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Araştırma Makalesi / Research Article

In silico Investigation of the Interactions of Thymol and Carvacrol on the Spike Protein of Omicron Variant and MPro Enzyme of Coronavirus

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Abstract

Many drug studies have been conducted against the coronavirus disease, which has affected the whole world since December 2019, and some studies have been carried out on natural treatment methods. Many ideas for curing coronavirus disease of *T. vulgaris* known as thyme plant have been presented, although there are gaps in the literature on the subject. In this work, the anti-severe acute respiratory syndrome coronavirus 2 potential of the major compounds of the *T. vulgaris* plant's essential oil was investigated *in silico*. The major components of the *T. vulgaris* plant's essential oil are thymol and carvacrol. Using molecular docking experiments, we evaluated the effects of thymol and carvacrol in thyme essential oil on Omicron variant spike protein and main protease enzyme (Mpro) of severe acute respiratory syndrome coronavirus 2. We also used online databases to investigate the adsorption, distribution, metabolism, absorption, and toxic (ADMET) aspects of these two compounds. It was determined that thymol and carvacrol have strong binding affinity to the spike protein of the Omicron variant and the main protease enzyme. The compounds interact with target proteins through electrostatic, hydrogen bonds, and hydrophobic interactions. More promising findings are obtained when the contacts of carvacrol with target proteins are assessed in terms of the structure-activity relationship.

Keywords: *T. vulgaris*, Thymol, Carvacrol, Severe acute respiratory syndrome coronavirus 2, Molecular Docking, ADMET

Timol ve Karvakrolün Koronavirüsün Ana Proteaz Enzimi ve Omicron Varyantının Spike Proteini ve Üzerindeki Etkileşimlerinin *In Silico* Araştırılması

Öz

Aralık 2019'dan beri dünyayı etkisi almış olan koronavirüs hastalığına karşı birçok ilaç çalışması yapılmış ve doğal tedavi yöntemleri üzerine bazı çalışmalar yapılmıştır. Kekik olarak bilinen *T. vulgaris'in* koronavirüs hastalığı tedavisi ile ilgili birçok öneride bulunulmuş ancak bu konu ile ilgili literatürde boşluklar bulunmaktadır. Bu çalışmada, *T. vulgaris*'in ana bileşenlerinin anti-şiddetli akut solunum sendromu koronavirüs 2 potansiyeli *in silico* olarak araştırılmıştır. Timol ve karvakrol, *T. vulgaris* bitkisinin uçucu yağının ana bileşenleridir. Kekik esansiyel yağının ana bileşenlerinin SARS CoV-2 ana proteaz ve Omicron varyantı Spike proteini üzerindeki etkilerini moleküler yerleştirme çalışmaları kullanılarak incelenmiştir. Ayrıca bu iki bileşiğin adsorpsiyon, dağılım, metabolizma, absorpsiyon ve toksik (ADMET) özelliklerini çevrimiçi veritabanlarının yardımıyla incelenmiştir. Timol ve karvakrol, şiddetli akut solunum sendromu koronavirüs 2'nin Omicron varyantının spike proteinine, ana proteaz enzimine kıyasla güçlü bir bağlanma afinitesine sahiptir. Bileşikler, hedef proteinler ile elektrostatik, hidrojen bağı ve hidrofobik etkileşimlerle bağlanmaktadır. Karvakrol bileşiğinin hedef proteinlerle olan etkileşimleri yapı-aktivite ilişkisi açısından değerlendirildiğinde daha umut verici bulgular göstermektedir.

Anahtar Kelimeler: *T. vulgaris*, Timol, Karvakrol, Şiddetli akut solunum sendromu koronavirüs 2, Moleküler Docking, ADMET

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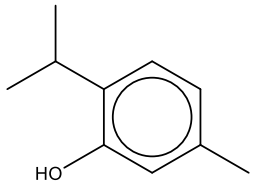
1. Introduction

The coronavirus disease has still caused many life-threatening pandemics worldwide. Numerous research have found that coronavirus variations are to blame for the disease's increasing infectiousness, effectiveness, and severity. Several investigations have found that changes in severe acute respiratory syndrome coronavirus 2 variants improve the potential of the Spike Receptor Binding Domain to bind to the angiotensin-converting enzyme 2 receptor. The need for treatment of this illness is obvious given its impact on mankind. The main protease enzyme (Mpro) is required for viral translation, transcription, and replication activities. Drug therapy, which is a typical issue with many viral infections, is nevertheless appropriate for this illness. As a result, in coronavirus research, it is critical to understand the interactions of therapeutic candidate compounds with the severe acute respiratory syndrome coronavirus 2 MPro enzyme and spike protein (Cheke 2020; Singh and Florez 2020; Daoud et al. 2021; Jain et al. 2021; Parmar et al. 2022).

