In Silico Prediction of High Potential Jararhagin Inhibitor: Comparison of Batimastat, EDTA and Hydroxytyrosol

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Abstract: One example of a group P-III SVMP is jararhagin which originates from a *Bothrops jararaca*. This study was conducted to compare the possibility of inhibitors that have the highest effectiveness and exact time of each compound to inhibit hemorragic effect of SVMP. Inhibition of hemorrhagic activity can be done with several types of compounds that have been known to be inhibitors for SVMP especially jararhagin (PDB ID: 1C9G) to bind with integrin $\alpha_2\beta_1$ (PDB ID: 1AOX). There are batimastat (PubChem ID: 5362422) as one of peptidomimetic compounds, EDTA (PubChem ID: 6049) as one of zinc chelating agents, and plant compounds such as hydroxytyrosol (PubChem ID: 82755). The batimastat inhibitory properties from value of binding energy, found that these inhibitor were more easily bound to jararhagin (-289.0 kcal/mol) compared to integrin $\alpha_2\beta_1$ (-277.1 kcal/mol). That inhibitor also more effectively inhibited by bounding to jararhagin spread in blood vessels after snakebite because of it's position and more positive binding energy (-784.1 kcal/mol). However, unfavorable bonds are formed in the interaction between batimastat inhibitors, jararhagin and integrin $\alpha_2\beta_1$. In inhibitor EDTA interaction, it was found that this compound also more easily bound to jararhagin (-227.23 kcal/mol), but this inhibitor are more effectively inhibited by bounding to integrin $\alpha_2\beta_1$ because of it's position and more positive binding energy (-721.57 kcal/mol). In other side it also has unfavorable bonds. While the interaction of hydroxytyrosol shows that inhibitor are easier to interact with jararhagin and more effectively acts as a jararhagin inhibitor by being consumed after the body is exposed to jararhagin (-781.33 kcal/mol) without showing an unfavorable bond. We can conclude that the natural inhibitors formed in hydroxytyrosol from olive oil are more stable and have highest possibility in preventing hemorrhagic symptoms due to snake bites that contain jararhagin venom.

1 INTRODUCTION

Envenomation is one of dangerous health problem because of the death risk. There are 5,5 millions envenomation cases annually. Snake venom contains mixture of various proteins or protein families with different bioactivities and tissue target (Williams et.al., 2010). The examples of protein families in snake venom are snake venom metalloproteinase (SVMP), snake venom serine proteinase (SVSP), cysteine-rich secretory protein (CRiSP), phospholipase A_2, phospholipase type B, C-type lectin-like protein, L-amino acid oxidase (LAAO), 3 finger toxin (3FTx), et cetera (Kunalan et.al., 2018).

SVMP (Snake Venom Metalloproteinase) is one of protein family in Elapidae and Viperidae snake venom. Up to 30% Viperidae venom is consist of SVMP (Silva et.al., 2016).

SVMP is a zinc-dependent hydrolase which has catalytic zinc ion in the active site. The catalysis process of this enzyme needs zinc ion (Zn^{2+}) as the mediator, zinc ion is coordinated with 3 side chain of histidine and water molecule binded with glutamate residue (Preciado et.al., 2018). SVMP has the hemorrhagic effect and fibrynogenolytic. It can cleave $A\alpha$ and $B\beta$ chain on fibrynogen. SVMP can degrade some of extracellular matrix proteins i.e. collagen IV, laminine, fibronectin, and proteoglycan perlecan. SVMP can act as the mediator of local tissue damage, and induce the endothelial cell hemorrhage as well. The damage of endothelial cell and basal membrane on blood vessels will helps the toxic protein spread to the tissue target (Pithayanukul et.al., 2009). Jararhagin is a member of SVMP protein family with high hemorrhagic effect. It is a 52 kDa PIIIb SVMP, the first

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metalloproteinase isolated from *B. jararaca* (Ferreira et.al., 2018).

The specific treatment of envenomation case is conducted by antivenom treatment. According to WHO (2010), antivenom or antivenin is consisted of pure immunoglobulin fragment from animal plasma which have been immunized by snake venom. Antivenom treatment has several disadvantages. Antivenom only neutralize any of the venoms used in its production, or from closely related species. It needs suitable storage condition because of the sensitive components in antivenom. It is unsuitable to neutralize the local tissue damage (Preciado et.al., 2018). Antivenom has high effectivity in neutralizing systemic effect of a venom, but it has low effectivity in neutralizing local effect of snake venom, i.e. effect of SVMP (Romero et.al., 2012).

