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The Determination of Molecular Electrostatic Potential and Anticancer Properties of Eugenol: A Theoretical Study

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Abstract

Cancer is one of the deadliest diseases worldwide, and for this reason, it is a prominent field of study in drug development. It has been reported in various studies that some of the plants and essential oils obtained from plants have high anticancer activities. This situation is related to the compound groups found in plants and essential oils. Studies on using essential oils in combination with synthetic drugs or aromatherapy are ongoing. Essential oils show cytotoxic properties and may play a role in the death of cancer cells. Eugenol is an important compound found in clove, laurel, and cinnamon essential oils that has anticancer activity in various types of cancer. Eugenol has the ability to reduce cyclooxygenase-2 (COX-2) activity and to inhibit cell proliferation through NF-κB suppression in various types of cancer. In this study, the binding profiles of eugenol with COX-2 and Human inhibitor of nuclear transcription factor κB (IkB) kinase beta, which plays a crucial role in the NF-κB signaling pathway, were examined by molecular docking study, which is one of the methods used in computer-aided drug design. A supporting study was performed to understand the electrostatic complementarity between ligand and receptor by molecular electrostatic potential (MEP) analysis. As a result of the study, it was comparatively presented that eugenol has similar interaction profiles with reference compounds.

Keywords: MEP, Molecular docking, Anticancer, Essential Oil

1. Introduction

Cancer is one of the world's deadliest diseases, known as uncontrolled cell proliferation caused by genetic and/or environmental factors. There are also plant-based studies in cancer treatment development studies, which have extensive scientific literature and are still being studied. Studies conducted with plants have reported anticancer effects [1-3]. It has been observed in the results of the study that various compounds in plants have properties that inhibit the growth of cancer cells and promote the apoptosis of cells. Since essential oils (EOs) have multiple effects such as antibacterial, antioxidant, and anticancer, their use with different treatment options has been reported to be popular in literature studies [4]. Plant essential oils have also been evaluated in cancer-related studies and have been reported to have cytotoxic effects on various cancer cell lines. For example, in a study conducted on Caco-2 colorectal cancer cell lines with EO of *Eucalyptus camaldulensis* Dehnh., it was reported that EO induced programmed cell death [5]. In a study conducted on breast cancer cell lines (MCF-7) with thyme and clove oils, it was reported that both essential oils inhibited the growth of cancer cell lines in a concentration-dependent manner [6]. However, more studies and results are needed for more reliable information about the cell target specificity of essential oils and their use in drug delivery systems [4]. Because various risks such as hydrophobicity, high volatility, and instability bring some limitations on their use. The encapsulation method is used to minimize these limitations [7]. In a study conducted by Ercin et al., encapsulation of *Laurus nobilis* L. EO was performed, and it was evaluated that it could be used as a phytotherapeutic agent-based controlled release system in cancer treatment [8].

Eugenol is a phenylpropanoid found in many essential oils (clove, laurel, cinnamon, etc.) and has been reported to have anticancer properties against many types of cancer, from lung cancer to prostate cancer, from breast cancer to leukemia [9-11]. The anticancer effect of eugenol can occur through different mechanisms, such as inducing cell death, cell cycle arrest, and metastasis [9,12,13]. Eugenol has the ability to reduce

cyclooxygenase-2 activity and inhibit NF-κB in various types of cancer [9]. COX-2 enzymes are important biological structures that provide the production of prostaglandins that are associated with inflammation. COX-2 inhibitors generally have inflammation-blocking properties [14]. Additionally, the increase in cyclooxygenase (COX-2) level due to chronic inflammation is associated with different stages such as tumor formation and proliferation. This is why the COX-2 enzyme is tried to be suppressed in cancer treatment studies [15]. It was reported by Jaganathan and Supriyanto that Eugenol may play a role in the inhibition of COX-2 expression [16]. The increase in NF-κB activity in tumor tissues is directly proportional to the release of factors such as pro-inflammatory cytokines, and this may cause the cancerous area to spread [17]. Human IkB kinase beta plays a crucial role in the NF-κB signaling pathway [18,19]. Some of the studies reported that eugenol potentiates the effect of cisplatin on cancer stem-like cells by targeting the NF-κB pathway [20], and that eugenol exhibits inhibitory properties on cell proliferation through NF-κB suppression in the rat gastric carcinogenesis model induced by N-methyl-N′-nitro-N-nitrosoguanidine [21].

