Egypt. J. Chem. **Vol. 66**, No. 2 pp. 151 – 166 (2023)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

In Silico and *In Vitro* Anticoagulant Activity Detection of Quercetin, Rutin, and Troxerutin as New Potential Inhibitors of Factor Xa

Ghalia Sabbagh [a*], Lara AlBeik [a] and Ibrahim Hadid [b]

[a] *Department of Pharmaceutical Chemistry and Quality Control, Faculty of Pharmacy, Aleppo University, Syria.*

[b] *Department of urological surgery, Faculty of Medicine, Aleppo University, Syria.*

Abstract

Factor Xa is an important target for the development of new anticoagulants. The present study aims to examine the effect of three compounds (Quercetin and its derivatives Rutin and Troxerutin) in comparison with Rivaroxaban on activated Human Clotting factor X (PDB ID: 2w26). Molecular docking was performed using iGemDock v2.1 software to predict their anti-activating factor X activity and Autodock vina software to check the bonds formed. Next, the *in vitro* biological effects on both PT and aPTT coagulation time were studied. Troxerutin and Rutin showed the best interaction values with 2w26 enzyme, and the binding energy was [(-185.743 kcal/mol) and (-178.034 kcal/mol)], respectively, using iGemDock; While the binding energy of Rivaroxaban was (-149.661 kcal/mol). The results of the *in vitro* study showed that the studied compounds have an effect in prolonging PT, aPTT compared to both negative and positive control. However, Troxerutin gave the highest prolongation of the evaluated clotting times, consistent with the *in silico* study the results..

Keywords: Anticoagulants; Factor Xa; Molecular docking; Rivaroxaban; Troxerutin

1 Introduction

Thrombotic disorders which may be inherited or acquired [1], greatly affect global health, being the leading cause of death and disability morbidity worldwide in both developed and developing countries [2–4] according to the most recent World Health Organization report [5–9]. In fact, thrombotic diseases, such as Ischemic Heart, Ischemic Stroke, and Venous Thromboembolism, could cause organ tissue ischemia, necrosis, and seriously endanger human health [10].

Prevention and treatment of thrombotic disorders and embolic events require anticoagulant therapy [11,12], there are types of multi-targeted anticoagulants [13], including Unfractionated Hepari and its derivatives [14–16], and Vitamin K antagonists [17–19] are widely used for many years over the previous decades. Even though their effects are well established, the deleterious life-threatening side effects or complications of these drugs have also been well documented [20,21] such as the narrow treatment window, necessity of continuous laboratory frequent monitoring, and a wide range of food and drug interactions, or possess many limitations such as slow onset, increased bleeding risk and causing bleeding, so that, they are associated with significant drawbacks that limit their use and acceptability in the clinical setting [22,23]. These deficiencies have encouraged the search for novel selective and safe alternatives in the clinical setting [24–26]. Indeed, several anticoagulant agents that specifically target a single clotting factor within the coagulation cascade, such as thrombin [27,28] or factor Xa (FXa) [29–31] are currently in development in an attempt to meet this need. Upon the release of thrombin inhibitors in the market, many disadvantages were reported for their direct action, resulting in undesirable effects, limiting their use and acceptability in the clinical setting. Therefore, the design of FXa inhibitors became the focus of many medicinal chemists for developing potent orally bioavailable anticoagulants with improved efficacy [32–36]. Therefore, FXa is recognized as an ideal, attractive model and smart target for anticoagulation therapy in the development of new drugs, which makes FXa inhibitors become a hot topic

*Corresponding author e-mail ghaliaaa@hotmail.com

Receive Date: 22 May 2022, Revise Date: 31 May 2022, Accept Date: 03 June 2022

DOI: 10.21608/EJCHEM.2022.140123.6144

of anticoagulant research [37]. Factor Xa has a critical role in the blood coagulation process by converting prothrombin to thrombin. Whereas, One factor Xa molecule stimulates several prothrombin molecules to thrombin molecules in the coagulation pathway through signal amplification [38]. Factor Xa is a serine protease, and it structurally belongs to the trypsin-like family that plays significant roles in many key biological processes [39]. Four functional sub-pockets form up the active site of human coagulation factor Xa. The target pockets of the Factor Xa are the S1 and S4 binding pockets. The S1 pocket describes the key selectivity and binding segment and it usually favors positively charged moieties such as benzamidine, amine, and guanidine. The second major binding pocket is S4 and is formed by different residues of amino acid residues. Usually, Factor Xa inhibitors bind in an L-shaped conformation with the active pocket [39,40].

In silico methods are good scientific tools for understanding biological processes [41]. Using molecular docking tools has become an important step in developing and discovering new drugs for many diseases, as it saves a lot of effort, time, and money as well [42–44]. Molecular docking is a computational method of searching for a suitable ligand, which matches the protein's binding position both energetically and geometrically. Molecular docking is routinely used to understand the interaction of a drug-receptor and is often used to predict the affinity and activity of a ligand or drug molecule to the binding site of target proteins [45]. So, many sampling algorithms have been developed and used widely in software for molecular docking [46]. Auto Dock Vina [47–49] and iGemdock [50–53] are among the most common and most broadly used molecular docking programs [54].

Because of the great sales achieved around the world by Rivaroxaban despite some severe side effects such as fatal bleeding and other symptoms as well as rebound thromboembolic effects when treatment is stopped, this has motivated us to investigate natural compounds that may be safe to suggest as an anticoagulant that acts similarly to it and has fewer side effects [55–57].

Natural compounds have over the centuries been a very attractive source of research on new effective, safe, and inexpensive drugs [58,59]. Flavonoids are a class of natural compounds with anti-biotic, anti-viral, anti‑inflammatory, anti-thrombus, anti‑oxidative, and anti‑tumoral effects [60,61]. Quercetin (Qur) is an aglycone; however, it is typically conjugated to monosaccharides (e.g., glucose, xylose, rhamnose, arabinose, or galactose) or disaccharide (e.g., Rutinose). In onions, Quercetin is hooked up to the glucose moiety and forms Quercetin-3-O-glucoside (Isoquercetin), while in apple and tea, Quercetin is conjugated to Rutinose to make Quercetin-3-O-rutinoside (Rutin). It has antiviral, immunomodulatory, and anticoagulant effects, It also proved good effects in the modern era disease, which is Covid 19 [62–64]. Whereas, Rutin (Rut) showed several pharmacological activities, including antioxidant, cellular protection, vascular protective, anticancer, neuroprotective, and cardioprotective activities [65,66]. Troxerutin (Trx) also known as vitamin P4, is one of the well-known and medicinally used flavonoids [67], it is a trihydroxyethylated derivative of Rutin. It has been used to treat chronic venous insufficiency (CVI) and many diseases. Troxerutin can be more easily absorbed by the digestive system in comparison with Rutin [68]. Troxerutin has proven to be of excellent safety profile and tolerable even at high doses in clinical trials [69].

In this research, Quercetin (aglycone) [70] and its derivatives of glycosidic flavonoids which are Rutin [71], and Troxerutin [72] were compared with Rivaroxaban as a positive reference [73], *in silico* and *in vitro* to detect the efficiency of their potential effect as factor Xa inhibitors.

2- Materials and Methods

All the experimental works were carried out using analytical-grade chemicals, reagents, and solvents. They were procured from commercial sources.

Chemicals: Factor Xa inhibitor Rivaroxaban was supplied by Ibn Al Haytham for the Pharmaceutical industry Company in Syria. Troxerutin was purchased from the Mediotic Pharmaceutical industry in Syria. Quercetin and Rutin were purchased from the UK Company Sigma Aldrich. Distilled water was used as a solvent for the compounds, in addition to a DMSO solution imported from Sigma Aldrich. Prothrombin Time (PT) and Activated Partial Thromboplastin Time (aPTT) kits were purchased from DIALAB, located in Austria.

Equipment: Sartorius TE214 precision balance (German make); HeraeusMegafuge (Centrifuge

Germany); 5CC disposable syringes; Sodium citrate tubes (Italian make); and Coagulyzer® 1 from Analyticon (German make) was used as an instrument in tests to measure clotting times.

1.1 *In silico* Docking Study

1.1.1 Protein Preparation

The crystal structure of the protein that we used for docking studies was obtained from the Protein Data Bank (PDB) [74][75], which is a free resource for 3D structures of proteins and other large molecules. We described the active site based on the binding model of the x-ray complex structure of the enzyme PDB ID:2w26 [76] and it had a resolution factor 2.08 A°. The enzyme was downloaded and saved in PDB format. **Figure 1** presents its 3D structure,

Fig. 1. This figure shows the 3D structure of the 2w26 enzyme.

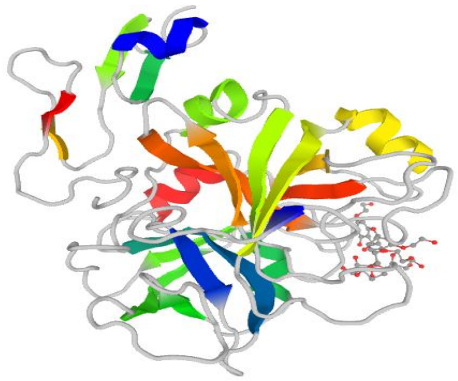
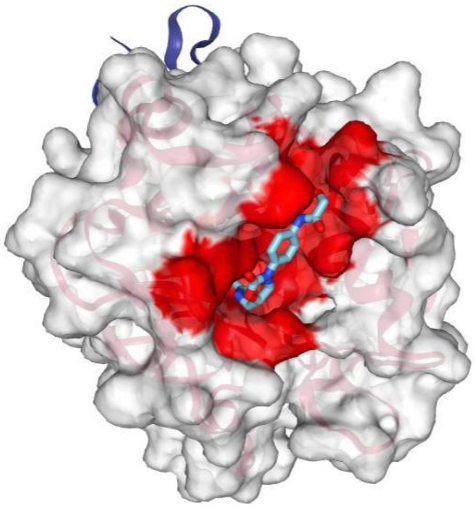


Fig. 2. This figure shows the 3D binding site of the 2w26 enzyme in red colour with reference inhibitor (Rivaroxaban) in blue.



while **Figure 2** presents the active site of the (2w26) as determined based on its complex x-ray structure and the binding ligand (RIV).