Enzyme inhibitor has become a potention to treat local tissue damage effect of SVMP. Some compounds which are known as inhibitor of SVMP are peptidomimetic, zinc chelating agent, and phenolic compound. Peptidomimetic such as Batimastat and Marimastat; zinc chelating agents such as EDTA, DTPA, TTD; and phenolic compounds are known as SVMP enzyme inhibitor (Preciado et.al., 2018). But there is no knowledge about which compound is most effective to inhibit SVMP hemorrhagic activity. The aim of this study is to compare Batimastat (peptidomimetic), EDTA (zinc chelating agent), and hydroxytyrosol (phenolic compound) as the most effective inhibitor of jararhagin SVMP.

2 MATERIALS AND METHODS

2.1 Protein and Ligand Structure Preparation

We use the structure of 2 proteins, the snake venom metalloproteinase (SVMP) jararhagin (PDB ID: 1C9G) and $\alpha_2\beta_1$ intregin (PDB ID: 1AOX). Protein structure was downloaded from RCSB PDB database (https://www.rcsb.org/). We also use structure of 3 ligands, Bastimastat (PubChem ID: 5362422), EDTA (PubChem ID: 6049), and Hydroxytyrosol (PubChem ID: 82755). Ligand structure was downloaded from PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Preparation of protein structure was conducted using *Discovery studio* 16.1.0 software. Each protein (jararhagin and $\alpha_2\beta_1$ intregin) was prepared by deleting the water and ligand molecule. Protein structures then saved as PDB format (.pdb). The ligand structure was prepared using PyRx software. Ligands was prepared by minimizing free energy and converted to PDB format.

2.2 Docking and Visualization

The docking of jararhagin and ligand with $\alpha_2\beta_1$ integrin collagen receptor on the cell-surface of platelet were carried out in two types of conditions to determine the effectiveness and efficiency of inhibitors. The first condition is interaction between jararhagin-ligand complex and $\alpha_2\beta_1$ integrin. The second condition is interaction between $\alpha_2\beta_1$ integrin-ligand complex and jararhagin. Since we used 3 kind of ligands (Batimastat, EDTA, and hydroxytyrosol), there are 6 types of interaction between jararhagin, ligand, and $\alpha_2\beta_1$ integrin. The docking was performed using HEX 8.0.0. Docking results were visualized using *Discovery studio* 16.1.0.

3 RESULT AND DISCUSSION

3.1 The Interaction of Jarharagin with Batimastat and A2β1 Integrin

Docking energy between jararhagin (green) and batimastat (red) ligand is -289.0 kcal/mol. Whereas if the results of jararhagin-batimastat docking are re-docked with the integrin receptor $\alpha_2\beta_1$ (α: blue, β: violet) , the docking energy decreases with a value of -784.1 kcal/mol. The interaction between (jararaghin + batimastat) and integrin $\alpha_2\beta_1$ are a first condition that indicated treatment of batimastat after envenomation. This condition has 10 of favorable bonds. Two conventional hydrogen bonds that binding the amino acid residues of Leu53 with atom H and O on Ligan 1. Then 2 carbon-hydrogen bonds between Ligand with the amino acid residues Leu53 and Tyr53. One Pi bond with sulfur with amino acid residue Tyr7. One Pi bond with a lone pair is Asn38. Three hydrophobic bonds with a Pi type with alkyl are all three Lig1 with Ala52, Pro202, and Ala37. One type hydrophobic bond between Pi is amino acid residue Tyr7. There are also appear 6 pieces that are unfavorable, which is appear in 2 kind of amino acid residue like Asn42 and His50.

The interaction between jararhagin and the docking results of batimastat and integrins $\alpha_2\beta_1$. The docking energy of the batimastat and integrin $\alpha2\beta1$ is -277.1 kcal/mol. If the result of the docking is docked again with a fault, the value of the docking energy becomes -857.5 kcal/mol. The interaction of

jararhagin and (Batimastat + Integrin $\alpha_2\beta_1$) are second condition that indicate treatment of batimastat before envenomation. This condition has 4 favorable binding bonds, each of which has a different type of bond. The first bond is a conventional hydrogen bond that binds with amino acid residue Asn27, then carbon bonds with hydrogen that binds atom H with amino acid residues Ala245, alkyl bonds that bind atom C with amino acid Leu276, sulfur bond with X which binds atom S with Glys338 amino acid residu. The last, there are also 3 unfavorable bond that appeat in 2 kinds of amino acid residues like Gly338 and Asn274. The docking results between jararhagin and integrin wich are a presumed condition if jararhagin envenomation occure. produce docking energy of -841.3 kcal/mol.