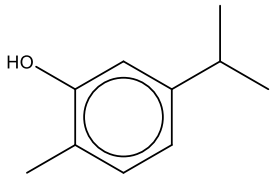
For the treatment of numerous disorders, using plant essential oils and medication containing natural herbs are both effective alternatives (Alp, M and Alp, A.S 2019). Many aromatic plant species in the Lamiaceae family are used by people for this purpose and are known as thyme. However, species that have components similar to thymol and carvacrol in their essential oils are regarded as thyme. This plant is used to treat asthma, as well as other inflammatory and infectious diseases. Thyme essential oil is known to have antibacterial, antifungal, antiviral, and antioxidant properties (Ipek et al. 2005; Amirghofran et al. 2012; Hadidi). The anti-severe acute respiratory syndrome coronavirus 2 abilities of some components of this biologically active plant have been investigated *in silico* with some studies and many promising results have been obtained (Sampangi-Ramaiah et al. 2020; Hadidi). It has previously been demonstrated that Thymus vulgaris EO (TEO) is effective against a variety of RNA viruses, including CoVs (Catella et al. 2021). Thyme has been shown by Sardari and his associates to have a beneficial impact on the recovery process from corona illness (Sardari et al. 2021). In this study, we examined the interactions of thymol and carvacrol, the major components of *T. vulgaris*, with the SARS-CoV-2 MPro enzyme and the spike protein of its final variant, Omicron, to contribute to the drug studies of coronavirus disease. Using internet databases, we looked into their toxicological and pharmacokinetic characteristics.

2. Materials and Methods

The structures of thymol and carvacrol are given in Figure 1.



2-isopropyl-5-methylphenol (Thymol)



5-Isopropyl-2-methylphenol (Carvacrol)

Figure 1. Structures of thymol and carvacrol.

2.1. Molecular Docking

The binding scores between the compounds and the MPro enzyme (PDB Code: 7BV2) (Fakhar et al. 2021) and the spike protein of the severe acute respiratory syndrome coronavirus 2 (PDB Code: 7T9J) (Bank) were calculated using the Autodock Vina (Trott and Olson 2009, p. vina) program. PDB extension files of the proteins were downloaded from the RCSB Protein Data Bank. (https ://www.rcsb.org). The structures of thymol and carvacrol were given in Figure 1. The proteins, which will be modelled, were first optimized with the help of BIOVA Discovery Studio Visualizer 2021 (BIOVA Software) software. With the AutoDockTools 1.5.7 software(Trott and Olson 2009), the active regions of proteins have interacted with thymol and carvacrol, and ligand-protein interactions were visualized.

2.2. Drug Likeness and Toxicity Studies

Early identification of the pharmaco- and toxicokinetic characteristics of drug candidate compounds reduces the need for additional testing, improves success rates, and saves time and money. The absorption, distribution, metabolism, excretion, and toxicity (ADMET) features describe a drug's properties. ADMET properties of carvacrol and thymol were computed by SwissADME (Daina et al. 2017) and ProTox-II (Banerjee et al. 2018).

3. Findings and Discussion

3.1. Molecular Docking

The binding energy value between thymol and carvacrol and the MPro enzyme was determined as -4.9 kcal/mol. Thymol interacts with MPro enzyme via π-alkyl interactions with methionine165 (Figure 2). Carvarol binds the active region of the MPro enzyme via the carbon-hydrogen bond with glutamine189, alkyl interactions with methionine165, and π-alkyl interactions with methionine165

and histidine41 (Figure 3). Although the binding energy is the same, considering the interaction types, it is thought that carvacrol interacts more strongly with the active region of the MPro enzyme.

