E., Zanchin G., Armanini, D., A history of the therapeutic use of liquorice in Europe, *Journal of ethnopharmacology,* 99(3) (2005) 317-324.

[8] Raus K., Pleschka S., Klein P., Schoop R., Fisher P., Effect of an Echinacea-based hot drink versus oseltamivir in influenza treatment: a randomized, double-blind, double-dummy, multicenter, noninferiority clinical trial, *Current Therapeutic Research,* (77) (2015) 66-72.

[9] San Chang J., Wang K. C., Yeh C. F., Shieh D. E., Chiang L. C., Fresh ginger (Zingiber officinale) has anti-viral activity against human respiratory syncytial virus in human respiratory tract cell lines, *Journal of ethnopharmacology,* 145(1) (2013) 146-151.

[10] Ehrhardt C., Hrincius E. R., Korte V., Mazur I., Droebner K., Poetter A., Ludwig S., A polyphenol rich plant extract, CYSTUS052, exerts anti influenza virus activity in cell culture without toxic side effects or the tendency to induce viral resistance, *Antiviral research,* 76(1) (2007) 38-47.

[11] Weber N. D., Andersen D. O., North J. A., Murray B. K., Lawson L. D., Hughes B. G., In vitro virucidal effects of Allium sativum (garlic) extract and compounds, *Planta medica,* 58(05) (1992) 417-423.

[12] Xu J., Xu Z., Zheng W., A review of the antiviral role of green tea catechins, *Molecules,* 22(8) (2017) 1337.

[13] Zhuang M., Jiang H., Suzuki Y., Li X., Xiao P., Tanaka T., Hattori T., Procyanidins and butanol extract of Cinnamomi Cortex inhibit SARS-CoV infection, *Antiviral research,* 82(1) (2009) 73-81.

[14] Praditya D., Kirchhoff L., Brüning J., Rachmawati H., Steinmann J., Steinmann E., Anti-infective properties of the golden spice curcumin, *Frontiers in microbiology,* (10) (2019) 912.

[15] Wen C. C., Kuo Y. H., Jan J. T., Liang P. H., Wang S. Y., Liu H. G., Yang N. S., Specific plant terpenoids and lignoids possess potent antiviral activities against severe acute respiratory syndrome coronavirus, *Journal of medicinal chemistry,* 50(17) (2007) 4087-4095.

[16] Stahl-Biskup E., Essential oil chemistry of the genus Thymus–a global view. *In Thyme* (2002) 89-138, CRC Press.

[17] Nickavar B., Mojab F., Dolat-Abadi R., Analysis of the essential oils of two Thymus species from Iran, *Food chemistry* (2005) 90(4) 609-611.

[18] Nikolic M., Glamoclija J., Ferreira I. C., Calhelha R. C., Fernandes A., Markovic T., Sokovic M., Chemical composition, antimicrobial, antioxidant and antitumor activity of Thymus serpyllum L., Thymus algeriensis Boiss. and Reut and Thymus vulgaris L. essential oils, *Industrial Crops and Products,* (52) (2014) 183-190.

[19] Kim S., Thiessen P. A., Cheng T., Yu B., Shoemaker B. A., Wang J., Bryant S. H., Literature information in PubChem: associations between PubChem records and scientific articles, *Journal of cheminformatics,* 8(1) (2016) 1-15.

[20] Hatada R., Okuwaki K., Mochizuki Y., Handa Y., Fukuzawa K., Komeiji Y., Tanaka S., Fragment molecular orbital-based interaction analyses on COVID-19 main protease− inhibitor N3 complex (PDB ID: 6LU7), *Journal of chemical information and modeling,* 60(7) (2020) 3593-3602.

[21] Verkhivker G. M., Agajanian S., Oztas D., Gupta G., Computational analysis of protein stability and allosteric interaction networks in distinct conformational forms of the SARS-CoV-2 spike D614G mutant: Reconciling functional mechanisms through allosteric model of spike regulation. *Journal of Biomolecular Structure and Dynamics,* (2021) 1-18.

[22] Bikadi Z., Hazai E., Application of the PM6 semi-empirical method to modeling proteins enhances docking accuracy of AutoDock. *Journal of cheminformatics,* 1(1) (2009) 1-16.

[23] Halgren T. A., Merck molecular force field. I. Basis, form, scope, parameterization, and performance of MMFF94. *Journal of computational chemistry,* 17(5-6) (1996) 490-519.

[24] Gasteiger J., Marsili M., Iterative partial equalization of orbital electronegativity—a rapid access to atomic charges, *Tetrahedron,* 36(22) (1980) 3219-3228.

[25] Tang Y., Zhu W., Chen K., Jiang H., New technologies in computer-aided drug design: Toward target identification and new chemical entity discovery, *Drug discovery today: technologies,* 3(3) (2006) 307-313.

[26] Kuntz I. D., Blaney J. M., Oatley S. J., Langridge R., Ferrin T. E., A geometric approach to macromolecule-ligand interactions, *Journal of molecular biology,* 161(2) (1982) 269-288.

[27] Onodera K., Satou K., Hirota H., Evaluations of molecular docking programs for virtual screening, *Journal of chemical information and modeling,* 47(4) (2007) 1609-1618.

[28] Kaya S., Erkan S., Karakaş D., Computational design and characterization of platinum-II complexes of some Schiff bases and investigation of their anticancer-antibacterial properties, *Applied Organometallic Chemistry,* (36) (2022) e6805.

[29] Kaya S., Erkan S., Karakaş D. Computational investigation of molecular structures, spectroscopic properties and antitumor-antibacterial activities of some Schiff bases, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy,* (244) (2021) 118829.

[30] Erkan, S., Karakaş, D. Computational investigation of structural, nonlinear optical and anti-tumor properties of dinuclear metal carbonyls bridged by pyridyl ligands with alkyne unit, *Journal of Molecular Structure,* (1199) (2020) 127054.