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RESEARCH ARTICLE

A Theoretical Study: DNA Binding and ADMET Profile of Some Hydroxycinnamic Acids

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ABSTRACT

Phytochemicals are important compounds produced by plants that have various beneficial effects on human health. These compounds are found in plant structures and are known to exhibit properties such as anticancer, antioxidant, and antiviral effects, making them valuable for medical studies as potential active ingredients for drugs. Phenolic compounds are compounds that have a protective effect in various disease classes such as cardiovascular diseases, cancer, and neurodegenerative diseases. Hydroxycinnamic acids are also phenolic compounds, and prominent groups of compounds include p-coumaric acid, caffeic acid, sinapic acid and ferulic acid. These compounds are known to have various therapeutic effects, from antioxidant to anticancer effects. DNA, one of the receptors used as a target in anticancer studies, is targeted by small molecules with therapeutic effect. Theoretically, an estimate of such interactions can be made at the atomic level with the molecular docking method. In addition, pharmacokinetic properties can be determined by making estimations of absorption, distribution, metabolism, excretion, and toxicity of drug candidate molecules with ADMET studies. In this study, optimized structures, chemical stability, interactions with DNA and ADMET profiles of hydroxycinnamic acids (caffeic acid, ferulic acid, p-coumaric acid, and sinapic acid) were elucidated.

Keywords: Molecular Docking; ADMET; Hydroxycinnamic acids; DNA Binding

1. INTRODUCTION

Phytochemicals are compounds that plants produce to protect themselves. Phytochemical compounds and the number of compounds in the content vary according to the plant species. Over 5000 phytochemicals have been identified to date, but many more remain unidentified (Tsao & Deng 2004). Phytochemicals are divided into five main groups as carotenoid, phenolic compounds, alkaloids, nitrogen-containing compounds, and organosulfur group. Among them, phenolic compounds are divided into five branches. These branches are tannins, phenolic acids, flavonoids, coumarins and stilbenes. Among them, phenolic acid is classified as hydroxycinnamic acid and hydroxybenzoic acid.

Hydroxycinnamic acids, which are compounds with a C6-C3 phenylpropane structure, differ according to the position and number of hydroxyl groups attached to the phenylpropane ring. The most common types of hydroxycinnamic acid found in fruits and vegetables are chlorogenic acid, caffeic acid, p-coumaric acid, ferulic acid, and sinapic acid.

P-Coumaric acid is a compound with antioxidant, anti-inflammatory, anti-cancer, anti-diabetic, and anti-melanogenic properties and the most dominant hydroxycinnamic acid in citrus fruits and pineapple (Roychoudhury et al. 2021). P-coumaric acid has a protective effect in atherosclerosis, oxidative cardiac injury, oxidative heart damage, UV-ocular tissue damage, neuron damage, anxiety, gout, and diabetes (Kianmehr et al. 2020). Caffeic acid, is the most dominant hydroxycinnamic acid in plums, apples, apricots, blueberries, and tomatoes, and has antioxidant, anti-inflammatory, anticancer, and neuroprotective effects (Alam et al. 2022). Caffeic acid has been found to be effective against various types of human cancer (Alam et al. 2022; Jung et al. 2007; Kang et al. 2011). Additionally, it is an antioxidant and anti-inflammatory agent (Alam et al. 2022; Korkina 2007). Ferulic acid has anti-inflammatory, antioxidant, antimicrobial activity, anticancer, and antidiabetic effect. Additionally, ferulic acid shows low toxicity and strong antioxidant effect (Zduńska et al. 2018). Sinapic acid has antioxidant, antimicrobial, anti-inflammatory, anticancer, and anti-anxiety properties (Nićiforović & Abramovič 2014).

DNA has two main functions as transcription and replication. These functions have an important place in the proper functioning of the cells living and multiplying, as well as other processes in the body. DNA is one of the targets of different therapeutics such as anticancer and antiviral drugs. Small molecules can act as a drug when activation or inhibition of DNA functions is required to exert a therapeutic effect (Bıçak et al. 2022). Various

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theoretical methods are used to evaluate interactions between target macromolecules and small compounds. One of them is the molecular docking method. The aim of this study is to examine the interactions of hydroxycinnamic acids with B-DNA and to elucidate their chemical stability, absorption, absorption distribution, metabolism, excretion, and toxicity profiles.

2. MATERIAL METHOD

2.1. Optimization and HOMO-LUMO Analysis

The initial structures of caffeic acid, ferulic acid, p-coumaric acid, and sinapic acid were taken from the PubChem site with the corresponding PubChem CID numbers 689043, 445858, 637542, and 637775 (Kim et al. 2023). The optimizations of the hydroxycinnamic acids were carried out by the Gaussian09 package program (Frisch et al. 2009) using the DFT method and B3LYP/6311++G(d, p) basis set. For the determination of frontier molecular orbitals required to obtain properties such as chemical stability, ionization potential, electron affinity, and chemical hardness of all molecules under a vacuum environment.

2.2. Molecular Docking Analysis

The molecular docking method, which has an important place in the discovery of molecules with the potential of being a drug, allows to reveal of the lowest energy binding score between ligand and and receptor complex structure by molecular interactions. All ligands (caffeic acid, p-coumaric acid, sinapic acid, ferulic acid) were prepared at AutoDockTools 1.5.6 software. The structure of a B-DNA Dodecamer (PDB Code: 1BNA; Drew et al. 1981) was selected as a receptor and downloaded from protein data bank[1]. All water molecules in pdb file were removed, polar hydrogen atoms were added, and grid boxes were adjusted at AutoDockTools 1.5.6 software. After all preparations were completed, the molecular docking studies were realized by AutoDock Vina 1.1.2 (Trott & Olson 2010) and obtained binding affinities and RMSD values. The receptor-ligand interactions were visualized by PyMOL (DeLano 2002) software program with the obtained interaction information.

2.3. ADMET Analysis

The ADMET analyses of the caffeic acid, p-coumaric acid, sinapic acid, and ferulic acid were carried out by the online server pkCSM (Pires et al. 2015).