Molecular docking and molecular electrostatic potential (MEP) analysis, which are molecular modeling methods, are used in drug design studies. With the molecular docking method, the interactions of the drug candidate, that is, the ligand, with the macromolecules, that is, the receptors, targeted for the disease are examined [22]. Molecular electrostatic potential (MEP) analysis is related to the charge distribution of the molecule. The charge distribution of the molecule is also an significant indicator of intermolecular interactions [23-25]. For example, the capability of a ligand to bind to the target receptor is determined through molecular electrostatic interactions. Both molecular docking and molecular electrostatic potential analyses are two important methods used to study interactions at the molecular level.

In this study, molecular electrostatic potential analysis of eugenol, one of the essential oil compounds known to have anticancer properties, and molecular docking studies with COX-2 and Human IkB kinase beta, which are important targets, were carried out.

2. Materials and Methods

Eugenol structure was obtained from Pubchem (CID: 3314) (https://pubchem.ncbi.nlm.nih.gov/compound/Eugenol). Eugenol was optimized at Gaussian09 package program [26] with DFT/B3LYP/6-311++G(d,p). For molecular docking studies, eugenol was prepared as ligand with AutoDock Tools. COX-2 (PDB: 5IKR) and human IkB kinase beta (PDB: 4KIK) structures were downloaded from PDB DataBank (https://www.rcsb.org/). Receptors were cleared of solutions and ions and added polar hydrogens. Grid boxes were adjusted as 30x30x30 Å, and molecular docking studies were carried out via AutoDock Vina program [27].

3. Results and Discussion

3.1 Molecular Docking

As a predictive tool for whether or not an effect will occur experimentally, molecular docking is an important method used in computer-aided drug design. It has been reported in literature studies that Eugenol may play a role in the inhibition of COX-2 expression and suppression of NF-κB [16,21]. In this study, considering that the mechanisms of action are active, the binding profiles and binding types that may occur in the event of the experimental effect were evaluated.

The first molecular docking study was performed with COX-2. The binding affinity of eugenol was calculated as -6.0 kcal/mol, in this study. When the RMSD values were examined, it was observed that they did not exceed values around 2 Å and gave values within an acceptable range (see Table 1). The interactions between eugenol and COX-2 were provided by amide-pi stacked, pi-alkyl, alkyl, and van der Waals (vdW) interactions (see Figure 1). Eugenol had an amide-pi stacked interaction with Gly-526. Pi-alkyl interactions of eugenol occurred with Val-523, Tyr-355, Val-349, Leu-352 and Ala-527 residues of COX-2. Additionally, there was another alkyl interaction with Ala-527. vdW interactions of eugenol occurred with Ser-353, Arg-120, Phe-518, Trp-387, Met-522, Phe-381 and Ser-530 residues of COX-2.

Considering the literature studies, the fact that similar amino acid residues play a role in the interaction is evidence that eugenol is in the active site. While it was observed in the literature that 4-Nitro-5-O-benzoylpinostrobin, 5-O-Benzoylpinostrobin, and flavanone interact with Arg-120 through different interaction types such as hydrogen bonding and pi-interaction [28], eugenol also interacted with Arg-120 in this study. While 4-Nitro-5-O-benzoylpinostrobin, flavonol, and chrysin molecules interacted with amide-pi stacked interaction with residue Gly-526 [28,29], eugenol also made the same interaction with the same residue in this study. In the study conducted with COX-2, quercetin, a potential anticancer agent [30], was reported to have pi interactions with Val-523, Val-349, and Leu-352 residues [31]. In this study conducted with eugenol, it was determined that Tyr-355 and Ala-527 residues were also involved in pi interactions in addition to these residues. Tyr-355 is an important amino acid residue involved in interactions. In another study, interactions in the active site of COX-2 were presented with the binding affinity value of eugenol. While there were interactions with residues similar to the results in

this study, it was determined that the binding affinity value in our study gave a better value [32].