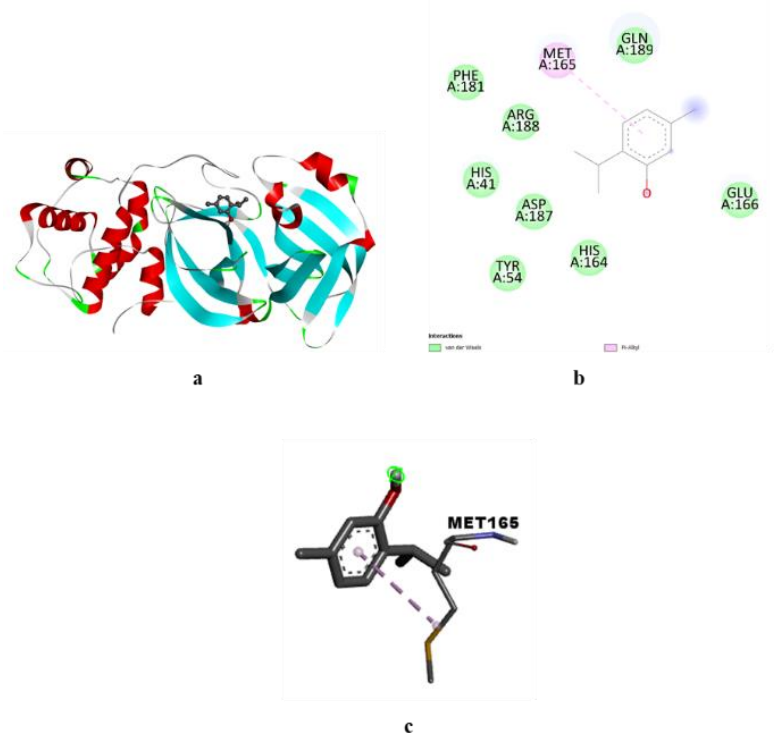


Figure 2. The Molecular docking results of thymol on the MPro enzyme (a), 2D interactions of thymol with the active region of the MPro enzyme (b), and ligand interactions between thymol and the active region of the MPro enzyme (c).

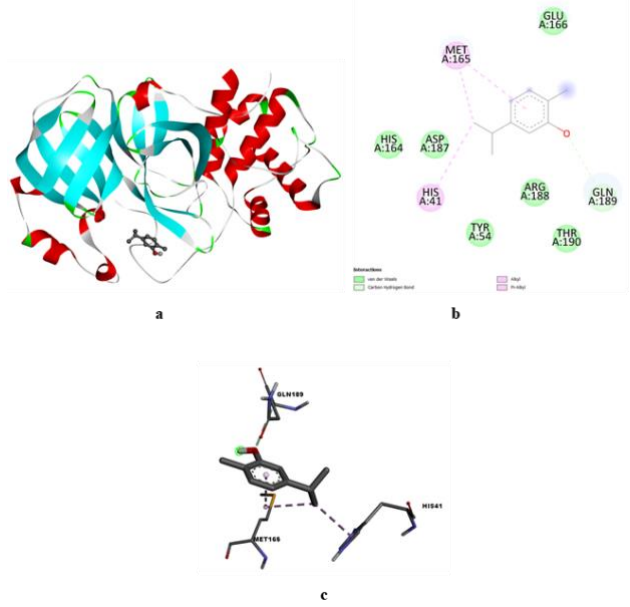


Figure 3. The Molecular docking results of carvacrol on the MPro enzyme (a), 2D interactions of carvacrol with the active region of the MPro enzyme (b), and ligand interactions between carvacrol and the active region of the MPro enzyme (c).

The binding energy values between thymol and carvacrol and the spike protein Omicron variant were found as -4.4 kcal/mol and -4.9 kcal/mol, respectively. Thymol interacts active region of the spike protein of Omicron variant by the conventional hydrogen bond with serine730, and

histidine1058, π-donor hydrogen bond with histidine1058, π-sigma interactions with histidine1058, π-alkyl interactions with phenylalanine872, isoleucine870 and alkyl interactions with isoleucine870 (Figure 4). The hydrogen bond distances of thymol and serine730 and histidine1058 amino acid residues were determined as 2.47 Å, and 2.44 Å, respectively. Carvacrol binds the active region of the spike protein of Omicron variant of severe acute respiratory syndrome coronavirus 2 by the conventional hydrogen bond with histidine1058 (2.76), alkyl interactions with proline863, π-alkyl interactions with histidine1058, isoleucine870 (Figure 5). The hydrogen bond distance of carvacrol and histidine1058 amino acid residue was found 2.76 Å. When the binding energy and interaction types were evaluated, it was determined that carvacrol interacted more strongly with spike protein. This indicates that the anti-severe acute respiratory syndrome coronavirus 2 potential of carvacrol is stronger when compared to the data obtained for thymol.

