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Chapter 5

Bioinformatics Approach to Screening and Developing Drug against Ebola

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Abstract

Ebola is an acute disease causing hemorrhagic fever marked with high mortality rate. The patients who suffer from Ebola only receive palliative care because there is no available drug which can consistently cure this disease. To date, no cure has been found to treat this disease. Bioinformatics and computer-aided drug discovery and development (CADDD) are employed to utilize the readily available genomic and proteomic data and enhance the hit rate of the novel and repurposed drug for Ebola therapy. Additionally, the time and cost of wet laboratory experiments can be drastically reduced by the support of bioinformatics approach. Our laboratory has succeeded not only in creating the bioinformatics research pipeline but also screening and developing the drug candidate to cure Ebola. Through pharmacophore-based virtual screening and molecular docking simulations, we discovered that about three Indonesian natural product compounds have noteworthy molecular interactions against EBOV VP35 protein, which are responsible for the RNA synthesis of the Ebola. These compounds can be reevaluated further through advances in in silico simulation and in vitro experiments.

Keywords: Ebola, *Ebola virus*, bioinformatics, CADDD, molecular docking

1. Introduction

Ebola, previously known as Ebola virus disease, is an acute viral infection causing hemorrhagic fever marked by high mortality rate in human and nonhuman primates [1]. It is a zoonotic disease transmitted by direct contact with mucosal tissue or bodily fluids (blood, feces, and other secreted fluids) of the infected living or dead human and animal [2–4]. The animal reservoir for this disease is still unknown. Fruit bat (*Hypsignathus monstrosus*, *Epomops*

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franqueti, and *Myonycteris torquata*) which belongs to Pteropodidae family is suspected as the most likely host of Ebola, although the linkage has not been confirmed [5–7].

Ebola is an enveloped, nonsegmented, negative-sense, single-stranded RNA virus which belongs to *Ebolavirus* genus, *Filoviridae* family, and *Mononegavirales* order [8, 9]. Ebola virus (EBOV), Tai Forest virus (TAFV), Reston virus (RESTV), Sudan virus (SUDV), and Bundibugyo virus (BDBV) are the virus making up the *Ebolavirus* genus [10]. EBOV and SUDV come as the most frequent outbreak-causing virus which has the case-fatality rate of 76 and 55% (CI 95%), respectively [11]. On the other hand, RESTV causes death in primates such as gorillas and chimpanzees but not known to have caused disease in humans [12, 13].

The Ebola virus genomic RNA is consisted of around 19,000 nucleotides [14]. It encodes seven structural protein, namely, nucleoprotein (NP), glycoprotein (GP), RNA-dependent RNA polymerase (L), matrix protein (VP40), and three nucleocapsid proteins (VP24, VP30, and VP35) [15, 16]. It also encodes one nonstructural protein, the secretory glycoprotein (sGP) [17]. The genome is linearly arranged as follows: 3′-leader-NP-VP35-VP40-GP/sGP-VP30-VP24-L-trailer-5′ [14, 17, 18].

The seven structural proteins and one nonstructural protein have an imperative role in Ebola virus life cycle [16]. NP: viral replication and scaffold for additional viral proteins. GP: binds to receptors on the cell surface and membrane fusion, pathogenicity. sGP: inhibits neutrophil function and adsorbs neutralizing antibodies. L: synthesis of positive-sense RNA. VP40: viral assembly and budding, structural integrity of viral particles, and maturation of the virion. VP24: nucleocapsid formation, encapsulates and shields viral genome from nucleases, viral replication. VP30: viral transcription activator. VP35: multi-virulence functionality, innate immune antagonist, and an RNAi silencing suppressor [16, 17].

The patient who suffers from Ebola shows no symptoms during the initial infection. After the incubation for about 4–10 days, the general symptoms such as fever, myalgia, and malaise and sometimes accompanied by chills appear. These symptoms often confused with dengue or malaria in tropical climates [3, 19]. As the infection progresses, the patient shows flu-like symptoms accompanied by gastrointestinal symptoms. In severe cases, Ebola developed into a conjunctival hemorrhage, epistaxis, melena, hematemesis, coagulation abnormalities, and a range of hematological irregularities. The neurological symptoms such as encephalopathy, convulsions, and delirium may also occur during the late stage of the infection [19, 20]. The patient dies around 6–9 weeks after the symptoms appear [21]. With the nonspecific symptoms, severe morbidity, and high mortality rate, the World Health Organization (WHO) has acknowledged Ebola as one of the most malignant diseases in the world [22].