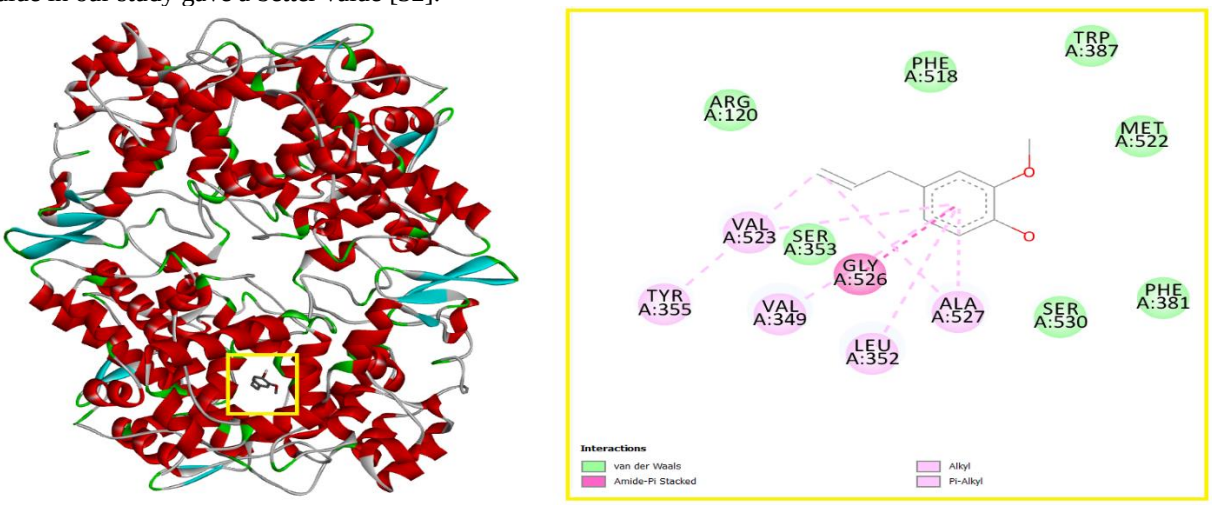

Figure 1. The binding profile of eugenol and COX-2.

Table 1. Binding affinities and RMSD values obtained from molecular docking study of Eugenol and COX-2.

Mode	Affinity (kcal/mol)	dist from best mode	
		rmsd l.b.	rmsd u.b.
1	-6.0	0.000	0.000
2	-5.9	1.326	1.782
3	-5.9	2.592	5.061
4	-5.9	1.440	2.761
5	-5.9	1.687	2.893
6	-5.6	2.443	4.660
7	-5.5	2.989	4.900
8	-5.4	2.518	4.685
9	-5.4	1.482	2.715

In the second part of the molecular docking study, eugenol was docked with human IkB kinase beta, and the binding energy was calculated as -6.3 kcal/mol. When the RMSD values were examined, it was observed that they gave values within the acceptable range in terms of suitability of the molecular docking study (see Table 2). The interactions between eugenol and human IkB kinase beta were provided by hydrogen bond, pi-sulfur, pi-sigma, pi-alkyl, alkyl, and vdW interactions (see Figure 2). Eugenol made hydrogen bond and pi-sulfur interactions with Cys-99 residue. Additionally, eugenol made pi-sigma interaction with Val-152 residue of human IkB kinase beta. Pi interactions were very active in the binding region. Another pi interaction (pi-alkyl) occurred with residues Ile-165, Val-29, and Leu-21. Ile-165, and Val-29 also made alkyl interactions. Met-96 was also observed as another residue participating in alkyl interactions. Considering the vdW interactions, it was determined that Val-74, Asp-166, Asp-103, Gly-102, and Tyr-98 residues interact with eugenol.

