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In silico evaluation of caffeic acid from coconut (*Cocos nucifera* L.) husks as a potential inhibitor of the human factor Xa

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Abstract
Selectively inhibiting the FXa has a broad therapeutic window as an anticoagulant target because of its starting position of the common pathway of the coagulation cascade which effectively blocks the coagulation. This study investigated the inhibiting capabilities of caffeic acid present in *C. nucifera* L. husks on the FXa and explored its ADMET profile using bioinformatic predicting tools. The caffeic acid and FXa structure was retrieved from PubChem database and RCSB Protein Data Bank, respectively. Binding geometries were illustrated with the use of AutoDock MGL Tools, AutoDock Vina, PyMol, and the ADMET profile was predicted with ADMETlab 2.0. Results of the *in silico* methods showed that caffeic acid interacted with residues within the active center of FXa, blocking the access of its native substrates, and demonstrated acceptable pharmacokinetics and drug-like effects, thus it can be recommended for the drug design and development of FXa inhibitors.

Keywords: Caffeic acid, coagulation factor Xa, coconut, coconut husks, anticoagulant, coagulation

1. Introduction
The coagulation cascade is a complex process where clotting factors connect through intrinsic, extrinsic, and common pathways to produce the enzyme responsible for clot formation, thrombin (Chaudhry *et al*., 2020) [3]. FXa is located at the coagulation cascade's common pathway, where the extrinsic and intrinsic pathways converge, and is considered as the site of amplification as it catalyzes the synthesis of 1000 thrombin molecules. Thus, selectively inhibiting FXa would effectively block coagulation (Ibrahim *et al*., 2020) [6]. Researchers have been searching for selective FXa inhibitors from natural products for its wide therapeutic range. Coconuts are rich in polyphenolic compounds that holds various medical effects (Turpie, 2007) [21]. However, the anticoagulant activity of caffeic acid present in coconut husks has not been studied yet. With this, the study aims to investigate the inhibiting capabilities of caffeic acid present in coconut (*C. nucifera* L.) husks on the FXa using a molecular docking tool and explore its ADMET profile and drug-likeness using a pharmacokinetic prediction tool.

2. Materials and Methods
2.1 Materials
Receptor (Coagulation Factor Xa Inhibitor Complex) 3D structure was obtained from RCSB PDB (https://www.rcsb.org/), which is a public database for the 3D structural data of large biological molecules. The chemical compound structure of caffeic acid and FXa inhibitor (Rivaroxaban) were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov), which is a public database of chemical molecules. The software that was used are AutoDock MGL Tools (http://mgltools.scripps.edu/) for the visualization and analysis of the molecular structure, AutoDock Vina (http://vina.scripps.edu/index.html) for molecular docking, and PyMol visualization (https://pymol.org/2/) and Discovery Studio (DS) Visualizer (https://discover.3ds.com/discovery-studio-visualizer-download) for rendering and animating the 3D structures. The software used for ADMET evaluation is the ADMETlab 2.0 (https://admetmesh.scbdd.com/) — a software used for rapid ADMET prediction of drug candidates.

2.2 Protein Preparation
The structure of the Coagulation Factor Xa Inhibitor Complex was retrieved in PDB format from RCSB Protein Data Bank (PDB: 2J4I). The structure was chosen owing to their fulfilment of the following three criteria: first, the source organism is Homo sapiens; where a non-mutated structure was selected. Second, X-ray resolution is better than 2Å. Lastly, the electron density map's availability for the structure was checked at Protein Data Bank in Europe (https://pdbe.org/). The goodness-of-fit between the map and the structure of both ligand and enzyme was inspected. AutoDock MGL Tools was then used to refine the structure by removing the water molecules and optimizing the hydrogen bonds of the protein.

2.3 Ligands Preparation
The caffeic acid and Rivaroxaban structures were downloaded from PubChem. The structures were downloaded in SDF file format and were converted to PDBQT format using the PyMol software. After converting the file, hydrogen bonds and Gasteiger charges were added using AutoDock MGL Tools.