[1] https://www.rcsb.org/

3. RESULT AND DISCUSSION

3.1. Structural Analysis

The hydroxycinnamic acids were optimized to obtain the values of bonds, angles, and dihedral angles with the DFT method B3LYP/6-311++ G(d, p) basis set using the Gaussian09 package program (see Tables **A1**, **A2**, **A3**, and **A4**). The energies of optimized structures were given in Table 1. Sinapic acid was determined as having the lowest energy among the hydroxycinnamic acids.

3.2. HOMO-LUMO Analysis

The highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) are related to the chemical reactivity and kinetic stability. Additionally, the HOMO is related to the ability to donate an electron and the LUMO is related to the ability to obtain an electron (Bıçak 2023; Saraç 2018).

The HOMO and LUMO energy values for p-coumaric acid in a vacuum environment were –6.40910 eV and -2.13582 eV, respectively. The HOMO and LUMO energy values for caffeic acid in a vacuum environment were –6.28610 eV and -2.1317 eV, respectively. The HOMO and LUMO energy values for ferulic acid in a vacuum environment were –6.351685 eV and -2.12439 eV, respectively. The HOMO and LUMO energy values for sinapic acid in a vacuum environment were –6.10978 eV and -2.08276 eV, respectively (see Table 2).

The large HOMO-LUMO energy gap is associated with high molecular stability. The smaller the difference (ΔE) in the energy levels, the easier the reaction will occur (Bıçak 2023; Saraç 2018). In this study, p-coumaric acid had the largest HOMO-LUMO gap, while sinapic acid had the smallest HOMO-LUMO gap. In addition, ionization potential, electron affinity, electronegativity, chemical potential, and chemical hardness information of the hydroxycinnamic acids were given in Table 2.

3.3. Molecular Docking Analysis

3.3.1. p-coumaric acid

To investigate the interaction of the p-coumaric acid molecule with B-DNA, nine different conformations of p-coumaric acid bound to B-DNA were defined. The most stable binding pose and binding affinity value among them were determined through molecular docking analysis. The best binding poses which corresponds to the best binding affinity value was calculated as −5.5 kcal mol^{-1}. The p-coumaric acid made six hydrogen bonds with B-DNA. The hydrogen bonding and close interactions between p-coumaric acid and B-DNA were given in Figure 1 and Table 3. At the close interactions, it was observed that the guanines belonging to DNA and the p-coumaric acid molecule had interacted eawith ch other. DG10 and DG16,

Table 1. The energies of the optimized structures of hydroxycinnamic acids.

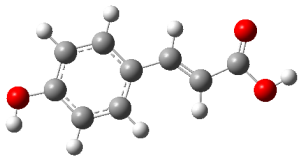
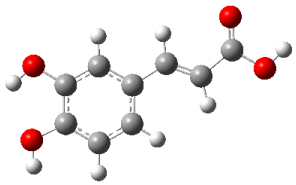
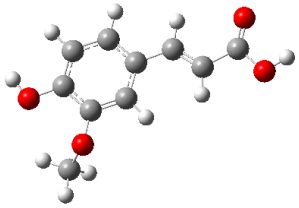
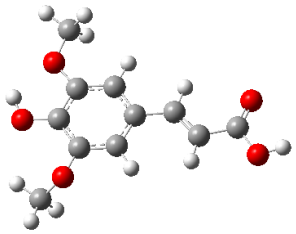
Hydroxycinnamic acids		Energy		
		a.u.	kcal mol^{-1}	eV
	**p-Coumaric acid**	-573.6201	-359952	-15609.00670
	**Caffeic acid**	-648.8678	-407171	-17656.60088
	**Ferulic acid**	-688.1695	-431833	-18726.05471
	**Sinapic acid**	-802.7282	-503720	-21843.35682

Table 2. The calculated values of ionization potential, electron affinity, electronegativity, chemical hardness, chemical softness and HOMO-LUMO gaps for hydroxycinnamic acids.

Hydroxycinnamic acids		p-Coumaric acid		Caffeic acid		Ferulic acid		Sinapic acid	
Vacuum	**TDDFT/B3LYP-6311++G(d, p)**	**(a.u.)**	**(eV)**	**(a.u.)**	**(eV)**	**(a.u.)**	**(eV)**	**(a.u.)**	**(eV)**
HOMO energy	E_{HOMO}	-0.23553	-6.40910	-0.23101	-6.28610	-0.23342	-6.35168	-0.22453	-6.10978
LUMO energy	E_{LUMO}	-0.07849	-2.13582	-0.07834	-2.13174	-0.07807	-2.12439	-0.07654	-2.08276
Ionization potential	$I = -E_{HOMO}$	0.23553	6.40910	0.23101	6.28610	0.23342	6.35168	0.22453	6.10978
Electron affinity	$A = -E_{LUMO}$	0.07849	2.13582	0.07834	2.13174	0.07807	2.12439	0.07654	2.08276
Electronegativity	$\chi = (I + A)/2$	0.15701	4.27246	0.15468	4.20906	0.15575	4.23818	0.15054	4.09640
Chemical potential	$\mu = -(I + A)/2$	-0.15701	-4.27246	-0.15468	-4.20906	-0.15575	-4.23818	-0.15054	-4.09640
Chemical hardness	$\eta = (I - A)/2$	0.07852	2.13664	0.07634	2.07732	0.07768	2.11378	0.07400	2.01364
ΔE (gap)	$E_{LUMO} - E_{HOMO}$	0.15704	4.27328	0.15267	4.15436	0.15535	4.22729	0.14799	4.02701

which have close interactions with p-coumaric acid, were determined to form hydrogen bonds with p-coumaric acid.

It was observed that especially DG10 and DG16 are involved in hydrogen bond interactions as both donors and acceptors. O2 and O3 atoms of p-coumaric acid and H1 bonded to O3 played the most active role in hydrogen bond interactions. The p-coumaric acid made very strong hydrogen bonds. Especially strong hydrogen bonds of 2.1 Å lengths of H1 and O3 atoms of coumaric acid with DG16 and DG10 have come to the fore. Information on all other hydrogen bond interactions between p-coumaric acid and B-DNA is given in Table 3.