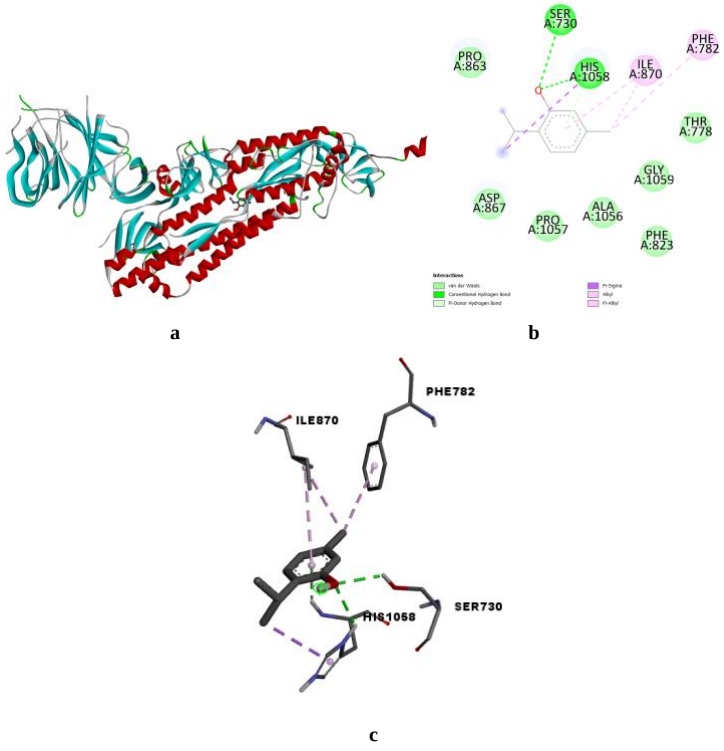


Figure 4. The Molecular docking results of thymol on the active region of the Omicron spike protein (a), 2D interactions of thymol with the active region of Omicron variant spike protein (b), and ligand interactions between thymol and the active region of active region of the Omicron spike protein (c).

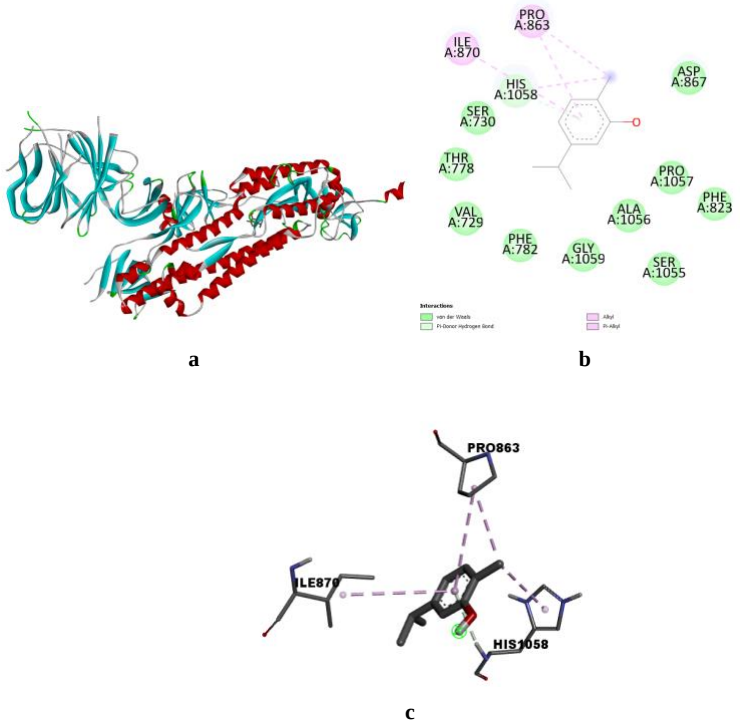


Figure 5. The Molecular docking results of carvacrol on the active region of the Omicron spike protein (a), 2D interactions of carvacrol with the active region of the Omicron variant spike protein (b), and ligand interactions between carvacrol and the active region of active region of the Omicron spike protein (c).

3.2. Prediction of Drug Likeness and Toxicity

Drug development is more likely to be effective if the pharmaco- and toxicokinetic characteristics of therapeutic candidate compounds can be predicted. Five rules of Lipinski were published by Lipinski and colleagues to determine if a chemical is an ideal pharmaceutical candidate (Lipinski et al. 1997). When the data were evaluated, thymol and carvacrol were determined to conform with Lipinski's five rules (Table 1). BOILED-Egg Models of thymol and carvacrol indicated their high gastrointestinal absorption, ability to cross the blood-brain barrier (BBB), and inability to function as P-glycoprotein substrates (Figure 6). The compounds were predicted to act as inhibitors of Cytochrome P450 enzyme 1A2, while not acting as inhibitors for Cytochrome P450 enzyme 2C19, Cytochrome P450 enzyme 2C9, Cytochrome P450 enzyme 2D6, Cytochrome P450 enzyme 3A4 with cytochrome P450 enzymes (CYPs). The compounds' hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity are all inactive, yet they have an active mitochondrial membrane potential. When all of these data are considered, thymol and carvacrol are believed to be candidate pharmaceuticals and compounds that can be employed in drug formulation research.