1.1.2 Ligands preparation

Quercetin (ID: ZINC3869685), Rutin (ID: ZINC4096846), Troxerutin (ID: ZINC85552699), and Rivaroxaban (ID: ZINC3964126) that their chemical structures are shown in **table 1**, were downloaded in SDF format from ZINC [45,46], a free-to-access database of compounds offering ready-formats for virtual screening. SDF files were then converted to MOL2 files using Open Babel for the virtual screening process on iGemdock v2.1 [79]. They were also converted into PDB files using Pymol to prepare the compound for docking on AutoDock Vina [80].

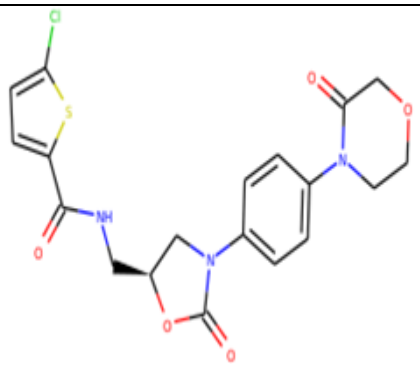
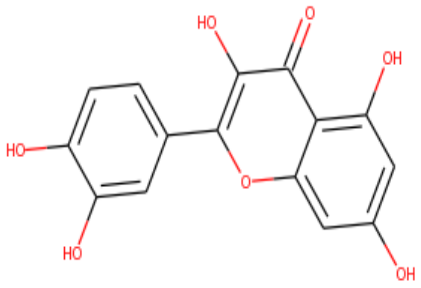
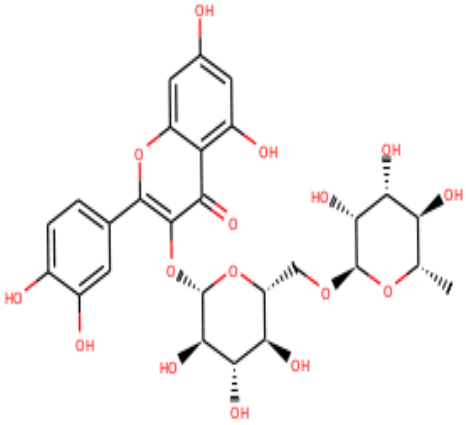
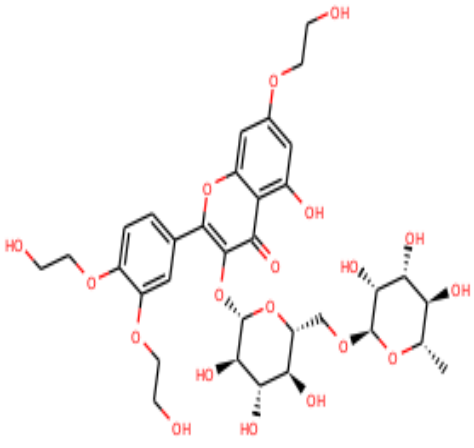
It is possible to exclude natural compounds from Lipinski and Veber rules because of their high complexity and special conformational features, which is supported by recent studies [81]. Troxerutin, for example, is used as a medicine for the treatment of hemorrhoids, varicose veins, and other chronic vascular insufficiency diseases [67].

1.1.3 Virtual screening using iGEMDOCK v2.1

iGemdock v2.1 was used to perform the docking study for the (2w26) that interacts with the compounds. Afterward, their effect on coagulation times PT, and aPTT *in vitro* has been evaluated. The program software is available for free and was used in previous research [52].

iGemdock is an integrated virtual screening (VS) environment for preparations through post-screening analysis with pharmacological interactions [82]. iGemdock was used as a molecular docking tool to dock the (2w26) with our selected compound. iGemdock provides interactive interfaces to prepare the screening compounds' library and the binding site of the targeted enzyme. Using iGemdock, each compound in the library was docked into the binding site. The protein-compound interaction was inferred by the software, which then clustered the screening compounds for the post-screening analysis based on profiles of hydrogen-bonding (H), electrostatic (E), and Van der Waal's (V) interactions. Furthermore, based on compound structures, iGemdock inferred the pharmacological interactions. Finally, the software ranked and visualized the screening compounds by combining the pharmacological interactions and energy-based scoring functions provided.

Table 1: This table shows the structure and information of the Quercetin, Rutin, Troxerutin and Rivaroxaban.

Chemical Name	IUPAC Name	Information	Compound Structure
Rivaroxaban	5-chloro-N-[[(5S)-2-oxo-3-[4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl]methyl]thiophene-2-carboxamide	MW: 435.889 g/mol MF: C19H18ClN3O5S H-bond donor: 1 H-bond acceptor: 6 Log p: 2.52	
Quercetin	2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one	MW: 302.23 g/mol MF: C15H10O7 H-bond donor: 3 H-bond acceptor: 7 Log p: 1.98	
Rutin	2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-[[(2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxymethyl]oxan-2-yl]oxychromen-4-one	MW: 610.52 g/mol MF: C27H30O16 H-bond donor: 10 H-bond acceptor: 16 Log p: -1.68	
Troxerutin	2-[3,4-bis(2-hydroxyethoxy)phenyl]-5-hydroxy-7-(2-hydroxyethoxy)-3-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-[[(2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxymethyl]oxan-2-yl]oxychromen-4-one	MW: 742.68 g/mol MF: C33H42O19 H-bond donor: 10 H-bond acceptor: 19 Log p: -2.691	

We used the protocol "accurate docking" with default parameters including a population size of 800 with 80 generations and 10 number of solutions. After the docking, the software performed the post docking analysis to find the docking pose and its energy values. The empirical scoring function of iGemdock was estimated using the following equation:

Energy = vdW + H-bond + Elec

whereas the vdW is van der Waal energy, H-bond is hydrogen bonding energy, and Elect is electro statistic energy.

1.1.4 Pre-molecular docking procedures, active site prediction, and molecular docking

Molecular docking was performed using AutoDock Vina [47]. In previous steps, water molecules and standard ligand were removed then hydrogen atoms and charges were added using the discovery studio visualization tool [83] and AutoDock tools. The PDB format of Macromolecule and studied compounds are converted to AutoDock's PDBQT format. The active pocket region of protein 2w26 is predicted using Discovery Studio 2021 [84,85], which provides information about the active site of the macromolecule in which the pre-loaded ligand binds to it.

The docking of small molecules to the protein was focused on the specified binding site. The total number of rotatable bonds of the ligands is calculated. The Grid was defined as the predicted binding site of the protein structure with the configurations of x/y/z coordinates was set to center_x=7.960828, center_y=5.317448, center_z=21.771931 in X, Y, Z dimensions and was set to size_x=30, size_y=20, size_z=30 of the grid box.

1.1.5 Protein-ligand interactions

Interactions like Hydrogen Bonding and other non-bonded terms between the three docked compounds and the protein are displayed and analyzed using Discovery Studio Visualizer [86].

1.2 In vitro Study

1.2.1 *In vitro* study with blood from healthy volunteers

Blood samples were collected in trisodium citrate tubes from 21 healthy volunteers who had not taken any medications (All volunteers were males aged 25 – 50 years to avoid the side effects of female hormones on coagulation). In order of registration, all blood samples were numbered and centrifuged and the coagulation times of each volunteer were measured before the experiment. Blood samples were mixed with negative control, stock solutions of Quercetin (Qur), Rutin (Rut), Troxerutin (Trx), and Rivaroxaban (Riv) as a positive control, in addition to a separate series of diluted solutions of Trx stock to confirm theirs *in vitro* anti-coagulation effects.

Quercetin, Rutin, Troxerutin, and Rivaroxaban were each dissolved in DMSO and then diluted with distilled water to yield stock solutions of 5 mM [87] with a proper amount in all of them. The stock solution of Trx was also diluted with distilled water to get concentrations of 4 mM, 3 mM, 2 mM, 1.5 mM, 1 mM, and 0.5 mM, respectively. Finally, each solution was mixed with a portion of plasma samples at the time of assay. The first 10 samples were used to test the effect of all stock solutions on PT, while the second 6 samples were used to test the effect of the series of diluted solutions of Trx stock solution on PT, while last 5 samples were used to determine the effect of all solutions on aPTT. After incubating the tested samples for 5 min. at a temperature of 37°C, we immediately registered the PT and aPTT effects.

1.3 Statistical Analysis

Statistical analysis was performed using SPSS 24 by one-way ANOVA with Tukey post-test for comparison. The p -value ≤ 0.05 was considered highly significant. Values are expressed as mean ± SD.

2 Results and Discussion

In this research, the effectiveness of several substances was studied in comparison to Rivaroxaban as an anticoagulant and its effects in prolonging clotting times. Firstly, we conducted an *in silico* study of the compounds as an anti-factor Xa in comparison to Rivaroxaban. Afterward, the results were also studied *in vitro*.

2.1 In silico Study

Molecular docking is the best method to investigate the suitability of any chemical as a remedy before proceeding continuing with any *in vivo* or *in vitro* studies. This is in order to shorten the time needed for experiments and to ensure cost savings. In this part of our study, Quercetin, Rutin, and Troxerutin were docked against human coagulation factor Xa (PDB ID: 2W26) in comparison to Rivaroxaban by using iGemdock to evaluate the *in silico* anticoagulant effect and conclude its interactive analysis by Autodock Vina software before investigating the *in vitro* effects.

2.1.1 Drug screening using iGEMDOCK v2.1

Trx showed good binding energy to 2w26 enzyme *in silico* compared to synthetic inhibitors that are available in the medication market. The kcal/mol results demonstrated that Trx had the highest binding energy (-185.743 kcal/ mol). This is demonstrated in **Table 2** compared to a positive control (Riv) (-149.661

kcal/mol) and the two rest compounds Rut and Qur. Therefore, these molecular docking analyses could express the potentially potent 2w26 inhibitors for the prevention and treatment of thrombosis.

Figure 3 shows the binding site of the compounds within the target pocket required for the molecular screening process, which proves the validity of the molecular docking method using iGEMDOCK v2.1.