2.4 Molecular Docking
Coagulation Factor Xa Inhibitor Complex acted as the protein and caffeic acid and Rivaroxaban acted as the ligand for the molecular docking. AutoDock MGL Tools was used to refine and optimize the structures. AutoDock Vina was used for the molecular docking which was performed with the settings: center x = 10.283; center y = 3.028; center z = 23.295; size x = 44; size y = 44; size z = 52; energy range = 4; and exhaustiveness = 8 (Bijak *et al*., 2014) [1]. The binding sites were computed and the ligand's binding affinity to the receptor was counted in kcal/mol. PyMol visualization and DS Visualizer was used for the visualization and analysis of the 3D conformer of the protein with the bound ligand.

2.5 ADMET Evaluation
The canonical simplified molecular input line entry system (SMILES) of caffeic acid was obtained from PubChem and was uploaded to ADMETlab 2.0 for ADMET screening using the default parameters. The drug-likeness character, and pharmacokinetic profile of caffeic acid was predicted by ADMETlab 2.0.

3. Results
In the present study, the inhibiting capabilities of caffeic acid present in coconut (*C. nucifera* L.) husks on the FXa were explored using a bioinformatic molecular docking method wherein the FXa (PDB: 2J4I) was used as the receptor protein. The resulting binding geometries of caffeic acid and FXa were visualized, and the stability of the interaction of the docked complex were evaluated on the basis of its binding affinity. The pharmacokinetic profile and drug-likeness of caffeic acid were also explored through the absorption, distribution, metabolism, excretion, and toxicity prediction of the compound.

3.1 Molecular docking of caffeic acid on FXa
Molecular docking is an algorithm wherein the structures of targets and ligands are examined to predict the potential binding geometries and binding energy of the ligand-target complex (Kumar & Kumar, 2019) [8]. Predicting the binding of a ligand to a pharmacological target is critical in the discovery of new medicines as their binding interaction provides the molecular basis for pharmaceutical activity and is thus used as an indicator of drug potency (Pantsar & Poso, 2018; Wan *et al*., 2020) [11, 17-18]. Thus, the binding geometries of caffeic acid and FXa were explored using molecular docking studies.

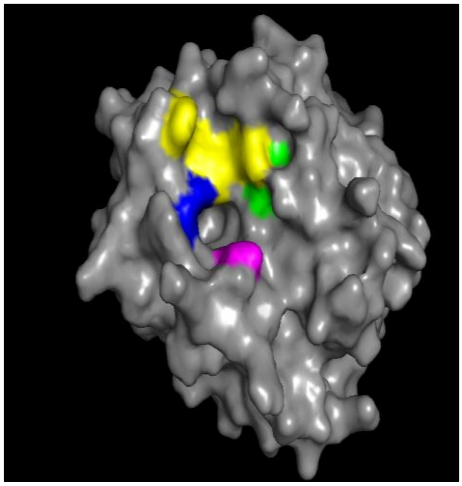

Fig 1: Illustration of the binding site of the FXa molecule (PDB: 2J4I) by authors. The binding site of the FXa has four pockets designated as the S1 pocket (green), S2 pocket (blue), S3 pocket (pink), and S4 pocket (yellow) of the FXa.