The first recorded Ebola outbreak emerged in Sudan between June and November 1976. It mainly affected Nzara, Maridi, Tembura, and Juba where 150 of 284 victims died (the mortality rate of 53%) [2, 23]. After the first outbreak, 19 other outbreaks have occurred in Africa with the mean fatality rate of 65.4% [11].

The last and the most extensive Ebola outbreak was announced by the WHO on March 23, 2014. This outbreak appears to have emerged in the Guéckédou district of the southeast region of Republic of Guinea [24–26]. The WHO announced the epidemic to be a Public

Health Emergency of International Concern (PHEIC) on August 8, 2014, due to the severe consequences if Ebola ever spread around the globe. PHEIC was disclosed because of the weak health services of Guinea, Liberia, Sierra Leone, and other neighboring countries at risk in combating Ebola and the continuing transmission with a high fatality rate of Ebola in West Africa [26]. When the outbreak ends in March 2016, Ebola has claimed 1310 lives out of 28,616 reported cases [27, 28]. Even though the damage caused by the last outbreak of Ebola is calamitous, there is still no FDA-approved antiviral drug to treat this disease.

Ebola is considered as one of the neglected tropical diseases because the outbreaks take place in the poor populations with limited resources, mostly in West Africa [29]. The research and drug development for Ebola have been neglected for decades because the drug developers regard it as a commercially unattractive project to invest their resource. The negligence occurs to all tropical diseases by only 13 out of 1393 new approved drugs between 1975 and 1999 that were indicated for tropical disease [30]. However, the frequent outbreaks in the last decade and the massive outbreak which was occurred in 2014 have drawn much attention to drug development for Ebola [16]. Without available treatment or vaccine, paramedic only relied on palliative care for the infected patients and barrier methods to prevent the transmission [31]. Hence, the researchers investigating ways for helping people just infected with Ebola (treatment) and preventing people to get infected when exposed to Ebola (vaccine) [32].

The conventional medical treatment for Ebola is a supportive care with intravenous fluids or oral rehydration with electrolyte solutions. The reason being that the virus interferes with blood clotting and disrupts electrolyte balance. Thus, such intervention can help to keep up the condition of the patient. However, such intervention is not enough for severely ill patients to sustain and recover [21, 32].

Zmapp, a combined humanized monoclonal antibody, was tested as a passive immunotherapy against Ebola. The preclinical test was conducted by Mapp Biopharmaceutical. This monoclonal antibody shows 100% efficacy in preventing lethal disease on cynomolgus macaques when treatment is initiated up to 5 days postinfection of EBOV [31].

Other experimental therapies developed a novel synthetic adenosine analog, BCX4430. This compound shows in vitro and in vivo activity by inhibiting viral RNA polymerase function, acting as a non-obligate RNA chain terminator. BCX4430 protects both mice and guinea pig models from a severe infection of Ebola virus and Marburg virus. In addition, this compound completely protects cynomolgus macaques from Marburg virus infection if administered as late as 48 h after infection [33].

Not only does the research focus on the development of a novel drug, but the research is also conducted to identify potential repurposed therapeutic agents for the treatment of Ebola [34, 35]. Toremifene and clomiphene, the selective estrogen reuptake modulators, are currently known as the drug to treat breast cancer and infertility, respectively. Both drugs inhibit Ebola virus entry into the cell by preventing the late stage membrane fusion. These drugs show an inhibition activity by more than 90% in vitro. Higher dose than the standard clinical range is needed to achieve the therapeutic effect on Ebola. However, a higher dose would increase the risk of serious side effect of toremifene and clomiphene, which are electrolyte derangements and ocular adverse effect, respectively [36].

Other experiments screen amiodarone, a multichannel ion blocker; sertraline, selective serotonin reuptake inhibitor; and bepridil, a calcium channel blocker as a repurposed therapeutic agent targeting Ebola. Amiodarone works by the induction of Niemann-Pick C-like phenotype that inhibits late endosomal entry of Ebola virus [37]. Sertraline and bepridil work in a similar fashion to amiodarone. Both drugs show inhibition activity in an in vitro test by more than 90% [35].

Several vaccines have also been developed to prevent the Ebola. ChAd3-ZEBOV, which has developed by GlaxoSmithKline in collaboration with the US National Institute of Allergy and Infectious Diseases, is a chimpanzee-derived adenovirus vector with an Ebola virus gene inserted. This vaccine induced uniform protection against acute lethal Ebola virus in cynomolgus macaques. However, the protection of this vaccine declines over several months [38].