Figure 4 below shows the predicted docking pose of each compound individually in comparison with the basic enzymatic ligand, and **Table 3** illustrates the predicted interacting amino acid residues that are involved in the binding of each compound.

Figure 5 shows the amino acid residues in the active pocket that are interaction with Troxerutin using iGEMDOCK.

Table 2: This table shows the values for the binding energies of the studied compounds resulting from the use of the iGEMDOCK v2.1.

S. No.	name Compound	Total binding energy (kcal/mol)	Vanderwaals force (kcal/mol)	H Bond (kcal/mol)	Electrostatic
1	Troxerutin	-185.743	-153.857	-31.8855	0
2	Rutin	-178.034	-143.594	-34.44	0
3	Rivaroxaban	-149.661	-135.591	-14.0703	0
4	Quercetin	-129.123	-87.8123	-41.3108	0

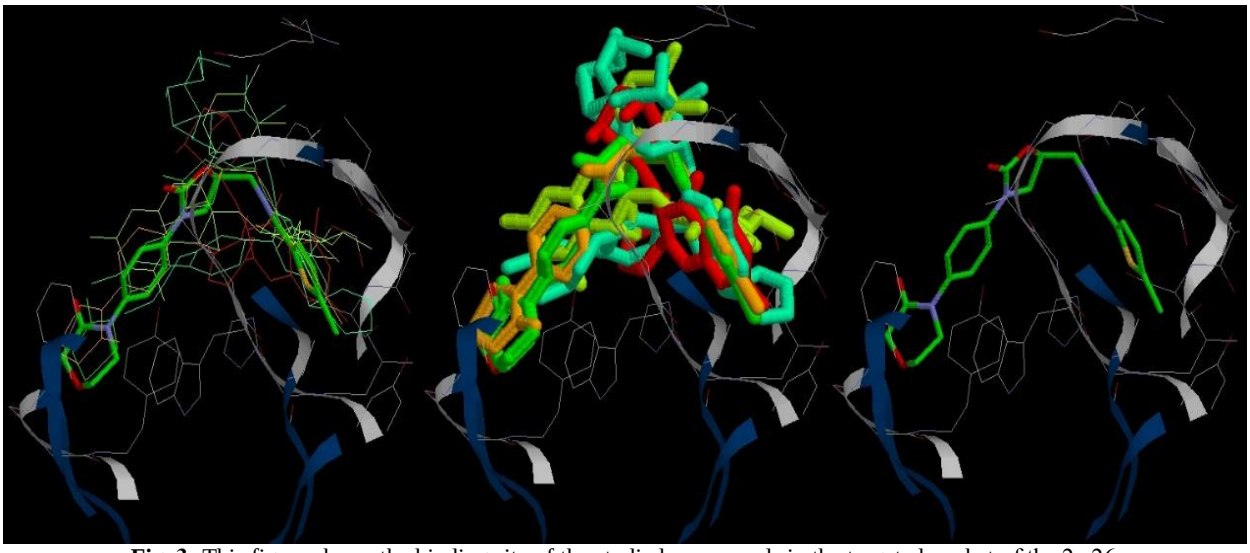


Fig. 3: This figure shows the binding site of the studied compounds in the targeted pocket of the 2w26.

Table 3: This table shows the predicted interacting amino acid residues stabilizing compounds in the binding site by iGEMDOCK .

Compound name and ID	hydrogen bond	Van der Waals interaction
Troxerutin	HIS-57, TYR-99, ARG-143, ASP-189, ALA-190, GLN-192, SER-195, TRP-215, GLY-216, CYS-220, ARG-222, ILE-227	HIS-57, TYR-99, ARG-143, PHE-174, ALA-190, CYS-191, GLN-192, SER-214, TRP-215, GLY-216, GLU-217, GLY-219, TYR-228
Rutin	GLU-97, THR-98, TYR-99, ASP-189, ALA-190, CYS-191, GLN-192, TRP-215, GLY-216, GLY-219, CYS-220,	HIS-57, TYR-99, PHE-174, ALA-190, CYS-191, GLN-192, SER-214, TRP-215, GLY-216, GLU-217, GLY-219, TYR-228
Quercetin	TYR-99, ASP-189, ALA-190, GLN-192, GLY-193, SER-195	HIS-57, TYR-99, ALA-190, CYS-191, GLN-192, SER-214, GLY-216
Rivaroxaban	GLN-192, GLY-219, CYS-220	TYR-99, PHE-174, ALA-190, CYS-191, GLN-192, SER-214, TRP-215, GLY-216, GLU-217, GLY-219, TYR-228

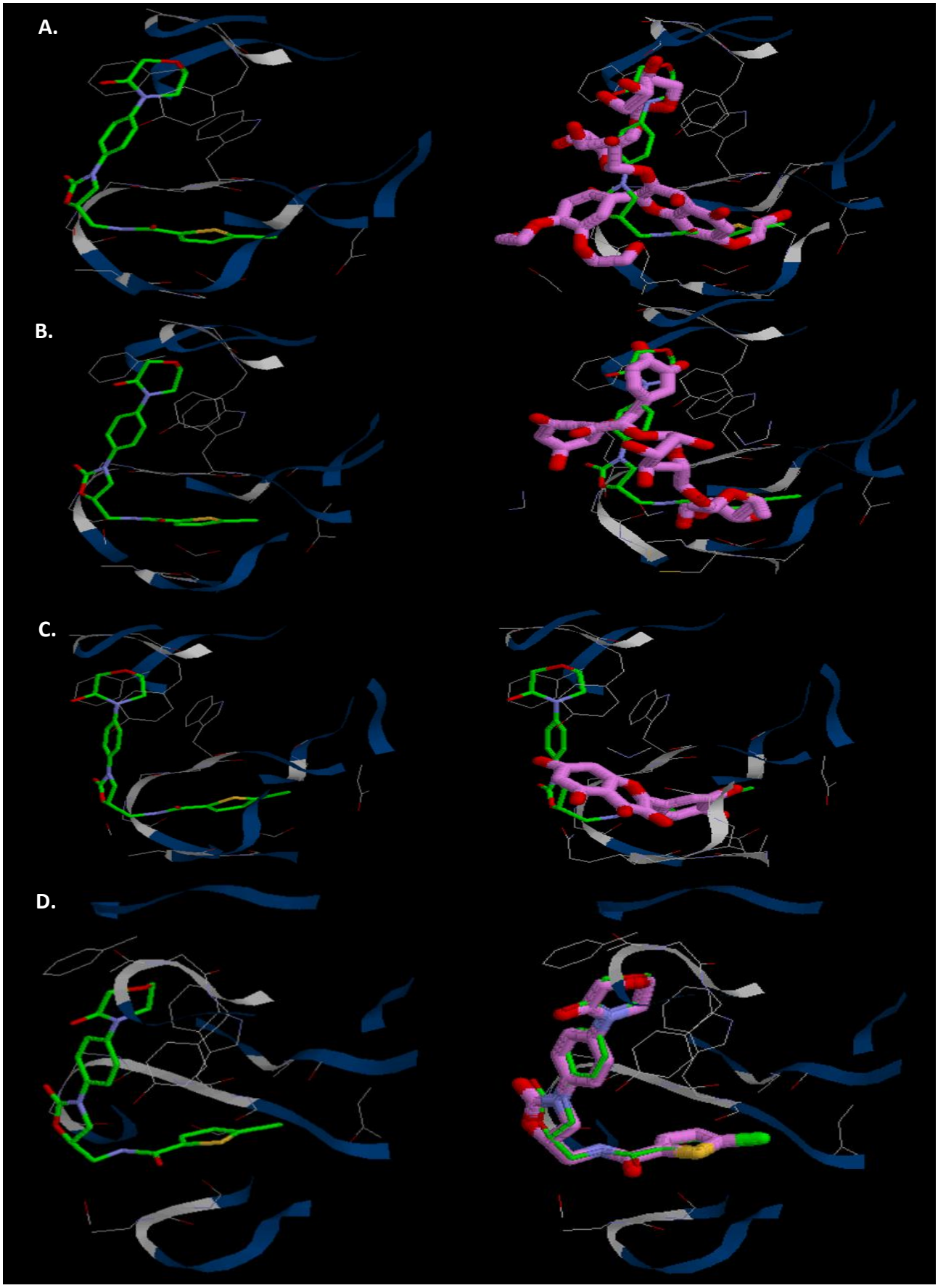


Fig. 4: This Figure shows the predicted docking pose of the studied compounds (A. Troxerutin, B. Rutin, C. Quercetin, and D. Rivaroxaban, respectively) within the active site of the target protein (PDB ID-2w26). The pink color represents the corresponding molecule and the green color represents the corresponding reference ligand that was loaded on the enzyme. Green and grey colors represent the amino acids involved in hydrogen bonding and Van der Waals interactions respectively.

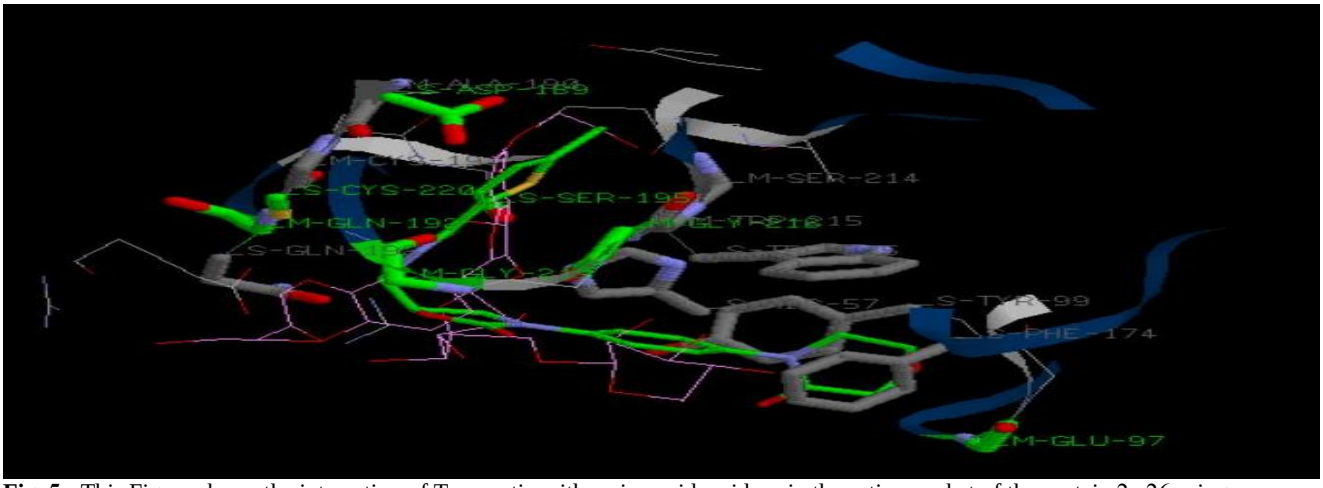


Fig. 5: This Figure shows the interaction of Troxerutin with amino acid residues in the active pocket of the protein 2w26 using iGEMDOCK.