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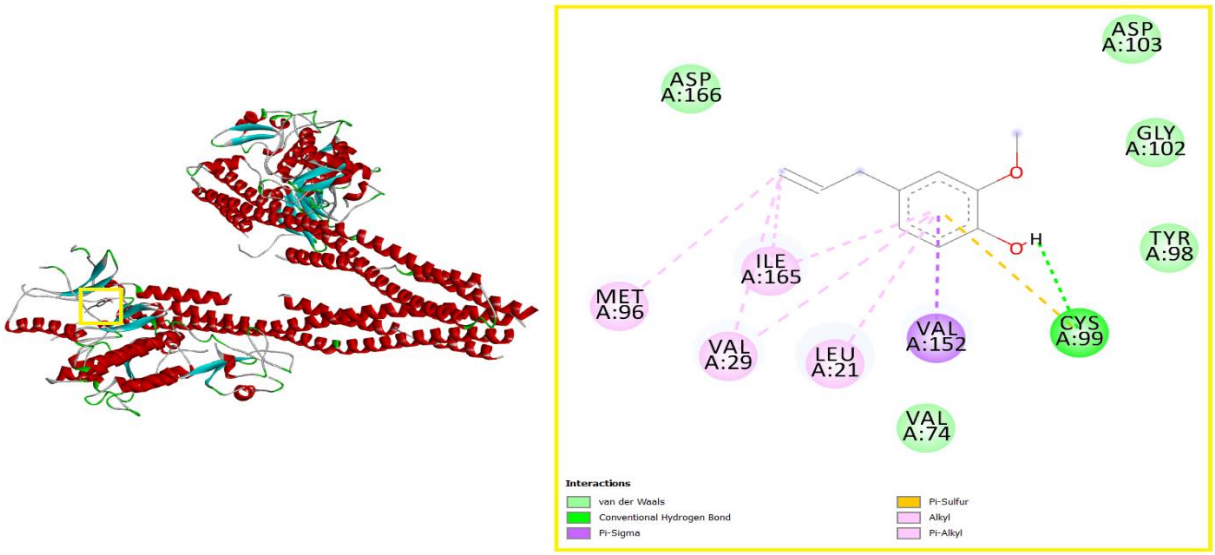

Figure 2. The binding profile of eugenol and human IkB kinase beta.

Table 2. Binding affinities and RMSD values obtained from molecular docking study of Eugenol and human IkB kinase beta.

Mode	Affinity (kcal/mol)	dist from best mode	
		rmsd l.b.	rmsd u.b.
1	-6.3	0.000	0.000
2	-6.2	1.231	1.602
3	-6.0	2.480	3.527
4	-5.8	2.032	3.597
5	-5.8	2.147	4.861
6	-5.8	2.586	4.542
7	-5.7	1.702	2.768
8	-5.7	1.116	2.618
9	-5.5	3.207	5.069

It has been determined that the prominent residues in the docking studies performed with human IkB kinase beta are quite compatible with the residues with which eugenol interacts in the active site of the receptor. In most of the literature studies, hydrogen bonding or hydrophobic interactions with Cys-99 have been observed [33,34]. In this study, eugenol made both hydrogen bond and pi-sulfur interaction with Cys-99. Terpenoids are plant defense molecules known to have anticancer effects [35]. In the docking study carried out with terpenoid plant components, it was reported that Asp-103 and Gly-102 came to the fore in hydrogen bond interactions or hydrophobic interactions [34]. Eugenol also had a van der Waals interaction with these residues. Leu-21, Val-152, Ile-165, and Val-29 residues are other important residues that are at the forefront in molecular interactions [33,34]. In this study, these amino acid residues were especially involved in pi interactions. Hematein is a molecular structure that has the role of preventing the proliferation of cancer cells and initiating cell apoptosis [36]. As a result of the molecular docking study of hematein, it was reported that this molecule makes hydrogen bonds with Cys-99, and also interacts closely with Leu-21, Val-152, Ile-165, and Val-29 [33]. Eugenol also interacted with these residues, making hydrogen bonds and pi-sulfur interactions with Cys-99. It was also determined that it made pi interactions with Leu-21, Val-152, Ile-165, and Val-29 residues. Genistein, which is reported to play a role in blocking NF-kB pathways [37-39], and apigenin, which targets pathways such as NF-kB [40], also interacted with similar residues with the eugenol molecule [41]. The Cys-99 residue, which is the most prominent and important indicator in all these literature studies, is observed in the foreground in this study.

3.2 MEP Analysis

Molecular electrostatic potential (MEP) is an important determinant of hydrogen bond interactions and is related to the charge distribution of the molecule. In studies such as molecular docking, the most appropriate interactions between drug candidates and target receptors begin with the existence of electrostatic potentials [42-44].

MEP analysis of eugenol was carried out in a vacuum environment using the Gaussian09 program. In the study carried out in a vacuum environment for the eugenol molecule, it was determined that the energy values of the red region, which is rich in electrons, and the blue region, which is poor in electrons, were between -0.06302 au and +0.06302 au, respectively (see Figure 3).