3.3.2. *Caffeic acid*

Molecular docking analysis of DNA, which is the main target of therapeutic molecules, and caffeic acid, which has therapeutic

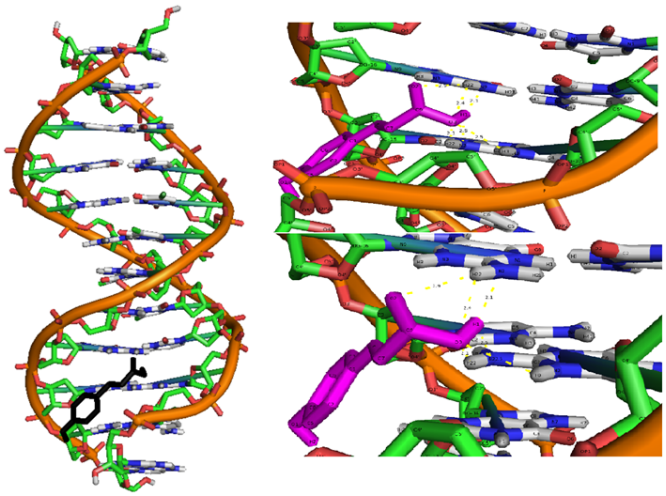


Figure 1. The close interactions of the p-coumaric acid (purple) with B-DNA.

Table 3. Hydrogen bond interactions of Coumaric acid-B-DNA complex.

Binding Affinity: -5.5 kcal mol^{-1}		
Donor Atom	**Acceptor Atom**	**Bond Length (Å)**
H1 of Coumaric acid	N2 of DG16 (Chain B)	2.1
H1 of Coumaric acid	N2 of DG10 (Chain A)	2.5
H22 of DG16 (Chain B)	O2 of Coumaric acid	2.9
H22 of DG16 (Chain B)	O3 of Coumaric acid	2.4
H22 of DG10 (Chain A)	O3 of Coumaric acid	2.1
H3 of DG10 (Chain A)	O3 of Coumaric acid	2.5

effects, was performed in this study. Molecular docking analysis identified nine different conformations of caffeic acid docked to DNA, yielding the most stable binding pose and binding affinity value. As a result of molecular docking analysis, the best binding affinity value was calculated as −6.2 kcal mol^{-1}. The hydrogen bonding and close interactions between caffeic acid and B-DNA were given in Figure 2 and Table 4. By looking at the close interactions, it was observed that the adenines, guanines, and cytosines belonging to DNA and the caffeic acid molecule interact. DC9, DG10, DG12, DG14, DC15, DG16, and DA17, which have close interactions with caffeic acid, were determined to form hydrogen bonds with caffeic acid.

Similar to literature studies (Ali et al. 2017; Sreejith, Mohan & Kurup 2017), it was determined that DA17, DG10, and DG16 of B-DNA were involved in hydrogen bond interactions as both donor and acceptor. Looking at the hydrogen bond interactions, it was seen that the H1, O1, and O4 atoms of caffeic acid played the most active role in binding to B-DNA. In docking studies, the stability of the complex structure and the strong bonding profile are directly proportional to the hydrogen bond interactions. In the hydrogen bond interactions, the strongest interaction occurred between the H1 of caffeic acid and the N2 atom of DG16, and its length was determined as 2.2 Å. Information on all other hydrogen bond interactions between caffeic acid and B-DNA is given in Table 4.

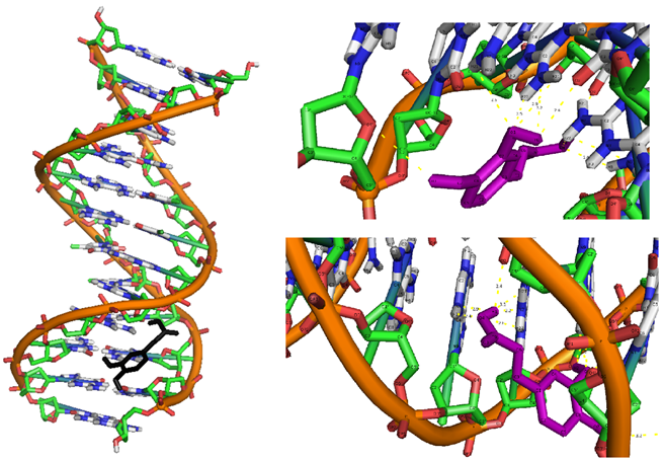


Figure 2. The close interactions of the caffeic acid (purple) with B-DNA.

Table 4. Hydrogen bond interactions of Caffeic acid with B-DNA.

Binding Affinity: -6.2 kcal mol^{-1}		
Donor Atom	**Acceptor Atom**	**Bond Length (Å)**
H3 of Caffeic acid	O4' of DG12 (Chain A)	3.2
H1 of Caffeic acid	N3 of DA17 (Chain B)	2.9
H1 of Caffeic acid	O2 of DC9 (Chain A)	2.4
H1 of Caffeic acid	N2 of DG16 (Chain B)	2.2
H2 of Caffeic acid	N2 of DG10 (Chain A)	3.2
H2 of Caffeic acid	O2 of DC15 (Chain B)	2.6
H3 of DA17 (Chain B)	O4 of Caffeic acid	2.4
H22 of DG16 (Chain B)	O4 of Caffeic acid	2.8
H21 of DG16 (Chain B)	O4 of Caffeic acid	3.1
H21 of DG14 (Chain B)	O1 of Caffeic acid	2.9
H22 of DG10 (Chain A)	O1 of Caffeic acid	2.5
H21 of DG10 (Chain A)	O1 of Caffeic acid	2.9

3.3.3. Sinapic acid

By docking study of sinapic acid and B-DNA, the most suitable binding poses and binding affinities were determined, and the best binding affinity value was calculated as −6.4 kcal mol^{-1} through the AutoDock Vina program. The sinapic acid made four hydrogen bonds with DG10, DA17, and DA18 of B-DNA. According to the literature, DG10, DA17, and DA18 are important residues in hydrogen bonding interaction with B-DNA (Ali et al. 2017; Sreejith, Mohan & Kurup 2017). The hydrogen bonding and close interactions between sinapic acid and B-DNA were given in Figure 3 and Table 5.