2.1.2 Molecular docking by AutoDock Vina

Molecular docking was performed using AutoDock Vina with the previous three compounds to verify their interactions with the target protein. The Predicted interacting amino acid residues were illustrated in **table 4** and **Figure 6**.

Figure 6 below show the interaction between 2w26 enzyme and compounds visualized using Discovery Studio Visualizer. They represent various residues corresponding to the different interactions of 2w26 enzyme with the molecule that is the subject of our study.

Table 4: This table shows the predicted interacting amino acid residues stabilizing compounds in the binding site by AutoDock Vina.

Compound name	Hydrogen bond		Hydrophobic	
	New bonds	Common bonds	New bonds	Common bonds
Troxerutin	GLU97 GLU147 GLY219	HIS57 TYR99 ASP189 ARG222	CYS220	TYR99 PHE174 TRP215
Rutin	PHE174 CYS191 SER195 ARG222	TYR99 GLN192 TRP215 GLY216 GLY219	-	TYR99 PHE174 TRP215
Quercetin	GLY216	ASP189 ALA190	CYS220	ALA190 CYS191 GLN192
Rivaroxaban	-	GLY219 CYS220	VAL213	TYR99 PHE174 ALA190 CYS191 GLN192 TRP215 GLY216 GLY217 TYR228

Based on the *in silico* study, it was suggested that Quercetin, Rutin, and Troxerutin are potentially effective inhibitors for human coagulation factor Xa.

2.2 Effect on *in vitro* Study

Based on the previous results of the *in silico* study, an *in vitro* study was conducted to confirm the theoretical results. Coagulation times were measured. These included: activated Partial Thromboplastin Time (aPTT), which is the measure of the functional activity of multiple factors in the intrinsic pathway, in addition to FII and FX. Furthermore, by measuring Prothrombin Time (PT), several factors in the extrinsic pathway such as VII and V are evaluated in addition to the common factors, such as factor Xa, which was done after preparing the specific concentrations of the studied compounds.

A negative control solution consisting of DMSO 20%, a positive control solution for Rivaroxaban, stock solutions for the studied compounds, and a series of diluted solutions from Trx stock solution were prepared. The measurements were made after mixing each solution separately with a portion of the plasma of each volunteer in a specified proportion, which were incubated at a temperature corresponding to the human body temperature for five minutes.

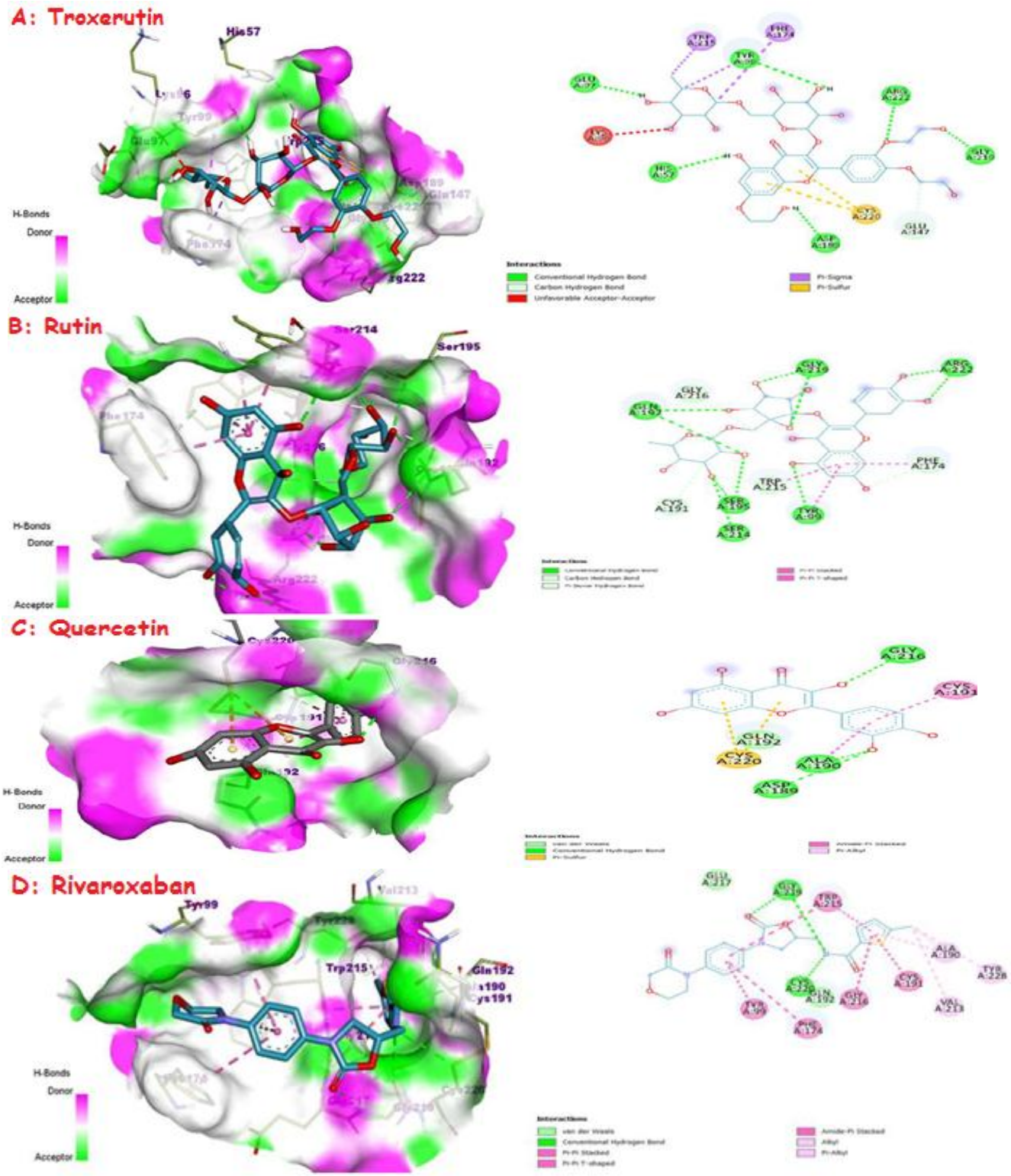


Fig. 6: This Figure shows the 3D and 2D binding poses of (a) Troxerutin, (b) Rutin, (c) Quercetin, (d) Rivaroxaban simulated by AutoDock Vina. For each ligand, hydrogen, carbon-hydrogen, unfavorable, as well as and–bonds are depicted as broken lines in green, light blue, purple, and magenta respectively.

Then the examinations were performed according to the directions/protocol on the reagent leaflet that was used.

All values are given in seconds as the mean of the measurements ± SD. Differences between controls and sample measurement were assessed by one-way ANOVA with Tukey post-test. A p -value ≤0.05 was considered statistically significant.

The results in **Table 5** and **Figure 7** showed that each solution was prepared with a concentration of 5 mM and was examined, had the effect of almost

prolonging the coagulation times, with statistically significant differences compared to the negative control

Solution groups	PT(Sec) n = 10 (means±SD)	aPTT (Sec) n = 5 (means±SD)
DMSO 20% (N.C.)	37.22 ± 1.05	52.06 ± 1.84
RIV (5 mM) (positive control)	102.03± 0.90*	219.00 ± 6.81*
Qur (5 mM)	38.51 ± 0.49*	82.52 ± 2.57*
Rut (5 mM)	105.13 ± 1.19*	221.33 ±6.67*
Trx (5 mM)	157.61 ± 0.74*	337.12 ± 10.49*
* The mean difference is significant at the 0.05 level with respect to the negative control group.		

Table 5: This table shows the effect of studied compounds solutions Qur, Rut, Trx, and Riv, as well as a negative control, on coagulation times.

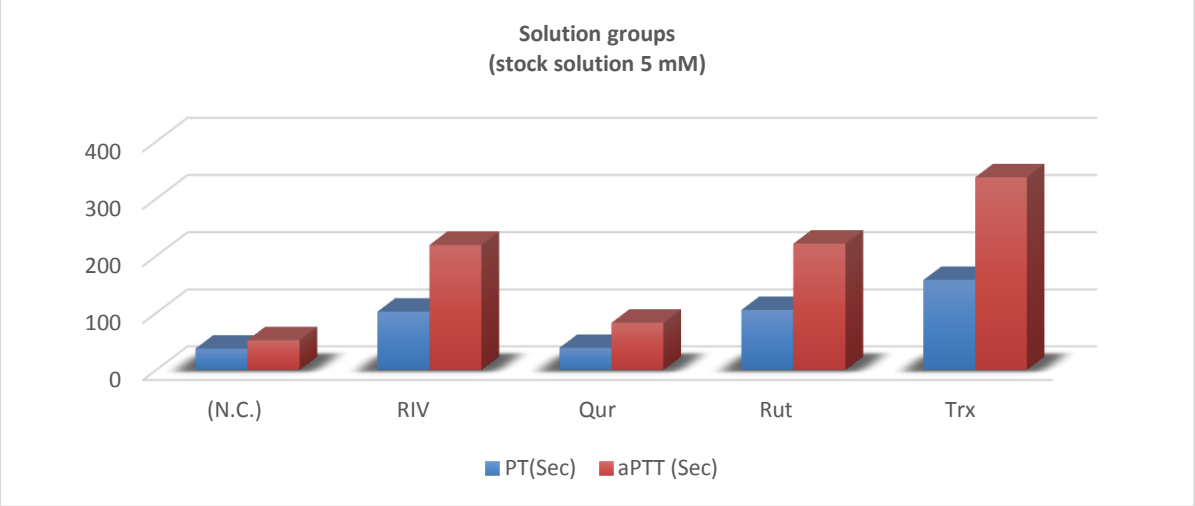


Fig. 7: This Figure represents the effect of the studied groups of solutions on PT and aPTT coagulation times

The results in **Table 6** and **Figure 8** showed that each solution prepared for the Troxerutin in the examined concentrations has prolonged the coagulation times almost directly proportional to the increase in the concentration. When studying the values statistically, there are significant differences for the studied concentrations groups with a p -value ≤ 0.05 compared to the negative control.