The result show that batimastat is easier to interact with jararhagin than the integrin receptor α2β1 because the energy used to interact with jararhagin is smaller than the interaction with the integrin receptor α2β1. Morover, the docking condition 2 that is batimastat-intgerine complex α2β1 interaction with jararhagin has a lower (negative) docking energy value of -841.3 kcal / mol compared with the jararhagin-batimastate complex interaction with the α2β1 integrin the value is higher (positive) which is -784.1 kcal / mol. So that the batimastatic-integrin α2β1complex that was interacted with jararahgin was less effective in inhibiting the enzyme formation of jararhagin because batimastate was easier to tie jararhagine than before binding to the integrin α2β1.Lower docking energy shows that the bonds between protein requires more energy to bind, so that it can be used to inhibit jararhagin for integrin receptors.

In addition, when viewed from a 3-dimensional structure, it can be seen from the 3-dimensional interaction that the batimastat ligand is positioned between jararhagin and the integrin domain α2 (Figure 1F). This is in accordance with the reference which states that jararhagin will bind to integrins in the α2 domain then integrin β1 cleavage, even with low docking energy that allows easy unbonding. Batimastat inhibits jararhagin which contains ZBG or zinc binding group by cleavage BaP1 protein between dermal-epidermal formed from basic membrane components. High docking energy also shows that the bonds between molecules are strong so they are not easily released (Jimenez et.al., 2008). Integrin-binding motif $\alpha_2\beta_1$ is located to or within the hyper-variable region of the cycstein-rich domain. Part that causes inhibition of platelet aggregation is the catalytic or proteolytic site that interacts with the integrin $\alpha_2\beta_1$ so that it will trigger signal transduction on platelets (Tanjoni et.al., 2010).

The atomic interactions between batimastats and jararhagin interact more than the atomic interactions between batimastats and integrins α2β1. The interaction of the jararhagin-batimastat complex with integrins α2β1 has more hydrophobic and hydrogen bonds than the complex interactions of jararhagin-integrin α2β1 with jararhagin. Conventional hydrogen bonds are stabilizing bonds in biomolecular structures. Hydrogen bonds occur between proton donor groups. The donor part is an electronegative element and the acceptor group is a free electron pair or phi bond, especially on oxygen and nitrogen atoms (Horowitz & Trievel, 2012).

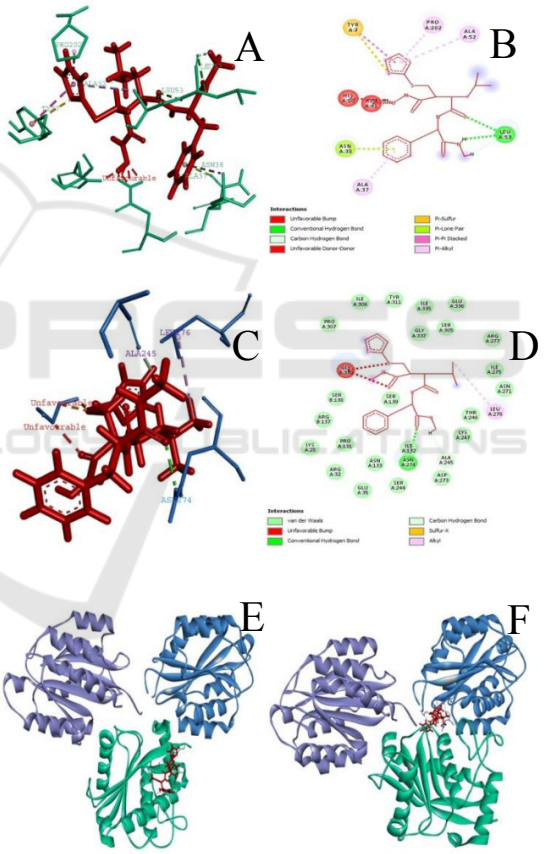

Figure 1. Interaction of Jararhagin, Batimastat, and Integrin $\alpha_2\beta_1$. A. Ligan interaction between complex jararhagin-batimastat and integrin $\alpha_2\beta_1$ (3D). B. Ligan interaction between complex jararhagin-batimastat and integrin $\alpha_2\beta_1$ (2D). C. Ligan interaction between complex integrin $\alpha_2\beta_1$ -batimastat and jararhagin (3D). D. Ligan interaction between complex integrin $\alpha_2\beta_1$ -batimastat and jararhagin (2D). E. Interaction complex jararhagin-batimastat and integrin $\alpha_2\beta_1$. F. Interaction complex integrin $\alpha_2\beta_1$-batimastat and jararhagin.

Table 1. Bonding interactions between the jararhagin batimastat complex and the integrin receptor α2β1.