The molecular docking revealed that caffeic acid was able to form a stable complex with FXa which were stabilized by various hydrophilic and hydrophobic bonding interactions. Caffeic acid was found to have directly formed hydrogen bonds with the catalytic amino acid residues of the binding pockets of FXa. The binding site of FXa has four pockets designated as S1, S2, S3, and S4 (Figure 1) The S1 and S4 pockets are the two pockets that mainly participate in the catalytic activity of FXa. S1, a negatively charged groove, is considered as the most important binding pocket and is regarded as one of the active centers as it greatly influences the binding energy of the protein-ligand complex. The bottom of the S1 pocket is lined by Asp189, which directly participates in the catalytic mechanism of FXa. The native substrate of FXa, prothrombin, forms an ionic hydrogen bond with Asp189 in the S1 pocket (Patel *et al*., 2016). The S2 pocket is a shallow and small depression that merges with the other pockets. While the limits of the S2 pocket are unclear and ill-defined, some authors consider S2 to be structurally formed by Gly216 and Gly218, while others have stated that the S2 pocket is lined with Tyr99. The S3 is a flat pocket at the edge of S1 and is thus similarly ill-defined. Nonetheless, the S3 is a molecular recognition site made up of Gln192 capable of forming hydrogen bonds with ligands (Patel *et al*., 2016; Sulimov *et al*., 2015) [13]. The S4 pocket, on the other hand, is primarily formed by Tyr99, Phe174, and Trp215. These residues have hydrophobic character and thus form hydrophobic and stacking interactions with ligands (Bijak *et al*., 2014; Sulimov *et al*., 2015) [1, 13].

The results show that caffeic acid was bound to residues within the S1 and S4 pockets of FXa (Figure 2). The catalytic Asp189 and Gly216 residues of the S1 pocket formed a hydrogen bond with the hydroxyl group attached to C3 and C4 of caffeic acid, respectively. The complex was further stabilized by hydrophobic interactions between the phenyl ring of the caffeic acid and the residues Cys191 and Ala190 of the S1 pocket, and Trp215 located in the S4 pocket of FXa, as well as eleven van der Waals interactions with His57,

Tyr99, Gln192, Ser195, Gly216, Val213, Ser214, Ala221, Cys220, Gly226, and Ile227. Observing the docking results of caffeic acid on FXa showed that hydrogen bonding with Gly219 and hydrophobic interactions with Cys191, Trp215 and Ala190 were important for the stability of the protein-ligand complex. Interestingly, similar docking interactions on FXa residues Gly219, Cys191, Trp215, and Ala190 were previously observed by Ibrahim *et al*. (2020) [6], while interactions with Gly219 and Trp215 were consistently observed by Bijak *et al*. (2014) [1]. Furthermore, docking results (Table 1) also show that caffeic acid exhibited roughly comparable binding affinity and interacted with similar residues that are prevalent in the interaction of FXa and Rivaroxaban, the first FDA-approved, orally-dosed direct FXa inhibitor and is the reference compound for anticoagulant activity against FXa (Ibrahim *et al*., 2020; Singh *et al*., 2021) [6, 12]. In the molecular docking of Rivaroxaban on FXa (Figure 3), two hydrogen bonds with Gln61 and Tyr99, five hydrophobic interactions with Ala190, Cys191, Cys220, and Trp215, a Pi-Sulfur interaction with Cys42, a salt bridge with Asp189, a carbon hydrogen bond with Gln192, and ten van der Waals interactions stabilized the protein-ligand complex. The same residues from the pockets of FXa were observed to interact with caffeic acid in various poses, as previously discussed. These observations suggest that caffeic acid is able to enter the vicinity of the FXa binding pocket and interact with its amino acid residues, comparatively with Rivaroxaban. This may be due to caffeic acid having a weak steric hindrance effect due to its small size and corresponding lack of substituents, therefore allowing the molecule to have an excellent opportunity to enter the pocket of the FXa and interact with its protein residues (Wang *et al*., 2020) [17-18]. Thus, the interaction of the ligand with residues that are located within the S1 and S4 pockets, and catalytic residue in the S1 pocket of the FXa indicate that caffeic acid has putative capacity to block the access of the native substrate of FXa to its active center (Bijak *et al*., 2014) [1].