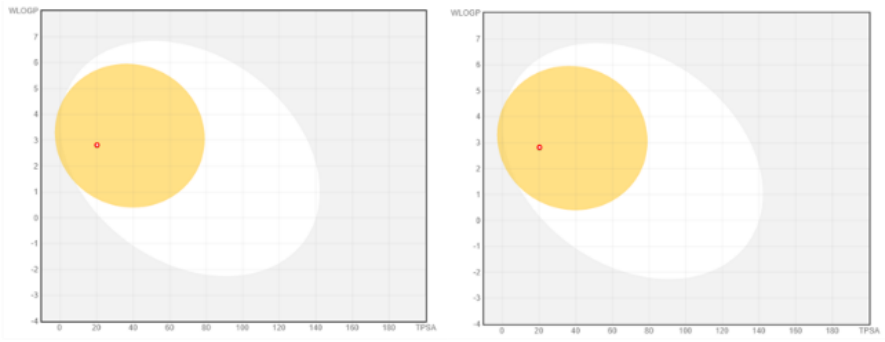


Figure 6. Boiled-Egg models of the thymol (a) and carvacrol (b).

Table 1. The pharmacokinetic properties of the thymol and carvacrol.

	Predicted Result	
Properties	Thymol	Carvacrol
Molecular weight	150.22 g/mol	150.22 g/mol
Heavy atoms' number	11	11
Aromatic heavy atoms' number	6	6
Rotatable bonds' number	1	1
Hydrogen bond acceptors' number	1	1
Hydrogen bond donors' number	1	1
Molar Refractivity	48.01	48.01
TPSA ($Å^2$)	20.23	20.23
Log $P_{o/w}$	2.80	2.82
Gastrointestinal absorption	High	High
The blood-brain barrier (BBB)	Yes	Yes
P-glycoprotein substrate	No	No
Cytochrome P450 enzyme 1A2 inhibitor	Yes	Yes
Cytochrome P450 enzyme 2C19 inhibitor	No	No
Cytochrome P450 enzyme 2C9 inhibitor	No	No
Cytochrome P450 enzyme 2D6 inhibitor	No	No
Cytochrome P450 enzyme 3A4 inhibitor	No	No
Log K_p (skin permeation)	-4.87 cm/s	-4.74 cm/s
Lipinski	Yes; 0 violation	Yes; 0 violation
Toxicity Class*	4	4
Predicted LD_{50}	640 mg/kg	810 mg/kg
Hepatotoxic	No	No
Carcinogenic	No	No
Immunotoxic	No	No
Mutagenic	No	No
Cytotoxic	No	No
Mitochondrial membrane potential	Yes	Yes
*Toxicity Class: 1-toxic; 6-non-toxic		

4. Conclusions and Recommendations

The studies show that thymol and carvacrol have a strong affinity for the spike protein of Omicron variant of severe acute respiratory syndrome coronavirus 2 as well as the MPro enzyme of severe acute respiratory syndrome coronavirus 2. The MPro enzyme of severe acute respiratory syndrome coronavirus 2 and the spike protein of severe acute respiratory syndrome coronavirus 2 Omicron variant have the best binding energy values for carvacrol. Carvacrol shows more promising findings when the interactions of the thymol and carvacrol with the proteins are assessed in terms of the structure-activity relationship. The thymol and carvacrol interact in electrostatic, hydrogen bonding, and hydrophobic interactions with the spike protein of the Omicron variant of severe acute respiratory syndrome coronavirus 2. Because it can exert an antagonistic impact by interacting with the amino acid residues in the active site of the spike protein of severe acute respiratory syndrome coronavirus 2. More research is needed to establish the effectiveness of thymol, carvacrol, and even *T. vulgaris* itself against severe acute respiratory syndrome coronavirus 2.

Authors' Contributions

The study's authors all contributed equally.

Statement of Conflicts of Interest

The authors do not have any conflicts of interest.

Statement of Research and Publication Ethics

The author declares that this study complies with Research and Publication Ethics.

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