Name	Distance (Å)	Category	Type	From Chemistry	To Chemistry
A:LEU53:HN - A:LIG1:O	2,64948Å	Hydrogen Bond	Conventional Hydrogen Bond	H-Donor	H-Acceptor
A:LIG1:H - A:LEU53:O	2,89807Å	Hydrogen Bond	Conventional Hydrogen Bond	H-Donor	H-Acceptor
A:ALA52:HA - A:LIG1:O	2,37972Å	Hydrogen Bond	Carbon Hydrogen Bond	H-Donor	H-Acceptor
A:LIG1:H - A:LEU53:O	2,13003Å	Hydrogen Bond	Carbon Hydrogen Bond	H-Donor	H-Acceptor
A:LIG1:S - A:TYR7	4,163Å	Other	Pi-Sulfur	Sulfur	Pi-Orbitals
A:ASN38:OD1 - A:LIG1	2,82212Å	Other	Pi-Lone Pair	Lone Pair	Pi-Orbitals
A:LIG1 - A:TYR7	4,23106Å	Hydrophobic	Pi-Pi Stacked	Pi-Orbitals	Pi-Orbitals
A:LIG1 - A:ALA52	5,49552Å	Hydrophobic	Pi-Alkyl	Pi-Orbitals	Alkyl
A:LIG1 - A:PRO202	4,09041Å	Hydrophobic	Pi-Alkyl	Pi-Orbitals	Alkyl
A:LIG1 - A:ALA37	4,39295Å	Hydrophobic	Pi-Alkyl	Pi-Orbitals	Alkyl
A:ASN41:ND2 - A:LIG1:O	1,84028	Unfavorable	Unfavorable Bump	Steric	Steric
A:ASN41:ND2 - A:LIG1:H	1,46595	Unfavorable	Unfavorable Bump	Steric	Steric
A:ASN41:HD21 - A:LIG1:O	1,76436	Unfavorable	Unfavorable Bump	Steric	Steric
A:ASN41:HD22 - A:LIG1:O	1,45353	Unfavorable	Unfavorable Bump	Steric	Steric
A:ASN41:HD22 - A:LIG1:H	0,646487	Unfavorable	Unfavorable Bump;Unfavorable Donor-Donor	Steric;H-Donor	Steric;H-Donor
A:HIS50:HD1 - A:LIG1:H	1,52363	Unfavorable	Unfavorable Donor-Donor	H-Donor	H-Donor

Table 2. Bonding interactions between the batimastat integrin α2β1 complex and jararhagin.

Name	Distance (Å)	Category	TYPE	From Chemistry	To Chemistry
A:ASN274:HD21 - B:LIG1:O	2,1888Å	Hydrogen Bond	Conventional Hydrogen Bond	H-Donor	H-Acceptor
B:LIG1:H - A:ALA245:O	1,6891Å	Hydrogen Bond	Carbon Hydrogen Bond	H-Donor	H-Acceptor
B:LIG1:S - A:GLY338:N	2,4186Å	Other	Sulfur-X	Sulfur	O,N,S
B:LIG1:C - A:LEU276	4,5753Å	Hydrophobic	Alkyl	Alkyl	Alkyl
A:ASN274:HB1 - B:LIG1:O	1,67052	Unfavorable	Unfavorable Bump	Steric	Steric
A:GLY338:CA - B:LIG1:O	2,23482	Unfavorable	Unfavorable Bump;Carbon Hydrogen Bond	Steric;H-Donor	Steric;H-Acceptor
A:GLY338:HN - B:LIG1:S	1,83166	Unfavorable	Unfavorable Bump	Steric	Steric

The results of research on hemorrhagic ability by jararhagin in the lungs and skin with experimental animals showed that batimastat was able to reduce hemorrhagic activity in these organs. Other results showed that jararhagin incubated with batimastat and inserted into intradermal mice showed that there was a reduction in hemorrhagic diameter in experimental animals compared to incubation of jararhagin with human 2-macroglobulin and normal serum mouse (Escalante et.al., 2003).

3.2 The Interaction of Jararhagin with A2β1 Integrin and EDTA

The first condition is the interaction between jararhagin-EDTA complex which requires an energy of -227.23 kcal/mol to bound each other. This interaction are made by $\alpha_2\beta_1$ integrin receptor which is a common target of the inhibition process by jararhagin. The energy needed from the ligand complex jararhagin-EDTA to bound with I domains of α_2 (colored blue) is -733.96 kcal/mol. While the position of EDTA is area that does not interact with integrin amino acid residues (jararhagin is green) (Figure 2E). The second condition is an alternative interaction between the $\alpha_2\beta_1$-EDTA complex which requires higher energy, which is -215.46 kcal/mol to bound with I domain of α_2. That interaction made with jararhagin being the natural inhibitor of the integrin receptor $\alpha_2\beta_1$. The energy required from the receptor complex $\alpha_2\beta_1$-EDTA to bound with the disintegrin-like domain of jararhagin (green) is -721.57 kcal/mol (Figure 2F). This condition showed that EDTA is more easily bound with jararhagin compared to $\alpha_2\beta_1$ integrin. In addition, when jararhagin-EDTA are the ligand complexed it required lower energy to bound to platelet integrin surface receptors, compared to the energy needed when EDTA in the form of receptor complexed with integrins. While the tendency of bond energy and bounding-site position between the receptor complex and jararhagin indirectly indicates that inhibition by EDTA is more effective by bounding to the integrin $\alpha_2\beta_1$ even before the venom spreads in the blood vessels after the envenomation.