When we examined the resulting profile of molecular docking, we observed that especially DA17 is involved in hydrogen

bond interactions as both donors and acceptors. O15 and H28 atoms of sinapic acid formed hydrogen bonds with N3 and H3 atoms of DA17. These hydrogen bonds have 2.4 Å and 2.3 Å lengths. Looking at other hydrogen bonds, H28 and O15 of sinapic acid formed a hydrogen bond with O4' atom of DA18 and H22 atom of DG 10, respectively. These hydrogen bonds have 2.5 Å and 2.4 Å lengths. In hydrogen bond interactions, an interaction with a length of around 2 Å indicates that a strong or medium hydrogen bond interaction is formed. Sinapic acid tends to have neither very strong nor very weak hydrogen bond interactions with DNA. Information on all other hydrogen bonding interactions between sinapic acid and B-DNA are given in Table 5.

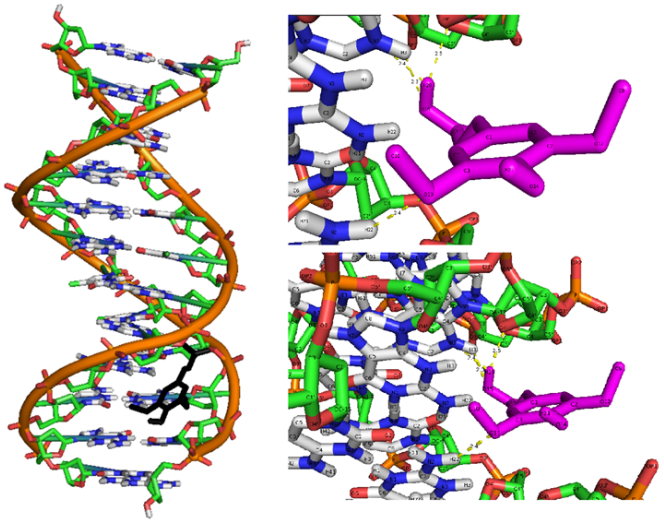


Figure 3. The close interactions of the sinapic acid (purple) with B-DNA.

Table 5. Hydrogen bond interactions of Sinapic acid with B-DNA.

Binding Affinity: -6.4 kcal mol^{-1}		
Donor Atom	**Acceptor Atom**	**Bond Length (Å)**
H28 of Sinapic acid	N3 of DA17 (Chain B)	2.4
H28 of Sinapic acid	O4' of DA18 (Chain B)	2.5
H22 of DG10 (ChainA)	O13 of Sinapic acid	2.4
H3 of DA17 (Chain B)	O15 of Sinapic acid	2.3

3.3.4. Ferulic acid

In the ferulic acid and B-DNA docking study, the best nine different binding conformations and their binding energies were determined, like other hydroxycinnamic acids. The binding energy of ferulic acid in its best binding conformation was determined as −6.4 kcal mol^{-1}. Ferulic acid made five hydrogen bonds with B-DNA, and in these interactions, DC9, DG10 in the A chain of B-DNA, and DG16 and DA17 in the B chain of B-DNA stood out. The hydrogen bonding and close interactions between ferulic acid and B-DNA were given in Figure 4 and Table 6.

In the molecular docking study, H24 atom of ferulic acid had more than one hydrogen bond. H24 atom of ferulic acid formed hydrogen bonds with O2 of DC9, N2 of DG16, and N3 of DA17. The lengths of these hydrogen bonds were 1.9 Å, 3.1 Å and 3.0 Å , respectively. Considering these bond lengths, it was determined that ferulic acid makes a strong hydrogen bond with DC9. O11 and O13 atoms of ferulic acid formed hydrogen bonds with H22 of DG10 (2.4 Å) and H3 of DA17 (2.3 Å), respectively. In a docking study conducted on Ferulic acid-B-DNA, it was reported that the best binding energy was −5.71 kcal mol^{-1} (Zhang et al. 2019). In this study, the binding energy was determined as −6.4 kcal mol^{-1}, and a detailed interaction profile was presented. Information on all other hydrogen bond interactions between ferulic acid and B-DNA were given in Table 6.

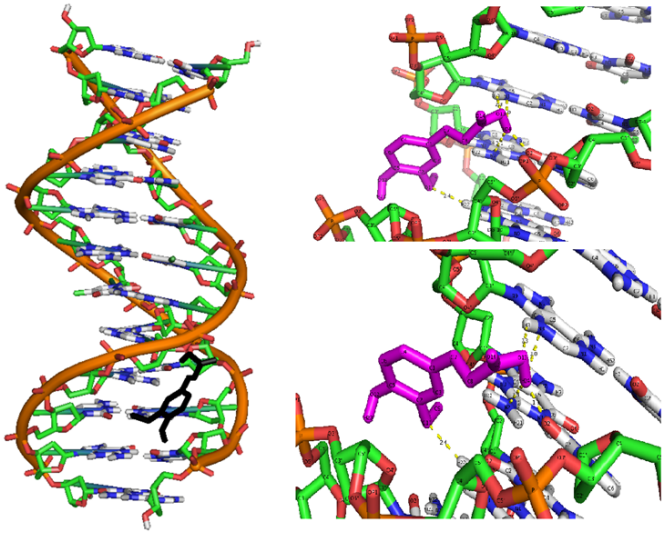


Figure 4. The close interactions of the ferulic acid (purple) with B-DNA.

Table 6. Hydrogen bond interactions of Ferulic acid with B-DNA complex.