The other vaccine, which is developed by the Public Health Agency of Canada in Winnipeg, is rVSV-ZEBOV. It uses an attenuated vesicular stomatitis virus which has been genetically modified to express glycoprotein of Ebola virus. The rVSV-ZEBOV has undergone a ring vaccination phase 3 efficacy trial which assesses the protective activity of rVSV-ZEBOV against Ebola virus in human beings. The result shows that rVSV-ZEBOV offers substantial protection against Ebola virus infection. Both randomized and a non-randomized clusters of vaccinated individuals show no disease development from the challenge performed 10 days postvaccination [39].

The Center for Disease Control and Prevention considered Ebola virus as a tier 1 select agent because it possesses a considerable risk of intentional misuse with a severe threat to public health and safety [40]. Researchers need to fill the APHIS/CDC Form 1 in order to register for possession, use, and transfer of Ebola virus. All requirements including the availability of Biosafety Level (BSL) 4 laboratory and certified personnel are needed to get access to Ebola virus sample [41]. Thus, to get a suitable sample, researchers tend to move their experiments on the genetically modified virus that can express part of known Ebola virus genome because it is not subjected to select agent [42].

Genomic and proteomic data of Ebola virus has been collected each time the outbreak occurred and stored in the open source database. Also, the Ebola virus protein interaction with the corresponding drug lead through in vitro test has also been increased in the past decades. To date, the protein three-dimensional (3D) structure of Ebola virus NP, VP35, VP40, GP, VP30, and VP24 has been available in Protein Databank (PDB). In addition, the active site residues of each protein have also been identified, except for NP. L is the only Ebola virus protein with unavailable 3D structure and unidentified active site [16]. Thus, researchers use a bioinformatics approach to utilize the readily available genomic and proteomic data to research drug design and discovery.

Computer-aided drug discovery and development (CADDD) is employed to accelerate hit identification, hit-to-lead selection, enhance absorption, distribution, metabolism, excretion, and toxicity (ADMET) profile and avoid another safety issue [43]. This approach is currently growing and adapted quickly by pharmaceutical industry and academia because it reduces the time and cost of drug research [44, 45]. Currently, 16 compounds (Aliskiren, Boceprevir, Captopril, Dorzolamide, Indinavir, LY-517717, Nolatrexed, NVP-AUY922, Oseltamivir,

Raltegravir, Ritonavir, Rupintrivir, Saquinavir, TMI-005, Tirofiban, and Zanamivir) are in clinical trial or have been approved for therapeutic use. These compounds are the examples of successful application of CADDD [46, 47]. Through CADDD, the hit rate of the novel and repurposed drug for Ebola therapy could be improved.

A consistently effective treatment for Ebola is currently not yet available. Present therapeutic options are directed at palliative and supportive care to maintain and prolong the patient life. The majority of treatment, novel or repurposed drug, have been developed, but none of them are entirely satisfactory. In attempts to find a drug in the treatment of Ebola, inhibitors targeting EBOV VP35 have received little attention even though it has a critical function in host immune evasion and viral RNA synthesis. Our objective is to find the optimal in silico Ebola therapeutic agents which later will be implemented in the wet laboratory.

2. Our in silico method

In this chapter, we will discuss the result of our in silico approach against EBOV VP35, one of the viral protein of EBOV which is responsible for the viral RNA synthesis and as the RNAi silencing suppressor agents [48, 49]. Moreover, this protein was also being studied by Brown et al. in 2014, which discovered the actual pose of their selected inhibitors against the EBOV VP35 in their perspective binding site and also deposited their work in RCSB Protein Databank (PDB) through several PDB IDs [50]. Thus, their proteins can be used as the template for pharmacophore mapping model for our docking simulation approach. Moreover, we also deployed the Indonesian natural product compounds for virtual screening purpose to find the suitable lead compounds for combating Ebola. The reason for choosing the Indonesian natural product compounds because of Indonesia, as one of the largest megadiversity countries, has no less than 38,000 flowering plants that grow around the nation, with 55% of them are endemic plants [51, 52].