Furthermore, the interaction between the jararaghin-EDTA ligand complex and $\alpha_2\beta_1$ integrins forms 5 kind of favorable bonds (Figure 2F). Four of them are conventional hydrogen bonds with an average distance of 2.3 Å to 2.9 Å that can be classify as strong until medium H-bond. Strong covalent H-bond have length 2.2 Å - 2.5 Å whereas moderate mostly electrostatic have length of distance 2.5 Å -3.2 Å (Baey, 2013). The hydrogen bond bound to asparagine amino acid residues (Asn194), proline (Pro195), tyrosine (Tyr5), and aspartic acid (Asp3). Then also formed one carbon hydrogen bond is formed at A LIG: 1. A hydrogen bond interaction span a large interval, ranging from tiny energies to large values when the acceptor is an anion that can devise interaction stability. Hydrogen bond is generally also stronger interaction, but still less stable than van der Waals interaction cause hydrogen bond have shorter distance (Mingos, 2004). A hydrogen bond can be called conventional or classical if it is formed between a partly positively charged hydrogen atom in proton-donor component and the lone electronic pair of electronegative element acting as a proton-accepting component. This conventional hydrogen bonds forming from weak to medium energy and accompanied by a remarkable interpenetration (Bakhmutov, 2008). In

addition, almost same as the results of interactions with batimastat inhibitors, the interaction of EDTA inhibitor ligand complex also forms 4 unfavorable bonds, that appear namely between ligands in 2 kind of amino acid residues like proline (Pro4) and lysine (Lys6). This unfavorable bump interaction bond in the wrong area that can cause the interaction unstable. Unfavorable bump are generally formed by tripled carbon interaction (Karimi & Nalapogaja, 2012).

The interaction of jararhagin with the EDTA-integrin receptor complex $\alpha_2\beta_1$ forms 8 kind of favorable bonds (Figure 2D). The first type of bond is a conventional hydrogen bond which consists of 5 bonds, namely ligand bonds with serine amino acid residues (Ser244), alanine (Ala245), lysine (Lys247), asparagine as a donor and H receptor (Asn274). The five bonds show a distance of 1.7 Å to 3 Å. The second type of bond is carbon hydrogen bonds with 1 bond between ligands with serine amino acid residues (Ser138) and 2 bonds between amino acids alanine (Ala245) with bond distances ranging from 1.7 Å to 3.7 Å that can be classify as strong until weak H-bond. Strong covalent H-bond have length 2.2 Å - 2.5 Å whereas moderate mostly electrostatic have length of distance 2.5 Å - 3.2 Å, and the weak electrostatic dispersed have length of distance more than 3.2 Å (Escalante et.al., 2003). While the last type of bond is 5 unfavorable bonds, that appear namely between ligands in 3 amino acid residues like alanine (Ala245), arginine (Arg243) and tyrosine (Try 235). This bond shows that the formed interaction is less stable even though it successfully inhibits the bounding of jararhagin to the position of domain I α_2 according to the form of the disturbance caused by the ability of jararhagin to block integrin interactions of $\alpha_2\beta_1$ with collagen by bounding to α_2 domain I or by cleavage of $\alpha_2\beta_1$ [16]. This inhibitor also proven to be the most effective *in vivo* and *in vitro* method to irreversibly inactivate the proteolytic activity of jararhagin by remove the active site zinc and structural calcium molecules from the protein using EDTA (Gallagher et.al., 2005).

3.3 The Interaction of Jararhagin with α2β1 Integrin and Hydroxytyrosol

The binding energy of jararhagin-hydroxytyrosol is -178.3 kcal/mol. The interaction between jararhagin-hydroxytyrosol and α2β1 integrin needs -781.33 kcal/mol to bind. Jararhagin-hydroxytyrosol is binding α2β1 integrin on α2-I domain (symbolized with blue coloration on Figure 3), the inhibitor is not attached on the interaction site between α2β1 integrin and jararhagin.