Table 6: This table shows the effect of diluent solutions from the stock solution of Trx and the negative control (only distilled water) on coagulation times.

Solution groups (Trx solutions with several concentrations)	PT(Sec) n = 6 (means±SD)	aPTT (Sec) n = 5 (means±SD)
Water (N.C.)	14.48 ± 1.07	26.24 ± 1.59
0.5 mM	24.84 ± 0.69*	50.62 ± 1.54*
1 mM	30.76 ± 0.63*	61.55 ± 1.85*
1.5 mM	31.54 ± 0.36*	63.25 ± 1.94*
2 mM	44.70 ± 0.54*	89.49 ± 2.71*

3 mM	63.30 ± 0.37*	125.30 ± 3.86*
4 mM	101.29 ± 0.73*	202.32 ± 6.27*
5 mM (stock solution of Trx)	157.80 ± 0.70*	337.04 ± 10.50*
* The mean difference is significant at the 0.05 level with respect to the negative control group.		

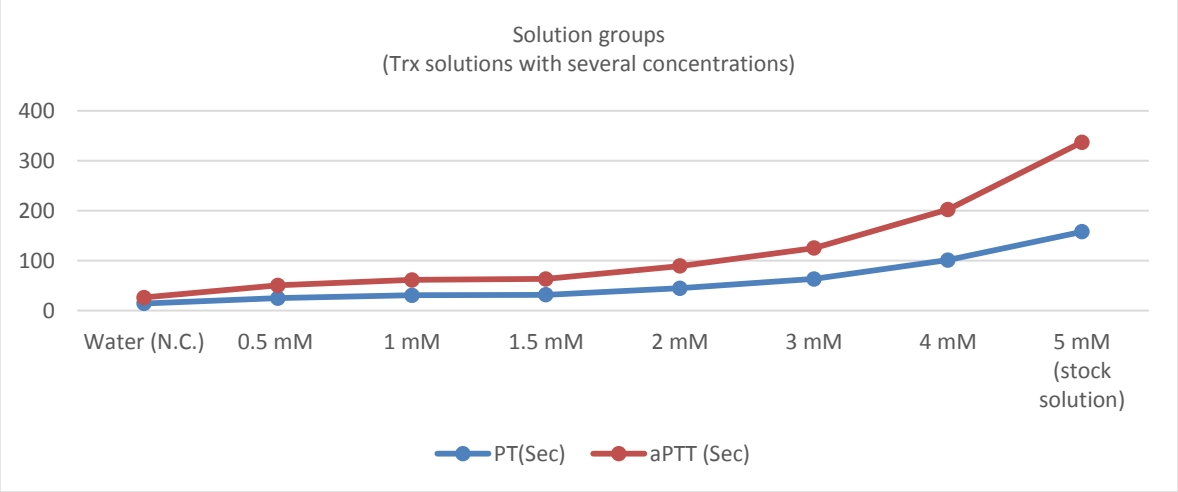


Fig. 8: This Figure represents the effect of the studied diluent solutions of Trx on PT and aPTT coagulation times.

The overarching results show that the application of Quercetin, Rutin, Troxerutin, and Rivaroxaban (as positive control) had an effect on the coagulation times of PT and aPTT in the *in vitro* study. They led to a prolongation of coagulation times compared to the negative control. The inhibitory effect of factor X of Quercetin has been confirmed by several recent studies [88,89]. Furthermore, this study examined the Structure-Activity Relationship (SAR). The results revealed the superiority of the flavonoid glucosides (Rutin and Troxerutin) over their aglycone part (Quercetin). Therefore, they can be considered agents with anti-factor Xa properties, but may also affect multiple targets other than factor Xa according to recent studies [66,90–93].

3 Conclusions

Recent significant developments in docking and screening have made them a valuable tool in the medication discovery and research processes. This study aimed to predict and determine the efficacy of the studied compounds as an inhibitor of the human coagulation factor Xa. The results obtained from *in silico* and *in vitro* studies revealed that Quercetin, Rutin, and Troxerutin may be candidates as inhibitors for the human coagulation factor Xa. When compared to medications with FDA approval for the inhibition of factor Xa, Rivaroxaban, in addition to the negative control, the compounds prolonged coagulation times when studied *in vitro* with specific concentrations. Thus, Rutin and Troxerutin can be considered good anticoagulants for inhibiting the human coagulation factor Xa as a suggested mechanism, with the possibility of having effects through other mechanisms as well.

4 DISCLAIMER

The products used for this research are commonly and predominantly products used in our area of research and country. There is no conflict of interest between the authors and sources of the products as we have no intention of using these products for any purposes other than the advancement of knowledge. The research was funded by Aleppo University, Faculty of Pharmacy as part of academic research done for the fulfillment of requirements for an MSc degree.

5 ACKNOWLEDGEMENTS

The authors would like to thank all of the Administrative Staff at Aleppo University and in particular the staff of the Faculty of Pharmacy and Aleppo University Hospital for their assistance.

There is no known conflict of interest associated with this publication and there has been no significant financial support provided for this work that could have influenced its outcome.

6 COMPETING INTERESTS

Authors have declared that no competing interests exist.

7 Abbreviations:

FXa: Factor X activity
RIV: Rivaroxaban**.**
Trx: Troxerutin**.**
Rut: Rutin**.**
Qur: Quercetin**.**

DMSO: Dimethyl Sulfoxide.
PDB: Protein Data Bank**.**
PT: Prothrombin Time.
aPTT: Activated Partial Thromboplastin Time.

References

[1] N.A. Alli, J. Vaughan, S. Louw, E. Schapkaitz, B. Jacobson, Thrombotic disorders (part 1), *South African Medical Journal*. 110 (2020) 83–87. https://doi.org/10.7196/SAMJ.2020.V110I2.14594.

[2] A.M. Wendelboe, G.E. Raskob, Global Burden of Thrombosis, *Circulation Research*. 118 (2016) 1340–1347. https://doi.org/10.1161/CIRCRESAHA.115.306841.

[3] J.W. Yau, K.K. Singh, Y. Hou, X. Lei, A. Ramadan, A. Quan, H. Teoh, W.M. Kuebler, M. Al-Omran, B. Yanagawa, H. Ni, S. Verma, Endothelial-specific deletion of autophagy-related 7 (ATG7) attenuates arterial thrombosis in mice, *J Thorac Cardiovasc Surg*. 154 (2017) 978-988.e1. https://doi.org/10.1016/J.JTCVS.2017.02.058.

[4] H. Mani, E. Lindhoff-Last, New oral anticoagulants in patients with nonvalvular atrial fibrillation: a review of pharmacokinetics, safety, efficacy, quality of life, and cost effectiveness, *Drug Des Devel Ther*. 8 (2014) 789–798. https://doi.org/10.2147/DDDT.S45644.

[5] M.A. Khan, M.J. Hashim, H. Mustafa, M.Y. Baniyas, S.K.B.M. al Suwaidi, R. AlKatheeri, F.M.K. Alblooshi, M.E.A.H. Almatrooshi, M.E.H. Alzaabi, R.S. al Darmaki, S.N.A.H. Lootah, Global Epidemiology of Ischemic Heart Disease: Results from the Global Burden of Disease Study., *Cureus*. 12 (2020) e9349. https://doi.org/10.7759/cureus.9349.

[6] Global Status Report on Noncommunicable Diseases 2014: Attaining the Nine Global Noncommunicable Diseases Targets; A Shared Responsibility - World | ReliefWeb, (n.d.). https://reliefweb.int/report/world/global-status-report-noncommunicable-diseases-2014-attaining-nine-global (accessed May 6, 2022).

[7] Global status report on noncommunicable diseases 2014, (n.d.). https://apps.who.int/iris/handle/10665/148114 (accessed May 6, 2022).

[8] Cardiovascular diseases (CVDs), (n.d.). https://www.who.int/news-room/fact-sheets/detail/cardiovascular-diseases-(cvds) (accessed May 6, 2022).

[9] G. Touloumi, A. Karakosta, N. Kalpourtzi, M. Gavana, A. Vantarakis, M. Kantzanou, C. Hajichristodoulou, G. Chlouverakis, G. Trypsianis, P. v. Voulgari, Y. Alamanos, K. Makrilakis, S. Liatis, S. Chatzipanagiotou, G. Stergiou, A. Yannis, B. Alexis, C. Grigoris, H. Christos, K. Anna, S. George, T. Giota, T. Grigoris, V. Apostolos, V. Paraskevi, K. Argiro, P. Nikos, V. Georgia, K. Natasa, K. Klea, K. Maria, C. Xenia, G. Magda, H. Bettina, H. Christos, R. George, V. v. Paraskevi, N. Ilias, P. Koustenis, M. Konstantinos, L. Stavros, High prevalence of cardiovascular risk factors in adults living in Greece: the EMENO National Health Examination Survey, *BMC Public Health*. 20 (2020) 1–10. https://doi.org/10.1186/S12889-020-09757-4/FIGURES/1.