Table 1: Results of the molecular docking studies of caffeic acid and Rivaroxaban on FXa using Autodock Vina

Ligand	Binding Affinity (kcal/mol)	Hydrogen-binding interactions	Hydrophobic interactions
Caffeic acid	-6.6	Asp189, Gly219	Ala190, Cys191, Trp215
Rivaroxaban	-8.7	Gln61, Tyr99	Cys42, Ala190, Cys191, Trp215, Cys220

Our findings are in conformity with the previous findings from the literature. It was previously reported that certain pharmacophoric features are necessary for FXa inhibitory effect. These features include two hydrogen bond donors, and an aromatic or a hydrophobic ring (Ibrahim *et al*., 2020) [6]. These features align with the features of caffeic acid, which is a hydroxycinnamic acid with hydroxyl groups in the 3 and 4 positions attached to its phenyl ring. In addition, other studies have demonstrated that derivatives of caffeic acid have exhibited particular anti- and pro-coagulant activities. The findings of the molecular docking demonstrate that caffeic acid is capable of inhibiting the activity of FXa by blocking the access of its native substrate to its active center with favourable affinity. This inhibitory capability is due to caffeic acid's weak steric hindrance and small size, which allows it to enter the vicinity of the FXa binding pocket, and due to the presence of structural features in caffeic acid, such as hydrogen bond donors, aromatic ring, phenolic hydroxyl groups, and carbonyl group, that aid in FXa inhibitory effect. These findings are consistent with the results of previous studies done by Wang *et al*. in 2020 [17-18], Bijak *et al*. in 2014 [1], and Ibrahim *et al*. in 2020 [6]. Hence, caffeic acid exhibits putative anticoagulant activity and can be used as a structural basis in developing selective FXa inhibitors.

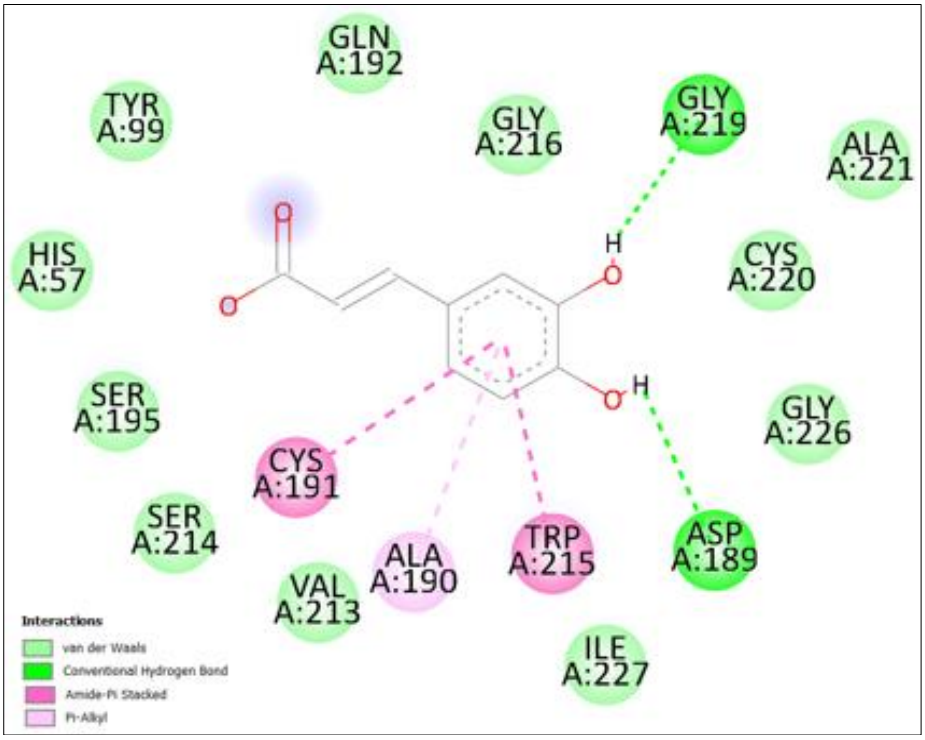

Fig 2: Captured image of the molecular docking of caffeic acid to the binding site of FXa (PDB: 2J4I) using Auto Dock Vina. Caffeic acid bonded via H-bond (dashed green lines) to the Gly219 and Asp189 of the S1 pocket, and via hydrophobic interactions (dashed pink lines) to the Cys191 and Ala190 of the S1 pocket, and Trp215 of the S4 pocket.