Binding Affinity: -6.4 kcal mol^{-1}		
Donor Atom	**Acceptor Atom**	**Bond Length (Å)**
H24 of Ferulic acid	O2 of DC9 (Chain A)	1.9
H24 of Ferulic acid	N3 of DA17 (Chain B)	3.0
H24 of Ferulic acid	N2 off DG16 (Chain B)	3.1
H22 of DG10 (Chain A)	O11 of Ferulic acid	2.4
H3 of DA 17 (Chain B)	O13 of Ferulic acid	2.3

Table 7. The absorption and distribution prediction of hydroxycinnamic acids.

		p-Coumaric Acid	Caffeic Acid	Ferulic Acid	Sinapic Acid	Unit
Property	**Model Name**		**Predict**	**Value**		**Numeric/Categorical**
Absorption	**Water solubility**	-1.839	-1.737	-2.823	-2.974	**(log mol/L)**
Absorption	**Caco2 permeability**	1.144	0.123	0.249	0.057	**log Papp in 10^{-6} cm/s**
Absorption	**Intestinal absorption (human)**	91.673	65.001	94.766	94.661	**% Absorbed**
Absorption	**Skin Permeability**	-2.366	-2.625	-2.730	-2.734	**log Kp**
Absorption	**P-glycoprotein substrate**	No	Yes	Yes	Yes	**Yes/No**
Absorption	**P-glycoprotein I inhibitor**	No	No	No	No	**Yes/No**
Absorption	**P-glycoprotein II inhibitor**	No	No	No	No	**Yes/No**
Distribution	**VDss (human)**	-0.607	-0.554	-1.171	-1.068	**log L/kg**
Distribution	**Fraction unbound (human)**	0.421	0.490	0.438	0.358	**Fu**
Distribution	**BBB permeability**	-0.239	-0.824	-0.284	-0.270	**log BB**
Distribution	**CNS permeability**	-2.413	-2.649	-2.535	-2.679	**log PS**

3.4. ADMET Analysis

The absorption, distribution, metabolism, excretion, and toxicity properties of caffeic acid, p-coumaric acid, sinapic acid, and ferulic acid were determined with the help of the online server (pkCSM 2015). The water solubility is important for the absorption of drugs. The predicted water solubility value of a drug candidate is given as log mol L^{-1}. The predicted water solubility of 4 compounds was given in Table 7. The absorption of oral drugs is predicted using the Caco-2 monolayer cells as an in vitro model of the human intestinal mucosa permeability values were predicted and determined that p-coumaric acid has a high Caco-2 permeability (> 0.90). It was determined that these four compounds exhibit high intestinal absorption properties. According to the estimated skin permeability for all compounds, caffeic acid, ferulic acid, and sinapic acid had low skin permeability because log Kp was greater than −2.5 (Pires et al. 2015; pkCSM 2015).

P-glycoprotein is a protein of the cell membrane that pumps many foreign substances out of cells. P-glycoprotein substrate and inhibitors information for all compounds were given in Table 7. The steady-state volume of distribution was predicted for all compounds, and it was determined that all compounds were more distributed in plasma rather than tissue. Drugs are in equilibrium at the point of bound-unbound to serum proteins. The fraction unbound estimate returns the fraction that is predicted to be unbound in the plasma. The predictions of fraction unbound for all compounds were given in Table 7. When looking at the BBB and CNS permeability of the compounds, they were predicted to have weak profiles.

The compounds were determined not to be CYP P450 inhibitors based on the predictions in the metabolism section of the ADMET analysis. The predictions of Organic Cation Transporter 2 substrate were realized, and it was determined that the compounds had no renal OCT2 substrate. Total clearance values as a combination of hepatic and renal clearance were given in Table 8. When the toxicity estimates of the compounds were examined, it was determined that they are not expected to lead to serious conditions such as skin sensitivity and hepatotoxicity. Based on Ames toxicity estimates, none of the compounds were mutagenic.

4. CONCLUSION

The study examined four hydroxycinnamic acids, and it was found that ferulic acid and sinapic acid had the lowest energy levels. The fact that ferulic acid and sinapic acid have better binding energy than the others was associated with the fact that they have more side groups for interactions. When the structure of the four compounds was examined, it was observed that the increase in binding energy was directly proportional to the increase in the groups attached to the ring. In the study of ferulic acid and sinapic acid, which have more side groups, although their binding energies were the same, their interaction sites and lengths in the DNA were different from each other. This situation was associated with the binding conformation.

In the ADMET study, it was determined that the intestinal absorption percentages of ferulic acid and sinapic acid were quite high, with values of 94.7% and 94.6%, respectively. These molecules were followed by the intestinal absorption percentages of p-coumaric acid and caffeic acid, respectively. When the toxic effects were examined with the help of pkCSM Webservers Predictor, it was determined that hydroxycinnamic acids did not have any toxic effects according to skin sensitization, hepatotoxicity, and AMES toxicity predictions.

Table 8. The metabolism, excretion, and toxicity prediction of hydroxycinnamic acids.

		p-Coumaric Acid	Cafeic Acid	Ferulic Acid	Sinapic Acid	Unit
Property	**Model Name**		**Predict**	**Value**		**Numeric/Categorical**
Metabolism	**CYP2D6 substrate**	No	No	No	No	**Yes/No**
Metabolism	**CYP3A4 substrate**	No	No	No	No	**Yes/No**
Metabolism	**CYP1A2 inhibitor**	No	No	No	No	**Yes/No**
Metabolism	**CYP2C19 inhibitor**	No	No	No	No	**Yes/No**
Metabolism	**CYP2C9 inhibitor**	No	No	No	No	**Yes/No**
Metabolism	**CYP2D6 inhibitor**	No	No	No	No	**Yes/No**
Metabolism	**CYP3A4 inhibitor**	No	No	No	No	**Yes/No**
Excretion	**Total Clearance**	0.696	0.544	0.619	0.760	**log ml/min/kg**
Excretion	**Renal OCT2 substrate**	No	No	No	No	**Yes/No**
Toxicity	**AMES Toxicity**	No	No	No	No	**Yes/No**
Toxicity	**Max. tolerated dose (human)**	0.758	-0.094	1.488	1.251	**log mg/kg/day**
Toxicity	**hERG I inhibitor**	No	No	No	No	**Yes/No**
Toxicity	**hERG II inhibitor**	No	No	No	No	**Yes/No**
Toxicity	**Oral Rat Acute Toxicity (LD50)**	2.070	2.281	2.491	2.411	**mol/kg**
Toxicity	**Oral Rat Chronic Toxicity (LOAEL)**	2.841	1.730	1.817	2.233	**log mg/kg_bw/day**
Toxicity	**Hepatotoxicity**	No	No	No	No	**Yes/No**
Toxicity	**Skin Sensitization**	No	No	No	No	**Yes/No**
Toxicity	***T.Pyriformis* toxicity**	0.211	0.018	0.271	0.280	**log ug/L**
Toxicity	**Minnow toxicity**	1.815	2.072	2.074	1.731	**log m**

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Table A1. The optimized parameters of p-Coumaric acid.