On the other hand, the docking in second condition showed that interaction between hydroxytyrosol and α2β1 integrin has binding energy -166.7 kcal/mol. Hydroxytyrosol as ligand bind at the □2-I domain of α2β1 integrin. The interaction between α2β1 integrin-hydroxytyrosol and jararhagin needs -809.3 kcal/mol to bind. The binding position of hydroxytyrosol is located near to binding target site of jararhagin generally. The energy yielded from interaction of α2β1 integrin-hydroxytyrosol is more than the interaction of jararhagin-hydroxytyrosol. It is showed that hydroxytyrosol is easier to bind with jararhagin than α2β1 integrin after the envenomation.

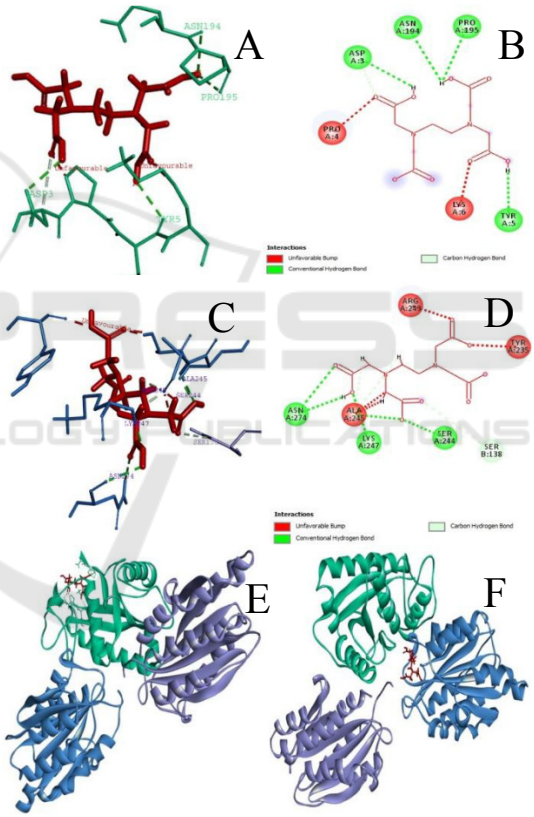

Figure 2. Interaction of Jararhagin, EDTA, and Integrin $\alpha_2\beta_1$. A. Ligand interaction between complex jararhagin-EDTA and integrin $\alpha_2\beta_1$ (3D). B. Ligand interaction between complex jararhagin-EDTA and integrin $\alpha_2\beta_1$ (2D). C. Ligand interaction between complex integrin $\alpha_2\beta_1$-EDTA and jararhagin (3D). D. Ligand interaction between complex integrin $\alpha_2\beta_1$-EDTA and jararhagin (2D). E. Interaction complex jararhagin-EDTA and integrin $\alpha_2\beta_1$. F. Interaction complex integrin $\alpha_2\beta_1$-EDTA and jararhagin.

Table 3. Detail information of H-Bond interaction between jararhagin-EDTA ligands complex with α2β1 integrin.

Name	Distance (Å)	Category	Types	From Chemistry	To Chemistry
A:LIG1:H - A:ASN194:O	2,98453	Hydrogen Bond	Conventional Hydrogen Bond	H-Donor	H-Acceptor
A:LIG1:H - A:PRO195:O	2,84653	Hydrogen Bond	Conventional Hydrogen Bond	H-Donor	H-Acceptor
A:LIG1:H - A:TYR5:O	2,81501	Hydrogen Bond	Conventional Hydrogen Bond	H-Donor	H-Acceptor
A:LIG1:H - A:ASP3:OD1	2,57797	Hydrogen Bond	Conventional Hydrogen Bond	H-Donor	H-Acceptor
A:ASP3:CA - A:LIG1:O	3,2238	Hydrogen Bond	Carbon Hydrogen Bond	H-Donor	H-Acceptor
A:PRO4:CD - A:LIG1:O	2,09393	Unfavorable	Unfavorable Bump;Carbon Hydrogen Bond	Steric;H-Donor	Steric;H-Acceptor
A:PRO4:HD1 - A:LIG1:O	1,53485	Unfavorable	Unfavorable Bump	Steric	Steric
A:LYS6:CG - A:LIG1:O	2,19754	Unfavorable	Unfavorable Bump	Steric	Steric
A:LYS6:CD - A:LIG1:O	2,19833	Unfavorable	Unfavorable Bump	Steric	Steric

Table 4. Detail information of H-Bond interaction between α2β1-EDTA receptors complex with jararhagin.