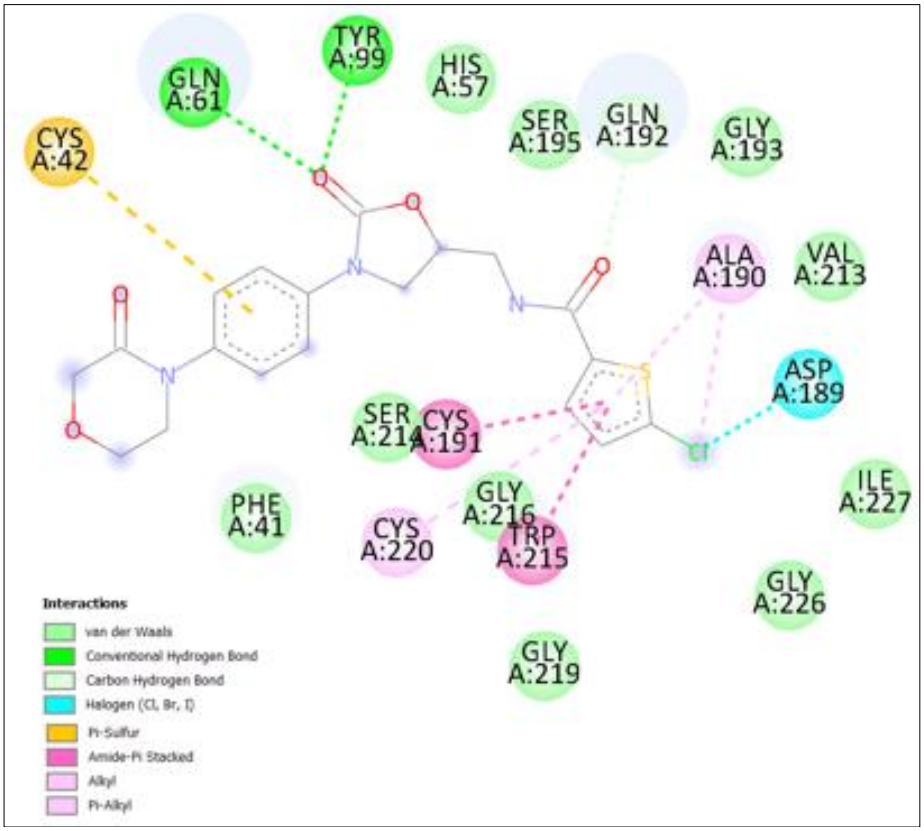

Fig 3: Captured image of the molecular docking of Rivaroxaban, the reference compound, to the binding site of FXa (PDB: 2J4I) using Auto Dock Vina. Rivaroxaban bonded via H-bond (dashed green lines) to Tyr99 of the S4 pocket and Gln61; via hydrophobic interactions (dashed pink lines) to the Cys191 and Ala190 of the S1 pocket, Trp215 of the S4 pocket, and Cys220; via salt bridge (dashed blue lines) to Asp189 of the S1 pocket; and via Pi-Sulfur interactions (dashed yellow lines) to Cys42.

3.2 ADMET prediction of caffeic acid

An ideal drug must possess good pharmacological activity in terms of its potency and selectivity, and good pharmacokinetic properties on the basis of its ADMET profile. The pharmacokinetic properties of candidate drugs are assessed using *in silico* predicted tools to reduce the expensive cost and risk of failure for drug discovery, as well as to avoid the development of drugs with un favourable efficacy and safety (Lin, 2003) [9]. As such, further assessment of caffeic acid was performed. The ADMET properties of caffeic acid were evaluated using ADMETlab 2.0 to explore the drug-likeness character, safety, and toxicity of the compound as a potential orally-administered anticoagulant against FXa. The ADMET prediction of caffeic acid as predicted by ADMETlab 2.0 are presented in Table 2.