[10] K.L. Furie, S.E. Kasner, R.J. Adams, G.W. Albers, R.L. Bush, S.C. Fagan, J.L. Halperin, S.C. Johnston, I. Katzan, W.N. Kernan, P.H. Mitchell, B. Ovbiagele, Y.Y. Palesch, R.L. Sacco, L.H. Schwamm, S. Wassertheil-Smoller, T.N. Turan, D. Wentworth, Guidelines for the prevention of stroke in patients with stroke or transient ischemic attack: a guideline for healthcare professionals from the american heart association/american stroke association, *Stroke*. 42 (2011) 227–276. https://doi.org/10.1161/STR.0B013E3181F7D043.

[11] T.M. Giglia, M.P. Massicotte, J.S. Tweddell, R.J. Barst, M. Bauman, C.C. Erickson, T.F. Feltes, E. Foster, K. Hinoki, R.N. Ichord, J. Kreutzer, B.W. McCrindle, J.W. Newburger, S. Tabbutt, J.L. Todd, C.L. Webb, American Heart Association Congenital Heart Defects Committee of the Council on Cardiovascular Disease in the Young, Council on Cardiovascular and Stroke Nursing, Council on Epidemiology and Prevention, and Stroke Council, Prevention and treatment of thrombosis in pediatric and congenital heart disease: a scientific statement from the American Heart Association., *Circulation*. 128 (2013) 2622–703. https://doi.org/10.1161/01.cir.0000436140.77832.7a.

[12] H.L. McRae, L. Militello, M.A. Refaai, Updates in Anticoagulation Therapy Monitoring, *Biomedicines*. 9 (2021). https://doi.org/10.3390/BIOMEDICINES9030262.

[13] M.K. Dabbous, F.R. Sakr, D.N. Malaeb, Anticoagulant therapy in pediatrics, *Journal of Basic and Clinical Pharmacy*. 5 (2014) 27. https://doi.org/10.4103/0976-0105.134947.

[14] B. Mulloy, J. Hogwood, E. Gray, R. Lever, C.P. Page, Pharmacology of Heparin and Related Drugs, *Pharmacol Rev*. 68 (2016) 76–141. https://doi.org/10.1124/PR.115.011247.

[15] J.I. Weitz, Low-molecular-weight heparins, *N Engl J Med*. 337 (1997) 688–698. https://doi.org/10.1056/NEJM199709043371007.

[16] J. Hirsh, S.S. Anand, J.L. Halperin, V. Fuster, Mechanism of action and pharmacology of unfractionated heparin, *Arterioscler Thromb Vasc Biol*. 21 (2001) 1094–1096. https://doi.org/10.1161/HQ0701.093686.

[17] A. Zirlik, C. Bode, Vitamin K antagonists: relative strengths and weaknesses vs. direct oral anticoagulants for stroke prevention in patients with atrial fibrillation, *J Thromb Thrombolysis*. 43 (2017) 365–379. https://doi.org/10.1007/S11239-016-1446-0.

[18] J.W. Suttie, G.L. Nelsestuen, Mechanism of action of vitamin K: Synthesis of y-carboxyglutamic aci, *Critical Reviews in Biochemistry and Molecular Biology*. 8 (1980) 191–223. https://doi.org/10.3109/10409238009105469.

[19] P. Griminger, Vitamin K antagonists: the first 50 years, *J Nutr*. 117 (1987) 1325–1329. https://doi.org/10.1093/JN/117.7.1325.

[20] K. Huber, E.R. Bates, M. Valgimigli, L. Wallentin, S.D. Kristensen, J.L. Anderson, J.L. Lopez Sendon, M. Tubaro, C.B. Granger, C. Bode, E.M. Ohman, P.G. Steg, Antiplatelet and anticoagulation agents in acute coronary syndromes: what is the current status and what does the future hold?, *Am Heart J*. 168 (2014) 611–621. https://doi.org/10.1016/J.AHJ.2014.06.014.

[21] J.L. Mega, T. Simon, Pharmacology of antithrombotic drugs: an assessment of oral antiplatelet and anticoagulant treatments, *Lancet*. 386 (2015) 281–291. https://doi.org/10.1016/S0140-6736(15)60243-4.

[22] J. Fareed, D.A. Hoppensteadt, D. Fareed, M. Demir, R. Wahi, M. Clarke, C. Adiguzel, R. Bick, Survival of heparins, oral anticoagulants, and aspirin after the year 2010, *Semin Thromb Hemost*. 34 (2008) 58–73. https://doi.org/10.1055/S-2008-1066025.

[23] J. Hirsh, M. O'Donnell, J.W. Eikelboom, Beyond unfractionated heparin and warfarin: current and future advances, *Circulation*. 116 (2007) 552–560. https://doi.org/10.1161/CIRCULATIONAHA.106.685974.

[24] S.M. Bates, J.I. Weitz, The status of new anticoagulants, *Br J Haematol.* 134 (2006) 3–19. https://doi.org/10.1111/J.1365-2141.2006.06134.X.

[25] K.A. Bauer, New Anticoagulants, *Hematology*. 2006 (2006) 450–456. https://doi.org/10.1182/ASHEDUCATION-2006.1.450.

[26] E. Saiah, C. Soares, Small molecule coagulation cascade inhibitors in the clinic, *Curr Top Med Chem*. 5 (2005) 1677–1695. https://doi.org/10.2174/156802605775009702.

[27] M.R. Patel, K.W. Mahaffey, J. Garg, G. Pan, D.E. Singer, W. Hacke, G. Breithardt, J.L. Halperin, G.J. Hankey, J.P. Piccini, R.C. Becker, C.C. Nessel, J.F. Paolini, S.D. Berkowitz, K.A.A. Fox, R.M. Califf, Rivaroxaban versus warfarin in nonvalvular atrial fibrillation, *N Engl J Med*. 365 (2011) 883–891. https://doi.org/10.1056/NEJMOA1009638.

[28] S.P. Jackson, R. Darbousset, S.M. Schoenwaelder, Thromboinflammation: challenges of therapeutically targeting coagulation and other host defense mechanisms, *Blood*. 133 (2019) 906–918. https://doi.org/10.1182/BLOOD-2018-11-882993.

[29] A.G.G. Turpie, Direct Factor Xa and direct thrombin inhibitors: a clinical trial update., *Curr Opin Drug Discov Devel*. 12 (2009) 497–508.

[30] Y.K. Lee, M.R. Player, Developments in factor Xa inhibitors for the treatment of thromboembolic disorders, *Medicinal Research Reviews*. 31 (2011) 202–283. https://doi.org/10.1002/med.20183.

[31] D. Kubitza, S. Haas, Novel factor Xa inhibitors for prevention and treatment of thromboembolic diseases, *Expert Opin Investig Drugs*. 15 (2006) 843–855. https://doi.org/10.1517/13543784.15.8.843.

[32] A. Polzin, L. Dannenberg, G. Wolff, C. Helten, A. Achilles, T. Hohlfeld, T. Zeus, M. Kelm, S. Massberg, T. Petzold, Non-vitamin K oral anticoagulants (NOAC) and the risk of myocardial infarction: Differences between factor IIa and factor Xa inhibition?, *Pharmacology & Therapeutics*. 195 (2019) 1–4. https://doi.org/10.1016/J.PHARMTHERA.2018.10.005.

[33] N.R. Patel, D. v. Patel, P.R. Murumkar, M.R. Yadav, Contemporary developments in the discovery of selective factor Xa inhibitors: A review, *Eur J Med Chem*. 121 (2016) 671–698. https://doi.org/10.1016/J.EJMECH.2016.05.039.

[34] D. Mccarty, A. Robinson, Factor Xa inhibitors: a novel therapeutic class for the treatment of nonvalvular atrial fibrillation, *Therapeutic Advances in Cardiovascular Disease*. 10 (2016) 37. https://doi.org/10.1177/1753944715605011.

[35] R. de Caterina, S. Husted, L. Wallentin, F. Andreotti, H. Arnesen, F. Bachmann, C. Baigent, K. Huber, J. Jespersen, S.D. Kristensen, G.Y.H. Lip, J. Morais, L.H. Rasmussen, A. Siegbahn, F.W.A. Verheugt, J.I. Weitz, General mechanisms of coagulation and targets of anticoagulants (Section I): Position paper of the ESC Working Group on Thrombosis - Task Force on anticoagulants in heart disease, *Thrombosis and Haemostasis*. 109 (2013) 569–579. https://doi.org/10.1160/TH12-10-0772/ID/JR0772-3.

[36] V. Laux, E. Perzborn, S. Heitmeier, G. von Degenfeld, E. Dittrich-Wengenroth, A. Buchmüller, C. Gerdes, F. Misselwitz, Direct inhibitors of coagulation proteins - the end of the heparin and low-molecular-weight heparin era for anticoagulant therapy?, *Thromb Haemost*. 102 (2009) 892–899. https://doi.org/10.1160/TH09-02-0134.

[37] T.J. Milling, S. Kaatz, Preclinical and clinical data for factor Xa and "universal" reversal agents, *Am J Emerg Med*. 34 (2016) 39–45. https://doi.org/10.1016/J.AJEM.2016.09.052.

[38] S.M. Abubacker, A. Pavanchand, S.B. Basheer, K. Sriveena, R. Paul, S. Enaganti, In silico Assessment of Factor Xa Inhibitors by Docking Studies, *Vedic Research International Bioinformatics and Proteomics*. 1 (2013) 9. https://doi.org/10.14259/bp.v1i1.43.

[39] V.B. Sulimov, I. V. Gribkova, M.P. Kochugaeva, E. V. Katkova, A. V. Sulimov, D.C. Kutov, K.S. Shikhaliev, S.M. Medvedeva, M.Yu. Krysin, E.I. Sinauridze, F.I. Ataullakhanov, Application of Molecular Modeling to Development of New Factor Xa Inhibitors, *BioMed Research International*. 2015 (2015) 1–15. https://doi.org/10.1155/2015/120802.

[40] M. Batool, A. Tajammal, F. Farhat, F. Verpoort, Z.A.K. Khattak, Mehr-Un-Nisa, M. Shahid, H.A. Ahmad, M.A. Munawar, M. Zia-Ur-Rehman, M.A.R. Basra, Molecular docking, computational, and antithrombotic studies of novel 1,3,4-oxadiazole derivatives, *International Journal of Molecular Sciences.* 19 (2018) 1–39. https://doi.org/10.3390/ijms19113606.