	Value		Value		Value
R(1, 2)	1.3948	A(2, 1, 3)	120.0022	D(3, 1, 2, 4)	0.0138
R(1, 3)	1.3949	A(2, 1, 7)	120.0110	D(3, 1, 2, 13)	-178.7482
R(1, 7)	1.4544	A(3, 1, 7)	119.9868	D(7, 1, 2, 4)	-179.9659
R(2, 4)	1.3949	A(1, 2, 4)	119.9949	D(7, 1, 2, 13)	1.2721
R(2, 13)	1.0861	A(1, 2, 13)	122.5790	D(2, 1, 3, 5)	-0.0050
R(3, 5)	1.3949	A(4, 2, 13)	117.4151	D(2, 1, 3, 14)	-179.9182
R(3, 14)	1.0874	A(1, 3, 5)	119.9978	D(7, 1, 3, 5)	179.9747
R(4, 6)	1.3948	A(1, 3, 14)	121.0590	D(7, 1, 3, 14)	0.0615
R(4, 15)	1.0868	A(5, 3, 14)	118.9431	D(2, 1, 7, 8)	20.0056
R(5, 6)	1.3948	A(2, 4, 6)	120.0055	D(2, 1, 7, 17)	-160.7266
R(5, 16)	1.0865	A(2, 4, 15)	119.3118	D(3, 1, 7, 8)	-159.9741
R(6, 10)	1.3608	A(6, 4, 15)	120.6821	D(3, 1, 7, 17)	19.2936
R(7, 8)	1.3516	A(3, 5, 6)	120.0008	D(1, 2, 4, 6)	-0.0133
R(7, 17)	1.0881	A(3, 5, 16)	119.6696	D(1, 2, 4, 15)	-179.7477
R(8, 9)	1.4767	A(6, 5, 16)	120.3296	D(13, 2, 4, 6)	178.8116
R(8, 18)	1.0841	A(4, 6, 5)	119.9988	D(13, 2, 4, 15)	-0.9229
R(9, 11)	1.3535	A(4, 6, 10)	119.9935	D(1, 3, 5, 6)	-0.0044
R(9, 12)	1.2194	A(5, 6, 10)	120.0077	D(1, 3, 5, 16)	-179.9777
R(10, 19)	0.9727	A(1, 7, 8)	125.3127	D(14, 3, 5, 6)	179.9107
R(11, 20)	0.9811	A(1, 7, 17)	115.4297	D(14, 3, 5, 16)	-0.0627
		A(8, 7, 17)	119.2537	D(2, 4, 6, 5)	0.0039
		A(7, 8, 9)	120.0806	D(2, 4, 6, 10)	179.9933
		A(7, 8, 18)	122.9350	D(15, 4, 6, 5)	179.7346
		A(9, 8, 18)	116.9719	D(15, 4, 6, 10)	-0.2760
		A(8, 9, 11)	108.6985	D(3, 5, 6, 4)	0.0050
		A(8, 9, 12)	126.4575	D(3, 5, 6, 10)	-179.9844
		A(11, 9, 12)	124.8440	D(16, 5, 6, 4)	179.9781
		A(6, 10, 19)	108.9074	D(16, 5, 6, 10)	-0.0113
		A(9, 11, 20)	111.9898	D(4, 6, 10, 19)	-0.0169
				D(5, 6, 10, 19)	179.9725
				D(1, 7, 8, 9)	179.5317
				D(1, 7, 8, 18)	0.8608
				D(17, 7, 8, 9)	0.2897
				D(17, 7, 8, 18)	-178.3812
				D(7, 8, 9, 11)	-179.4305
				D(7, 8, 9, 12)	0.6322
				D(18, 8, 9, 11)	-0.6822
				D(18, 8, 9, 12)	179.3806
				D(8, 9, 11, 20)	-179.9700
				D(12, 9, 11, 20)	-0.0315

Table A2. The optimized parameters of Caffeic acid.