Name	Distance (Å)	Category	Types	From Chemistry	To Chemistry
A:SER244:HN - B:LIG1:O	1,85267	Hydrogen Bond	Conventional Hydrogen Bond	H-Donor	H-Acceptor
A:ALA245:HN - B:LIG1:O	2,62916	Hydrogen Bond	Conventional Hydrogen Bond	H-Donor	H-Acceptor
A:LYS247:HN - B:LIG1:O	1,76284	Hydrogen Bond	Conventional Hydrogen Bond	H-Donor	H-Acceptor
A:ASN274:HD21 - B:LIG1:O	3,033	Hydrogen Bond	Conventional Hydrogen Bond	H-Donor	H-Acceptor
B:LIG1:H - A:ASN274:O	2,45232	Hydrogen Bond	Conventional Hydrogen Bond	H-Donor	H-Acceptor
B:SER138:CB - B:LIG1:O	3,76774	Hydrogen Bond	Carbon Hydrogen Bond	H-Donor	H-Acceptor
B:LIG1:H - A:ALA245:O	2,24188	Hydrogen Bond	Carbon Hydrogen Bond	H-Donor	H-Acceptor
B:LIG1:H - A:ALA245:O	1,73094	Hydrogen Bond	Carbon Hydrogen Bond	H-Donor	H-Acceptor
A:TYR235:O - B:LIG1:O	1,93483	Unfavorable	Unfavorable Bump	Steric	Steric
A:ARG243:N - B:LIG1:O	1,99267	Unfavorable	Unfavorable Bump	Steric	Steric
A:ARG243:HN - B:LIG1:O	1,6825	Unfavorable	Unfavorable Bump;Conventional Hydrogen Bond	Steric;H-Donor	Steric;H-Acceptor
A:ALA245:O - B:LIG1:C	2,07184	Unfavorable	Unfavorable Bump	Steric	Steric
B:LIG1:H - A:ALA245:O	1,27798	Unfavorable	Unfavorable Bump;Carbon Hydrogen Bond	Steric;H-Donor	Steric;H-Acceptor

When α2β1integrin-hydroxytyrosol interacted to jararhagin, the binding energy is less than energy of interaction between jararhagin-hydroxytyrosol and α2β1 integrin. It is showed that hydroxytyrosol as the inhibitor would inhibit effectively if it is consumed after envenomation.

Hydroxytyrosol is one of phenolic compound which has the high level of antioxidant (Obied et.al., 2012). Interaction of phenolic compound and SVMP will form the hydrogen bond with three histidine residue on zinc binding motive area. Thus, zinc ion will be chelated from SVMP complex. Zinc ion is an important component of SVMP, because SVMP is categorized as zinc-dependent hydrolase. When zinc ion is chelated from SVMP, its enzimatic activity is inhibited (Pithayanukul et.al., 2009).

Interaction of jararhagin-hydroxytyrosol and $\alpha_2\beta_1$ integrin is consist of 1 hydrogen bond and 2 hydrophobic bonds. The first bond is hydrogen bond which formed from hydrogen atom on ligand to Pro202 residue as hidrogen receptor. The distance of this bond is 2,88431. The second bond is hydrophobic bond (Pi-Pi Stacked) which formed from Tyr7 residue of jararhagin protein (to an atom of ligand. The distance of this bond is 4,14095. The third bond has same type as the second bond, which formed from His50 residue to an atom of hydroxytyrosol. The distance of this bond is 4,82085.

Pi-pi (π-π) stack is a type of non-covalent bond. That type of bond is formed between two aromatic ring from different compound. It has acquainted for its role to stabilize the macromolecular structures such as nucleic acid, protein, and other material (Boehr et.al., 2002). Thus, the presence of two Pi-Pi stacked hydrophobic bond, indicate the strong interaction between jararhagin-hydroxytyrosol and $\alpha_2\beta_1$ integrin.

On the other hand, interaction between $\alpha_2\beta_1$ integrin-hydroxityrosol with jararhagin has only one hydrogen bond. This bond is formed from hydrogen atom on ligand with Asn274 residue. The distance of this bond is 1,90548. Hydrogen bond is the interaction between hydrogen atom and electronegative atom group, it has stronger bond than van der Waals interaction, and weaker than covalent or ionic bond. Hydrogen bond cosidered to be the regulator of protein-ligand binding. This bond can create stronger protein-ligand interaction but causing absence of net gain binding affinity, but this bond is also reported to enhance ligand binding affinity by displacing protein-bound water molecule to the bulk solvent (Chen et.al., 2016).

Since there is no any unfavorable bond, the interaction with phenolic compound is better than other ligand because of the stability. Besides, this natural inhibitor is more efficient because it include the metal chelator activity, high level of antioxidant which can support the cell regeneration, free radical scavenger, enzyme activity modulator, and anticancer (Pithayanukul et.al., 2009).