[41] R. Sharmila, K.M. Subburathinam, S. Aishwarya, A.A. Margret, In-Silico Analysis of Andrographolide against Cancer, 5 (2013) 56–61.

[42] X.-Y. Meng, H.-X. Zhang, M. Mezei, M. Cui, Molecular Docking: A powerful approach for structure-based drug discovery, *Curr Comput Aided Drug Des*. 7 (2011) 146.

[43] F.H. Mohamed, A.M. Shalaby, H.A. Soliman, A.Z. Abdelazem, M.M. Mounier, E.S. Nossier, G.O. Moustafa, Design, Synthesis and Molecular Docking Studies of Novel Cyclic Pentapeptides Based on Phthaloyl Chloride with Expected Anticancer Activity, *Egyptian Journal of Chemistry*. 63 (2020) 1723–1736. https://doi.org/10.21608/EJCHEM.2019.18452.2137.

[44] A.M. El-Maghraby, A.H. Abdelmonsef, Synthesis, Characterization and In silico Molecular Docking Studies of Novel Chromene Derivatives as Rab23

Inhibitors, *Egyptian Journal of Chemistry*. 63 (2020) 1341–1358. https://doi.org/10.21608/EJCHEM.2019.15013.1911.

[45] A.M. Vijesh, A.M. Isloor, S. Telkar, T. Arulmoli, H.K. Fun, Molecular docking studies of some new imidazole derivatives for antimicrobial properties, *Arabian Journal of Chemistry*. 6 (2013) 197–204. https://doi.org/10.1016/j.arabjc.2011.10.007.

[46] I.A. Guedes, C.S. de Magalhães, L.E. Dardenne, Receptor-ligand molecular docking, *Biophysical Reviews*. 6 (2014) 75–87. https://doi.org/10.1007/s12551-013-0130-2.

[47] A.J. Trott, O., Olson, AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading, *Journal of Computational Chemistry*. 31 (2010) 455–461. https://doi.org/10.1002/jcc.21334.AutoDock.

[48] R.A. Alsuhaimat, Q. Abualassal, Z.H. Abudayeh, S.S. Ebada, A. Albohy, Synthesis and Docking Studies of a Novel Tetrahydroquinazoline Derivative as Promising Scaffold for Acetylcholine Esterase Inhibition, *Egyptian Journal of Chemistry*. 63 (2020) 4797–4804. https://doi.org/10.21608/EJCHEM.2020.27985.2588.

[49] A.A. El-Sayed, M.M.T. El-Saidi, R.R. Khattab, Unexpected Reactions of Azido-p-Benzoquinone Derivatives Towards Lawesson's Reagent and Molecular Docking Study as a Promising Anticancer Agent, *Egyptian Journal of Chemistry*. 62 (2019) 315–326. https://doi.org/10.21608/EJCHEM.2019.17488.2074.

[50] G. Sabbagh, N. Berakdar, Docking studies of flavonoid compounds as inhibitors of β-ketoacyl acyl carrier protein synthase I (Kas I) of Escherichia coli, *Journal of Molecular Graphics and Modelling*. 61 (2015) 214–223. https://doi.org/10.1016/j.jmgm.2015.07.005.

[51] F. Sangande, E. Julianti, D.H. Tjahjono, Ligand-Based Pharmacophore Modeling, Molecular Docking, and Molecular Dynamic Studies of Dual Tyrosine Kinase Inhibitor of EGFR and VEGFR2, *International Journal of Molecular Sciences*. 21 (2020) 7779. https://doi.org/10.3390/ijms21207779.

[52] G. Sabbagh, B. Kurdi, W. Khayata, R. Lahdo, A Study on the Inhibitory Potential of Dpp-Iv Enzyme by Lobeline through In silico and In vivo Approaches, *International Research Journal of Pure and Applied Chemistry*. 22 (2021) 79–91. https://doi.org/10.9734/irjpac/2021/v22i130376.

[53] G. Sabbagh, T. Murad, In Silico Study of Novel Fluoroquinolones as Inhibitors of Topoisomerase IV of Acinetobacter Baumannii, *International Journal of Pharmaceutical Sciences and Nanotechnology*. 9 (2016) 3287–3298. https://doi.org/10.37285/IJPSN.2016.9.3.2.

[54] A. Rauf, S. Bawazeer, J. Herrera-Bravo, M. Raza, H. Naz, S. Gul, N. Muhammad, Z.M. Almarhoon, Y.N. Mabkhot, M.F. Ramadan, W.N. Setzer, S.D. Daştan, S. Mahmud, J. Sharifi-Rad, Potent In Vitro Phosphodiesterase 1 Inhibition of Flavone Isolated from Pistacia integerrima Galls, *BioMed Research International*. 2022 (2022) 1–6. https://doi.org/10.1155/2022/6116003.

[55] P. Fan, Y. Gao, M. Zheng, T. Xu, P. Schoenhagen, Z. Jin, Recent progress and market analysis of anticoagulant drugs., *J Thorac Dis*. 10 (2018) 2011–2025. https://doi.org/10.21037/jtd.2018.03.95.

[56] C.M. Kessler, J.N. Goldstein, Clinical Roundtable Monograph A New Strategy for Uncontrollable Bleeding After Treatment With Rivaroxaban or Apixaban Discussants, *Clinical Advances in Hematology & Oncology*. 17 (2019) 20.

[57] N. Vene, A. Mavri, M. Gubenšek, G. Tratar, T. Vižintin Cuderman, M. Pohar Perme, A. Blinc, Risk of Thromboembolic Events in Patients with Non-Valvular Atrial Fibrillation After Dabigatran or Rivaroxaban Discontinuation - Data from the Ljubljana Registry., *PLoS One*. 11 (2016) e0156943. https://doi.org/10.1371/journal.pone.0156943.

[58] J.A. Beutler, Natural Products as a Foundation for Drug Discovery., *Curr Protoc Pharmacol*. 46 (2009) 9.11.1-9.11.21. https://doi.org/10.1002/0471141755.ph0911s46.

[59] D.J. Newman, G.M. Cragg, Natural Products as Sources of New Drugs over the Nearly Four Decades from 01/1981 to 09/2019, Jo*urnal of Natural Products.* 83 (2020) 770–803. https://doi.org/10.1021/acs.jnatprod.9b01285.

[60] S. Rashid, H. Iftikhar, Molecular docking studies of flavonoids for their inhibition pattern against β-catenin and pharmacophore model generation from experimentally known flavonoids to fabricate more potent inhibitors for Wnt signaling pathway, *Pharmacognosy Magazine*. 10 (2014) 264. https://doi.org/10.4103/0973-1296.133269.

[61] G. Sabbagh, N. Berakdar, Molecular docking study of flavonoid compounds as inhibitors of β-ketoacyl acyl carrier proteinsynthase II (KAS II) of pseudomonas aeruginosa, *International Journal of Pharmacy and Pharmaceutical Sciences*. 8 (2016) 52–61.

[62] S.H. Manjunath, R.K. Thimmulappa, Antiviral, immunomodulatory, and anticoagulant effects of quercetin and its derivatives: Potential role in prevention and management of COVID-19, *Journal of Pharmaceutical Analysis*. (2021). https://doi.org/10.1016/j.jpha.2021.09.009.

[63] Ş. Taştemur, H. Ataseven, Quercetin in the treatment and prevention of COVID-19 tedavi ve profilaksisinde Quercetin, 939856 (2021) 100–116.

[64] N.M. Abd El-Aziz, O.M. Eldin Awad, M.G. Shehata, S.A. El-Sohaimy, Inhibition of COVID-19 RNA-Dependent RNA Polymerase by Natural Bioactive Compounds: Molecular Docking Analysis., *Egyptian Journal of Chemistry.* 64 (2021) 1989–2001. https://doi.org/10.21608/EJCHEM.2021.45739.2947.

[65] K. Patel, D.K. Patel, The Beneficial Role of Rutin, A Naturally Occurring Flavonoid in Health Promotion and Disease Prevention: A Systematic Review and Update, in: Bioactive Food as Dietary Interventions for Arthritis and Related Inflammatory Diseases, *Elsevier*, 2019: pp. 457–479. https://doi.org/10.1016/B978-0-12-813820-5.00026-X.

[66] A. Ganeshpurkar, A.K. Saluja, The Pharmacological Potential of Rutin., *Saudi Pharm J.* 25 (2017) 149–164. https://doi.org/10.1016/j.jsps.2016.04.025.

[67] D. Sahu, S.C. Bishwal, Z. Malik, S. Sahu, S.R. Kaushik, S. Sharma, E. Saini, R. Arya, A. Rastogi, S. Sharma, S. Sen, R.K. Brojen Singh, R.K. Nanda, A.K. Panda, Troxerutin acts on complement mediated inflammation to ameliorate arthritic symptoms in rats., *BioRxiv*. (2020). https://doi.org/10.1101/2020.08.18.253427.

[68] S. Sampath, B. Karundevi, Effect of troxerutin on insulin signaling molecules in the gastrocnemius muscle of high fat and sucrose-induced type-2 diabetic adult male rat, *Molecular and Cellular Biochemistry*. 395 (2014) 11–27. https://doi.org/10.1007/s11010-014-2107-2.

[69] H. Malinska, M. Hüttl, O. Oliyarnyk, I. Markova, M. Poruba, Z. Racova, L. Kazdova, R. Vecera, Beneficial effects of troxerutin on metabolic disorders in non-obese model of metabolic syndrome, *PLOS ONE*. 14 (2019) e0220377. https://doi.org/10.1371/journal.pone.0220377.

[70] M. Bijak, R. Ziewiecki, J. Saluk, M. Ponczek, I. Pawlaczyk, H. Krotkiewski, B. Wachowicz, P. Nowak, Thrombin inhibitory activity of some polyphenolic compounds, *Medicinal Chemistry Research*. 23 (2014) 2324–2337. https://doi.org/10.1007/s00044-013-0829-4.