Table 2: The ADMET properties of caffeic acid predicted by ADMETlab 2.0.

ADMET Properties	Caffeic Acid	Comment
Intestinal absorption in human	High	
Blood-brain barrier penetration (BBB)	No	Excellent: </- 90%; Otherwise: poor
CYP1A2 inhibitor	No	
CYP1A2 substrate	No	
CYP2C19 inhibitor	No	
CYP2C19 substrate	No	
CYP2C9 inhibitor	No	
CYP2C9 substrate	Yes	
CYP2D6 inhibitor	No	
CYP2D6 substrate	No	
CYP3A4 inhibitor	No	
CYP3A4 substrate	No	
Total clearance of drug (mL/min/kg)	17.44	High: >15 mL/min/kg; Moderate: 5-15 mL/min/kg; Low: <5 mL/min/kg
hERG blockers	Inactive	
Human hepatotoxicity	Inactive	
AMES Toxicity	Inactive	
Rat oral acute toxicity	Inactive	
Skin sensitization	Inactive	

Drug absorption and bioavailability is a vital aspect in pharmacokinetics as it greatly affects the effectiveness and safety of a drug candidate. Furthermore, ideal drug candidates undergo rapid absorption with minimal side effects (Lin, 2003) [9]. The human intestine is considered a primary site for the absorption of drugs. The intestinal absorption of a drug is also linked to its bioavailability to some extent, thus the human intestinal absorption is predicted to estimate the apparent efficacy of a candidate drug (Xiong *et al*., 2021) [20]. The predicted human intestinal absorption of caffeic acid indicates that caffeic is highly absorbed in the intestines.

The transport of the drugs starting from the systemic circulation leading to the extravascular space after the absorption of the drug is referred to as drug distribution. A drug that has a specific target organ, must have sufficient drug concentrations on the factors or properties that influence the distribution process in the body to attain the desired pharmacological activity (Lin *et al*., 2003) [9]. For drugs with a peripheral target, the BBB permeability is predicted to avoid the possible toxicities or side effects of a drug in the central nervous system (Xiong *et al*., 2021; Bucao & Solidum, 2022) [20, 2]. The ADMET results for the BBB permeability of caffeic acid demonstrate that caffeic acid exhibits low probability of crossing the BBB.

The process of drug metabolism, according to the chemical nature of biotransformation, involves phase I (oxidative reactions), and phase II (conjugative reactions). The human cytochrome P450 (CYP) family, also known as the phase I enzymes, house the enzymes that are responsible for the metabolism of roughly two-thirds of the drugs available in the market. Five CYP isozymes, which are 1A2, 3A4, 2C9, 2C19, and 2D16, account to 80% of drug metabolism. The activity of the CYP enzymes can greatly influence the ADMET properties of drugs, thus it is necessary to evaluate whether a drug is a substrate or inhibitor of these enzymes (Xiong *et al*., 2021) [20]. The prediction results indicate that caffeic acid is not an inhibitor of CYP1A2, CYP2C19, CYP2C9, CYP2D6, and CYP3A4. Results also demonstrate that caffeic acid is not a substrate of CYP1A2, CYP2C19, CYP2D6, and CYP3A4. In contrast, the ADMET prediction indicates that caffeic acid is a substrate of CYP2C9, suggesting that caffeic acid may bind to the active site of CYP2C9 and may be metabolized or transformed to metabolites.