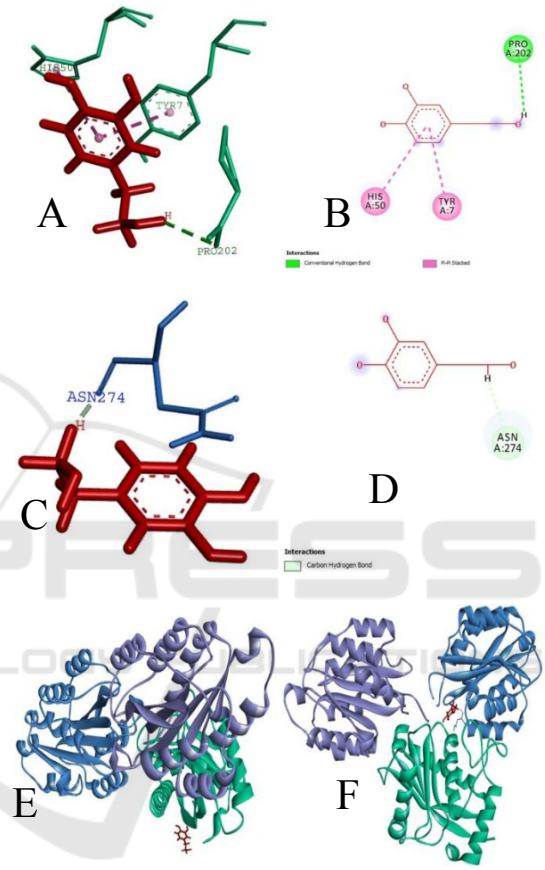

Figure 3. Interaction of Jararhagin, *Hydroxytyrosol*, and Integrin $\alpha_2\beta_1$. A. Ligan interaction between complex jararhagin-*hydroxytyrosol* and integrin $\alpha_2\beta_1$ (3D). B. Ligan interaction between complex jararhagin-*hydroxytyrosol* and integrin $\alpha_2\beta_1$ (2D). C. Ligan interaction between complex integrin $\alpha_2\beta_1$ - *hydroxytyrosol* and jararhagin (3D). D. Ligan interaction between complex integrin $\alpha_2\beta_1$ -*hydroxytyrosol* and jararhagin (2D). E. Interaction complex jararhagin-*hydroxytyrosol* and integrin $\alpha_2\beta_1$. F. Interaction complex integrin $\alpha_2\beta_1$-*hydroxytyrosol* and jararhagin.

Table 5. Interaction between jararhagin, hydroxytyrosol, and $\alpha_2\beta_1$ integrin.

	Name	Distance (Å)	Category	Types	From Chemistry	To Chemistry
Interaction between jararhagin-hydroxytyrosol ligands complex with $\alpha_2\beta_1$ integrin	A:LIG1:H - A:PRO202:OXT	2,88433	Hydrogen Bond	Conventional Hydrogen Bond	H-Donor	H-Acceptor
	A:TYR7 - A:LIG1	4,1407	Hydrophobic	Pi-Pi Stacked	Pi-Orbitals	Pi-Orbitals
	A:HIS50 - A:LIG1	4,8207	Hydrophobic	Pi-Pi Stacked	Pi-Orbitals	Pi-Orbitals
Interaction between $\alpha_2\beta_1$ integrin - hydroxytyrosol ligands complex with jararhagin	B:LIG1:H - A:ASN274:O	1,90548	Hydrogen Bond	Carbon Hydrogen Bond	H-Donor	H-Acceptor

4 CONCLUSIONS

Inhibition of hemorrhagic activity can be done with several types of compounds that have been known to be inhibitors for SVMP especially jararhagin can done by batimastat (peptidomimetic compounds), EDTA (zinc-chelating agents), and plant compounds such as hydroxytyrosol. Based on the results of the in silico analysis from batimastat, EDTA and hydroxytyrosol inhibitory properties, found that these inhibitor were more easily bound to jararhagin compared to integrin $\alpha_2\beta_1$. But only two of them are more effectively inhibited by bounding to jararhagin spread in blood vessels after snakebite cases. However, unfavorable bonds are formed during interaction between batimastat inhibitors, jararhagin and integrin $\alpha_2\beta_1$. These inhibitor are Batimastat and hydroxytyrosol. Furthermore, in the second inhibitor EDTA, it was found that this compound more effective doing inhibition by inhibiting integrin $\alpha_2\beta_1$. In other side inhibitor batimastat and EDTA also has unfavorable bonds. While the last alternative of phenolic compounds in the form of hydroxytyrosol shows that inhibitor are interact with jararhagin and integrin $\alpha_2\beta_1$ without showing an unfavorable bond. From that result we can conclude that the natural inhibitors formed in hydroxytyrosol from olive oil are more stable and have highest effectiveness and efficiency in preventing hemorrhagic symptoms due to snake bites that contain jararhagin venom.

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