[71] N.A. Al-Shabib, J.M. Khan, A. Malik, M.A. Alsenaidy, M.T. Rehman, M.F. AlAjmi, A.M. Alsenaidy, F.M. Husain, R.H. Khan, Molecular insight into binding behavior of polyphenol (rutin) with beta lactoglobulin: Spectroscopic, molecular docking and MD simulation studies, *Journal of Molecular Liquids*. 269 (2018) 511–520. https://doi.org/10.1016/j.molliq.2018.07.122.

[72] S. Cristina, G. Gomes, L. Tainá, T. Tobias, Cardioprotective Effect of Troxerutin : An In silico Study, *Asian Journal of Emerging Research*, 2 (2020) 35–42. https://doi.org/ajerpk.

[73] S. Roehrig, A. Straub, J. Pohlmann, T. Lampe, J. Pernerstorfer, K.-H. Schlemmer, P. Reinemer, E. Perzborn, Discovery of the Novel Antithrombotic Agent 5-Chloro- *N* -({(5 *S*)-2-oxo-3- [4-(3-oxomorpholin-4-yl)phenyl]-1,3-oxazolidin-5-yl}methyl)thiophene- 2-carboxamide (BAY 59-7939): An Oral, Direct Factor Xa Inhibitor, *Journal of Medicinal Chemistry*. 48 (2005) 5900–5908. https://doi.org/10.1021/jm050101d.

[74] Y. Rose, J.M. Duarte, R. Lowe, J. Segura, C. Bi, C. Bhikadiya, L. Chen, A.S. Rose, S. Bittrich, S.K. Burley, J.D. Westbrook, RCSB Protein Data Bank: Architectural Advances Towards Integrated Searching and Efficient Access to Macromolecular Structure Data from the PDB Archive, *Journal of Molecular Biology*. 433 (2021) 166704. https://doi.org/10.1016/j.jmb.2020.11.003.

[75] C. Zardecki, S. Dutta, D.S. Goodsell, R. Lowe, M. Voigt, S.K. Burley, PDB-101: Educational resources supporting molecular explorations through biology and medicine., *Protein Sci*. 31 (2022) 129–140. https://doi.org/10.1002/pro.4200.

[76] F.A. Klok, M.J.H.A. Kruip, N.J.M. van der Meer, M.S. Arbous, D.A.M.P.J. Gommers, K.M. Kant, F.H.J. Kaptein, J. van Paassen, M.A.M. Stals, M. V. Huisman, H. Endeman, Incidence of thrombotic complications in critically ill ICU patients with COVID-19, *Thrombosis Research*. 191 (2020) 145–147. https://doi.org/10.1016/j.thromres.2020.04.013.

[77] J.J. Irwin, K.G. Tang, J. Young, C. Dandarchuluun, B.R. Wong, M. Khurelbaatar, Y.S. Moroz, J. Mayfield, R.A. Sayle, ZINC20—A Free Ultralarge-Scale Chemical Database for Ligand Discovery, *Journal of Chemical Information and Modeling*. 60 (2020) 6065–6073. https://doi.org/10.1021/acs.jcim.0c00675.

[78] J.J.I.K. Shoichet, (1) ZINC − A Free Database of Commercially Available Compounds for Virtual Screening | Request PDF, *Journal of Chemical Information and Modeling*. (2005). https://doi.org/10.1021/ci049714+.

[79] N.M. O'Boyle, M. Banck, C.A. James, C. Morley, T. Vandermeersch, G.R. Hutchison, Open Babel: An Open chemical toolbox, *Journal of Cheminformatics*. 3 (2011) 1–14. https://doi.org/10.1186/1758-2946-3-33/TABLES/2.

[80] Mohd.A. Rauf, S. Zubair, A. Azhar, Ligand docking and binding site analysis with pymol and autodock/vina, *International Journal of Basic and Applied Sciences*. 4 (2015) 168. https://doi.org/10.14419/ijbas.v4i2.4123.

[81] T.H. Keller, A. Pichota, Z. Yin, A practical view of "druggability," *Current Opinion in Chemical Biology*. 10 (2006) 357–361. https://doi.org/10.1016/j.cbpa.2006.06.014.

[82] K.C. Hsu, Y.F. Chen, S.R. Lin, J.M. Yang, Igemdock: A graphical environment of enhancing gemdock using pharmacological interactions and post-screening analysis, *BMC Bioinformatics*. 12 (2011). https://doi.org/10.1186/1471-2105-12-S1-S33.

[83] S. Yuan, H.C.S. Chan, Z. Hu, Using PyMOL as a platform for computational drug design, *WIREs Computational Molecular Science*. 7 (2017). https://doi.org/10.1002/wcms.1298.

[84] S.S. Pawar, S.H. Rohane, Review on Discovery Studio: An important Tool for Molecular Docking, *Asian Journal Of Research in Chemistry*. 14 (2021) 1–3. https://doi.org/10.5958/0974-4150.2021.00014.6.

[85] S. Shaweta, S. Akhil, G. Utsav, Molecular Docking studies on the Anti-fungal activity of Allium sativum (Garlic) against Mucormycosis (black fungus) by BIOVIA discovery studio visualizer 21.1.0.0, *Annals of Antivirals and Antiretrovirals*. (2021) 028–032. https://doi.org/10.17352/aaa.000013.

[86] Y. Dinakarkumar, J.R. Rajabathar, S. Arokiyaraj, I. Jeyaraj, S.R. Anjaneyulu, S. Sandeep, C.S. Karthik, J.N. Appaturi, L.D. Wilson, Anti-Methanogenic Effect of Phytochemicals on Methyl-Coenzyme M Reductase—Potential: In Silico and Molecular Docking Studies for Environmental Protection, *Micromachines (Basel)*. 12 (2021). https://doi.org/10.3390/MI12111425.

[87] H. Sugihara, Y. Idemoto, T. Kuwano, Y. Nagata, J. Morii, M. Sugihara, M. Ogawa, S.-I. Miura, K. Saku, Evaluation of the Antithrombotic Effects of Rivaroxaban and Apixaban Using the Total Thrombus Formation Analysis System®: In Vitro and Ex Vivo Studies, *Original Article J Clin Med Res*. 8 (2016) 899–907. https://doi.org/10.14740/jocmr2773w.

[88] M. Bijak, M.B. Ponczek, P. Nowak, Polyphenol compounds belonging to flavonoids inhibit activity of

coagulation factor X, *International Journal of Biological Macromolecules*. 65 (2014) 129–135. https://doi.org/10.1016/j.ijbiomac.2014.01.023.
[89] J.H. Choi, K.J. Kim, S. Kim, Comparative Effect of Quercetin and Quercetin-3-O-β-d-Glucoside on Fibrin Polymers, Blood Clots, and in Rodent Models, *Journal of Biochemical and Molecular Toxicology*. 30 (2016) 548–558. https://doi.org/10.1002/jbt.21822.
[90] L.T.T. Tobias, S.C.G. Gomes, L.L. Borges, T.M. Alquimim, L.S.F. Sá, M.H.D.S. Pitta, L.C. Pina, L.R. Pedro, R.M.M.O. Filho, G.C. Pina, TROXERUTIN AS A POTENTIAL THROMBIN INHIBITING DRUG, *Hematology, Transfusion and Cell Therapy*. 42 (2020) 100–101. https://doi.org/10.1016/j.htct.2020.10.170.
[91] S. Cristina, G. Gomes, L. Tainá, T. Tobias, L.L. Borges, Cardioprotective Effect of Troxerutin: An In silico Study, *Asian Journal of Emerging Research*. 2 (2020) 35–42. https://doi.org/10.3923/ajerpk.2020.35.42.
[92] M.A. Dar, N. Tabassum, Rutin-potent natural thrombolytic agent, 2012. http://www.icpjonline.com/documents/Vol1Issue12/07.pdf.
[93] X. Wang, Z. Yang, F. Su, J. Li, E.O. Boadi, Y. xu Chang, H. Wang, Study on Structure Activity Relationship of Natural Flavonoids against Thrombin by Molecular Docking Virtual Screening Combined with Activity Evaluation In Vitro, *Molecules*. 25 (2020). https://doi.org/10.3390/MOLECULES25020422.

الكشف عن نشاط مضادات التخثر في السيليكو وفي المختبر لمادة الكويرسيتين والروتين والتروكسيروتين كمثبطات العاشر المُنشط محتملة جديدة لعامل التخثر(FXa)

غالية صباغ [أ*]، لارا البيك [أ] ، إبراهيم حديد [ب]

[أ] قسم الكيمياء الصيدلية و المراقبة الدوائية، كلية الصيدلة، جامعة حلب، سوريا.
[ب] قسم جراحة المسالك البولية، كلية الطب، جامعة حلب، سوريا

الملخص:

يُعتبر العامل العاشر المُنشط هدف مهم لتطوير مضادات تخثر جديدة. تهدف الدراسة الحالية إلى فحص تأثير ثلاثة مركبات (الكويرستين و مشتقيه الروتين و التروكسيروتين) بالمقارنة مع الريفاروكسبان على عامل التخثر البشري العاشر المنشط (PDB ID: 2w26). تم إجراء الإرساء الجزيئي باستخدام برنامج iGemDock v2.1 للتنبؤ بنشاطهم المثبط للعامل العاشر المنشط وبرنامج Autodock vina للتحقق من الروابط المتكونة. بعد ذلك، تمت دراسة التأثيرات البيولوجية في المختبر على كل من زمن تخثر PT و aPTT. أظهر التروكسيروتين و الروتين أفضل قيم ارتباط مع إنزيم 2w26، وكانت طاقة الربط [(-185.743 kcal / mol) و (-178.034 kcal / mol)] على التوالي باستخدام iGemDock؛ بينما كانت طاقة ربط الريفاروكسابان (-149.661 kcal / mol). أظهرت نتائج الدراسة في المختبر أنَّ المركبات المدروسة لها تأثير في إطالة أزمنة الـ PT، aPTT مقارنة بكل من الشاهد السلبي والإيجابي. ومع ذلك، أعطى التروكسيروتين أعلى إطالة في أوقات التخثر المُقيّمة، بما يتوافق مع نتائج الدراسة في السيليكو.

الكلمات الرئيسية: مضادات التخثر؛ العامل العاشر؛ الارساء الجزيئي ؛ الريفاروكسابان؛ التروكسيروتين.