The clearance of a drug (CL) is a crucial pharmacokinetic variable that is linked to drug bioavailability. It is also an essential parameter used to determine the frequency of drug dosage to achieve steady-state concentrations (Xiong *et al*., 2021; Bucao & Solidum, 2022) [20, 2]. The predicted clearance of caffeic acid is 17.444mL/min/kg (Table 2), which is greater than 15mL/min/kg (high clearance). This estimates that caffeic acid exhibits high clearance and is thus more rapidly excreted from the human body.

The toxicity characteristics of a drug candidate is estimated to assess the safety of drug candidates and to reduce the failure of candidate drugs in the later stages and clinical trials of drug discovery (Wu *et al*., 2020; Lin *et al*., 2003) [19, 9]. The human ether-a-go-go-related gene (hERG) plays a key responsibility in the passage of potassium ions through the cellular membrane and is important in cardiac action and resting potentials. The blockade of hERG may cause long QT syndrome (LQTS), arrythmia, torsades de pointes (TdP), which may even lead to death (Xiong *et al*., 2021) [20]. Prediction of a drug candidate's ability to inhibit the hERG is necessary to the aforementioned adverse effects.

Drug induced liver injury is among the greatest concerns in patient safety and is a great determinant in the withdrawal of a drug in the market. As such, the human hepatotoxicity characteristic of drug candidates are assessed to reduce the late and costly termination of drugs in its late stages (Xiong *et al*., 2021; Wu *et al*., 2020) [20, 19].

AMES toxicity is a test for mutagenicity. Mutagenicity is an important end point of toxicity and has a close relationship to carcinogenicity, as such it is essential to estimate the AMES toxicity to avoid the development of harmful mutagenic and potentially carcinogenic drugs (Xiong *et al*., 2021) [20].

Lastly, rat oral acute toxicity estimates the acute toxicity of a candidate drug on mammals (e.g. rats or mice) and provides insight on the potential adverse effects that may arise from the prolonged and repeated exposure to the drug candidate (Xiong *et al*., 2021) [20].

The toxicity prediction revealed that caffeic acid exhibited inactive remarks in all toxicity parameters and is thus predicted to be a non-toxic compound and is considered a safe drug candidate.

3.3 Drug-likeness prediction of caffeic acid

Drug-likeness assesses the likeliness of a drug candidate to become an oral compound on the basis of its bioavailability and evaluates the resemblance of the drug candidate to existing drugs available in the market (Diana *et al*., 2017) [4]. ADMETlab 2.0 utilizes the Lipinski rule-of-five, which assesses the drug-likeness of a drug based on the molecular weight, the total number of hydrogen bond donors and acceptors, and logarithm of the n-octanol/water distribution coefficient. A drug candidate that violates two of the aforementioned properties indicates that a drug candidate may exhibit poor absorption or poor permeability. In the drug-likeness prediction of caffeic acid presented in Table 3, caffeic acid satisfies all the properties of the Lipinski rule with 0 violations. Thus, caffeic acid shows good potential for drug-likeness, absorption, and permeability and may therefore be recommended for drug development and design.

Table 3: The drug-likeness of caffeic acid predicted by ADMETlab 2.0.

Property	Caffeic acid
Molecular weight (</-500 g/mol)	182.06
H-bond donor (</-5)	3
H-bond acceptor (</-10)	4
Log P (</-5)	0.783
No. of violations	0
Lipinski Rule	Accepted

4. Conclusion

The results of the *in silico* evaluation of caffeic acid using molecular docking and ADMET prediction tools demonstrate that caffeic acid exhibits putative anticoagulant activity and exhibits drug-like effects, and can therefore be recommended for the drug design and development of FXa inhibitors. However, these results are based on *in silico*-based predictive methods, which have their own limitations and drawbacks. Thus, the researchers recommend conducting *in vitro* and *in vivo* analyses for the confirmation of the inhibitory action of caffeic acid against FXa and to further optimize its assets. Nevertheless, the results of the *in silico* analyses conducted in this research may be integrated with *in vitro* and *in vivo* studies to develop a rational framework for the development of a natural FXa inhibitor.

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