In the MEP map obtained for the vacuum environment, it was observed that the region where the O1 (-0.0445078 au) atom is located is the electrophilic region. When looking at the nucleophilic regions, the region with H22 bonded to the O2 atom stands out (+0.0626075 au). In both docking studies, it was observed that hydrophobic interactions were dominant, especially in regions where the electrostatic potential was close to neutral. In the study conducted with human IkB kinase beta, it was observed that the nucleophilic region played a role in the hydrogen bond interaction with the Cys-99 residue. In another molecular docking study of eugenol, it was observed that electrophilic and nucleophilic regions take part in hydrogen bond interactions, while other regions make hydrophobic interactions [45]. These types of interactions are formed as a result of the amino acid residues in the active site of the targeted receptor and eugenol having the most suitable pose in the active site (key-lock compatibility).

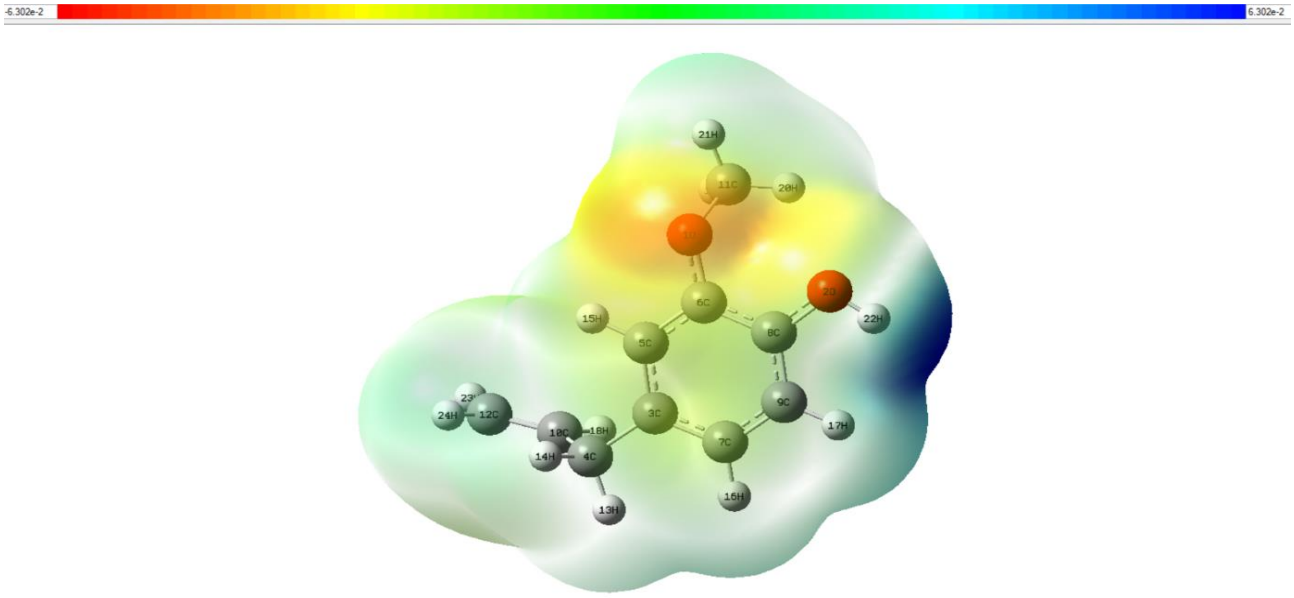

Figure 3. Molecular electrostatic potential (MEP) map of eugenol.

4. Conclusion

An attempt was made to elucidate how eugenol, which has been found to be effective in various types of cancer and is among the major compounds in the essential oils of plants such as clove and cinnamon, interacts with certain targets used in cancer studies at the molecular level. Considering that eugenol reduces COX-2 activation and inhibits NF-kB, molecular docking studies were performed. The study determined that eugenol had a similar interaction profile with potential therapeutic compounds in the literature. In addition, the electrophilic and nucleophilic regions of eugenol and the electrostatic potential values of these regions were determined by molecular electrostatic potential analysis. When molecular docking study and MEP analysis were evaluated together, it was observed that the regions where the electrostatic potential was close to neutral played a role in hydrophobic interactions. It was evaluated in this study that hydrophobic interactions may play a role in the therapeutic effect of eugenol. It is anticipated that the role of eugenol in the cancer mechanism will be elucidated in more detail in future experimental studies.

Author's Contributions

Bilge Bıçak: Drafted and wrote the manuscript, performed the all analysis.

Ethics

There are no ethical issues after the publication of this manuscript.

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