	Value		Value		Value
R(1, 2)	1.3949	A(2, 1, 3)	120.000	D(3, 1, 2, 4)	-0.0092
R(1, 3)	1.3949	A(2, 1, 7)	119.999	D(3, 1, 2, 14)	179.9170
R(1, 7)	1.4543	A(3, 1, 7)	120.001	D(7, 1, 2, 4)	179.9519
R(2, 4)	1.3948	A(1, 2, 4)	120.001	D(7, 1, 2, 14)	-0.1219
R(2, 14)	1.0880	A(1, 2, 14)	120.673	D(2, 1, 3, 6)	-0.0044
R(3, 6)	1.3949	A(4, 2, 14)	119.326	D(2, 1, 3, 15)	178.3207
R(3, 15)	1.0858	A(1, 3, 6)	119.993	D(7, 1, 3, 6)	-179.9660
R(4, 5)	1.3948	A(1, 3, 15)	122.998	D(7, 1, 3, 15)	-1.6404
R(4, 10)	1.3622	A(6, 3, 15)	116.989	D(2, 1, 7, 8)	160.0399
R(5, 6)	1.3949	A(2, 4, 5)	120.001	D(2, 1, 7, 17)	-19.2341
R(5, 11)	1.3621	A(2, 4, 10)	119.494	D(3, 1, 7, 8)	-19.9990
R(6, 16)	1.0869	A(5, 4, 10)	120.505	D(3, 1, 7, 17)	160.7270
R(7, 8)	1.3298	A(4, 5, 6)	120.001	D(1, 2, 4, 5)	0.0230
R(7, 17)	1.0870	A(4, 5, 11)	120.532	D(1, 2, 4, 10)	179.9211
R(8, 9)	1.4776	A(6, 5, 11)	119.467	D(14, 2, 4, 5)	-179.9040
R(8, 18)	1.0831	A(3, 6, 5)	120.004	D(14, 2, 4, 10)	-0.0061
R(9, 12)	1.3536	A(3, 6, 16)	119.279	D(1, 3, 6, 5)	0.0041
R(9, 13)	1.2194	A(5, 6, 16)	120.716	D(1, 3, 6, 16)	179.6570
R(10, 19)	0.9730	A(1, 7, 8)	122.838	D(15, 3, 6, 5)	-178.4190
R(11, 20)	0.9727	A(1, 7, 17)	116.184	D(15, 3, 6, 16)	1.2334
R(12, 21)	0.9811	A(8, 7, 17)	120.974	D(2, 4, 5, 6)	-0.0233
		A(7, 8, 9)	118.549	D(2, 4, 5, 11)	179.9629
		A(7, 8, 18)	124.626	D(10, 4, 5, 6)	-179.9200
		A(9, 8, 18)	116.797	D(10, 4, 5, 11)	0.0658
		A(8, 9, 12)	108.688	D(2, 4, 10, 19)	-179.9570
		A(8, 9, 13)	126.492	D(5, 4, 10, 19)	-0.0597
		A(12, 9, 13)	124.819	D(4, 5, 6, 3)	0.0097
		A(4, 10, 19)	108.332	D(4, 5, 6, 16)	-179.6380
		A(5, 11, 20)	109.015	D(11, 5, 6, 3)	-179.9770
		A(9, 12, 21)	111.987	D(11, 5, 6, 16)	0.3757
				D(4, 5, 11, 20)	-179.9790
				D(6, 5, 11, 20)	0.0075
				D(1, 7, 8, 9)	-179.2020
				D(1, 7, 8, 18)	-1.2206
				D(17, 7, 8, 9)	0.0379
				D(17, 7, 8, 18)	178.0194
				D(7, 8, 9, 12)	179.2113
				D(7, 8, 9, 13)	-0.7861
				D(18, 8, 9, 12)	1.0721
				D(18, 8, 9, 13)	-178.9250
				D(8, 9, 12, 21)	-179.9950
				D(13, 9, 12, 21)	0.0024

Table A3. The optimized parameters of Ferulic acid.

	Value		Value		Value
R(1, 3)	1.3950	A(3, 1, 4)	120.0001	D(4, 1, 3, 2)	-0.0123
R(1, 4)	1.3949	A(3, 1, 7)	120.0122	D(4, 1, 3, 15)	-178.7161
R(1, 7)	1.4543	A(4, 1, 7)	119.9877	D(7, 1, 3, 2)	-179.9620
R(2, 3)	1.3949	A(3, 2, 5)	119.9983	D(7, 1, 3, 15)	1.3342
R(2, 5)	1.3949	A(3, 2, 11)	119.4636	D(3, 1, 4, 6)	0.0076
R(2, 11)	1.3623	A(5, 2, 11)	120.5381	D(3, 1, 4, 16)	-179.8972
R(3, 15)	1.0866	A(1, 3, 2)	119.9925	D(7, 1, 4, 6)	179.9573
R(4, 6)	1.3948	A(1, 3, 15)	122.1636	D(7, 1, 4, 16)	0.0525
R(4, 16)	1.0875	A(2, 3, 15)	117.8318	D(3, 1, 7, 8)	19.9509
R(5, 6)	1.3949	A(1, 4, 6)	120.0083	D(3, 1, 7, 18)	-160.7625
R(5, 12)	1.3623	A(1, 4, 16)	121.0485	D(4, 1, 7, 8)	-159.9988
R(6, 17)	1.0867	A(6, 4, 16)	118.9431	D(4, 1, 7, 18)	19.2878
R(7, 8)	1.3516	A(2, 5, 6)	120.0113	D(5, 2, 3, 1)	0.0115
R(7, 18)	1.0881	A(2, 5, 12)	120.5463	D(5, 2, 3, 15)	178.7706
R(8, 10)	1.4765	A(6, 5, 12)	119.4424	D(11, 2, 3, 1)	-179.9685
R(8, 19)	1.0841	A(4, 6, 5)	119.9896	D(11, 2, 3, 15)	-1.2093
R(9, 11)	1.4262	A(4, 6, 17)	119.2501	D(3, 2, 5, 6)	-0.0059
R(9, 20)	1.0946	A(5, 6, 17)	120.7603	D(3, 2, 5, 12)	-179.9288
R(9, 21)	1.0946	A(1, 7, 8)	125.3211	D(11, 2, 5, 6)	179.9738
R(9, 22)	1.0934	A(1, 7, 18)	115.4272	D(11, 2, 5, 12)	0.0509
R(10, 13)	1.3536	A(8, 7, 18)	119.2480	D(3, 2, 11, 9)	-90.0288
R(10, 14)	1.2195	A(7, 8, 10)	120.0888	D(5, 2, 11, 9)	89.9913
R(12, 23)	0.9726	A(7, 8, 19)	122.9422	D(1, 4, 6, 5)	-0.0020
R(13, 24)	0.9810	A(10, 8, 19)	116.9558	D(1, 4, 6, 17)	-179.9938
		A(11, 9, 20)	110.4884	D(16, 4, 6, 5)	179.9048
		A(11, 9, 21)	110.6345	D(16, 4, 6, 17)	-0.0870
		A(11, 9, 22)	108.2689	D(2, 5, 6, 4)	0.0012
		A(20, 9, 21)	110.0868	D(2, 5, 6, 17)	179.9928
		A(20, 9, 22)	108.6520	D(12, 5, 6, 4)	179.9250
		A(21, 9, 22)	108.6468	D(12, 5, 6, 17)	-0.0834
		A(8, 10, 13)	108.7071	D(2, 5, 12, 23)	-179.9637
		A(8, 10, 14)	126.4598	D(6, 5, 12, 23)	0.1130
		A(13, 10, 14)	124.8331	D(1, 7, 8, 10)	179.5313
		A(2, 11, 9)	117.0371	D(1, 7, 8, 19)	0.8986
		A(5, 12, 23)	109.0069	D(18, 7, 8, 10)	0.2697
		A(10, 13, 24)	111.9924	D(18, 7, 8, 19)	-178.3630
				D(7, 8, 10, 13)	-179.4288
				D(7, 8, 10, 14)	0.6317
				D(19, 8, 10, 13)	-0.7161
				D(19, 8, 10, 14)	179.3444
				D(20, 9, 11, 2)	61.5627
				D(21, 9, 11, 2)	-60.6100
				D(22, 9, 11, 2)	-179.5631
				D(8, 10, 13, 24)	-179.9668
				D(14, 10, 13, 24)	-0.0260

Table A4. The optimized parameters of Sinapic acid.

	Value		Value		Value
R(1, 5)	1.3948	A(5, 1, 6)	119.9937	D(6, 1, 5, 2)	0.0003
R(1, 6)	1.3948	A(5, 1, 7)	120.0033	D(6, 1, 5, 17)	179.8788
R(1, 7)	1.4545	A(6, 1, 7)	120.0029	D(7, 1, 5, 2)	179.9946
R(2, 4)	1.3949	A(4, 2, 5)	119.9939	D(7, 1, 5, 17)	-0.1269
R(2, 5)	1.3949	A(4, 2, 12)	120.5790	D(5, 1, 6, 3)	-0.0004
R(2, 12)	1.3623	A(5, 2, 12)	119.4271	D(5, 1, 6, 18)	178.8619
R(3, 4)	1.3948	A(4, 3, 6)	120.0036	D(7, 1, 6, 3)	-179.9946
R(3, 6)	1.3948	A(4, 3, 13)	120.5715	D(7, 1, 6, 18)	-1.1324
R(3, 13)	1.3624	A(6, 3, 13)	119.4249	D(5, 1, 7, 8)	160.0005
R(4, 14)	1.3635	A(2, 4, 3)	119.9978	D(5, 1, 7, 19)	-20.5850
R(5, 17)	1.0854	A(2, 4, 14)	119.9973	D(6, 1, 7, 8)	-20.0052
R(6, 18)	1.0867	A(3, 4, 14)	120.0050	D(6, 1, 7, 19)	159.4092
R(7, 8)	1.3516	A(1, 5, 2)	120.0075	D(5, 2, 4, 3)	-0.0186
R(7, 19)	1.0883	A(1, 5, 17)	117.0108	D(5, 2, 4, 14)	179.9913
R(8, 11)	1.4766	A(2, 5, 17)	122.9816	D(12, 2, 4, 3)	-179.9688
R(8, 20)	1.0841	A(1, 6, 3)	120.0035	D(12, 2, 4, 14)	0.0411
R(9, 12)	1.4262	A(1, 6, 18)	122.0969	D(4, 2, 5, 1)	0.0092
R(9, 22)	1.0953	A(3, 6, 18)	117.8902	D(4, 2, 5, 17)	-179.8618
R(9, 23)	1.0954	A(1, 7, 8)	125.3163	D(12, 2, 5, 1)	179.9599
R(9, 24)	1.0951	A(1, 7, 19)	115.7052	D(12, 2, 5, 17)	0.0890
R(10, 13)	1.4261	A(8, 7, 19)	118.9759	D(4, 2, 12, 9)	179.9979
R(10, 25)	1.0946	A(7, 8, 11)	120.0815	D(5, 2, 12, 9)	0.0474
R(10, 26)	1.0946	A(7, 8, 20)	123.0911	D(6, 3, 4, 2)	0.0185
R(10, 27)	1.0935	A(11, 8, 20)	116.7585	D(6, 3, 4, 14)	-179.9913
R(11, 15)	1.3536	A(12, 9, 22)	111.6668	D(13, 3, 4, 2)	-179.9790
R(11, 16)	1.2192	A(12, 9, 23)	111.6539	D(13, 3, 4, 14)	0.0111
R(14, 21)	0.9730	A(12, 9, 24)	107.0681	D(4, 3, 6, 1)	-0.0091
R(15, 28)	0.9811	A(22, 9, 23)	112.471	D(4, 3, 6, 18)	-178.9186
		A(22, 9, 24)	106.7918	D(13, 3, 6, 1)	179.9885
		A(23, 9, 24)	106.7903	D(13, 3, 6, 18)	1.0790
		A(13, 10, 25)	110.4906	D(4, 3, 13, 10)	90.0047
		A(13, 10, 26)	110.6405	D(6, 3, 13, 10)	-89.9929
		A(13, 10, 27)	108.2629	D(2, 4, 14, 21)	0.0531
		A(25, 10, 26)	110.0823	D(3, 4, 14, 21)	-179.9371
		A(25, 10, 27)	108.6523	D(1, 7, 8, 11)	179.5311
		A(26, 10, 27)	108.6486	D(1, 7, 8, 20)	-3.5866
		A(8, 11, 15)	108.6941	D(19, 7, 8, 11)	0.1342
		A(8, 11, 16)	126.4593	D(19, 7, 8, 20)	177.0165
		A(15, 11, 16)	124.8465	D(7, 8, 11, 15)	150.5662
		A(2, 12, 9)	117.0263	D(7, 8, 11, 16)	-29.3597
		A(3, 13, 10)	117.0277	D(20, 8, 11, 15)	-26.5087
		A(4, 14, 21)	108.3761	D(20, 8, 11, 16)	153.5655
		A(11, 15, 28)	111.9876	D(22, 9, 12, 2)	63.3908
				D(23, 9, 12, 2)	-63.4953
				D(24, 9, 12, 2)	179.9521
				D(25, 10, 13, 3)	61.6522
				D(26, 10, 13, 3)	-60.5204
				D(27, 10, 13, 3)	-179.4756
				D(8, 11, 15, 28)	-179.9644
				D(16, 11, 15, 28)	-0.0371