First, we prepared the Indonesian natural product compounds by searching the molecular structures through several journals and databases [53–69], after which we were drawing them using ChemBioDraw 14.0 software. From this step, we obtained about 3429 compounds in the process. All of these ligands were then protonated, washed, and minimized by using MOE 2014.09 software [70]. These ligands were saved for the docking simulation purpose. For the EBOV VP35 protein, we selected the PDB ID: 4IBC as our protein, and we determined the pharmacophore site through standard protein-ligand interaction fingerprints (PLIF) protocol of MOE 2014.09 software. This step generated the pharmacophore model around the binding site of EBOV VP35 after we performed the protonating process of EBOV VP35 through "LigX" feature of MOE 2014.09 software. Later on, we conducted molecular docking simulation using the modification of our current approach [71, 72]. Instead of using "Triangle Matcher" and "London dG," we used "Pharmacophore" and "Affinity dG" for "Placement" and "Rescoring 1" parameters to accommodate the pharmacophore model that created in an earlier phase, while the rest of parameters were set according to the default setup. First, the STD1 ligand (IUPAC name: 2-(4-(4-(2-chlorobenzoyl)-5-(2-chlorophenyl)-2,3-dioxo-2,3-dihydro-1H-pyrrol-1-yl)

phenyl)acetic acid) and 100 decoy ligands were docked into the binding site to validate the pharmacophore model. "Rigid Receptor" and "Induced-Fit" protocols were performed against the Indonesian natural product compounds and STD1 ligand later on.

In an attempt of searching the proper pharmacophore site in the binding site of EBOV VP35, we utilized the PLIF protocol from MOE 2014.09 software by using STD1 ligand as the template. From this approach, we figured out that the binding site of EBOV VP35 protein consists of three pharmacophore sites, as it displayed in **Figure 1**. One hydrophobic spot is affiliated with Lys248 residues through arene-hydrogen interaction, while two H-bond acceptors, lone-pair sites, are connected with Gln241 and Lys251. These sites were responsible for the binding attachment of the STD01 ligand when bound to EBOV VP35 protein. Thus, it can be predicted that any ligands that bind to these residues may exhibit the same antiviral activities like STD01 ligand.

The pharmacophore sites were later validated by having the STD01 ligand and 100 decoys to be screened through molecular docking simulation. In this phase, we deployed "virtual screening" approach as our docking protocol, with pharmacophore model that was included in the simulation. After the screening was conducted, we discovered that all of the decoys did not pass the test, indicating this method was validated and did not create the "false-positive" ligand that may result during docking simulation. Furthermore, the STD01 ligand passed this test with a $\Delta G_{binding}$ score of −5.2778 kcal/mol and RMSD value of 1.5487 Å. This result was shown that the parameters that were set earlier were decent enough to be reproduced in the next simulation. The comparison of the initial and screened poses of STD01 ligand is shown in **Figure 2**.

The pharmacophore-based docking simulation of EBOV VP35 protein was later performed against the 3429 Indonesian natural product ligands that were already prepared. From the simulations, we acquired 20 ligands that matched with the pharmacophore model of EBOV VP35, which means that other 3409 ligands did not possess the properties that needed to pass the initial pharmacophore screening. In the first docking simulation (Rigid Docking protocol),

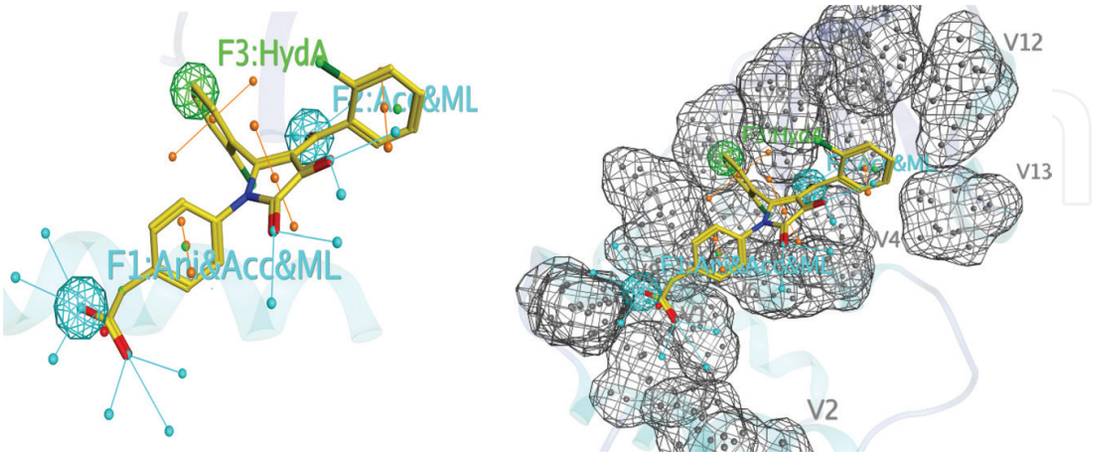

Figure 1. The pharmacophore model of the STD01 ligand in the binding site of EBOV VP35 protein. According to the PLIF feature of MOE 2014.09 software, the STD01 ligand comprises three pharmacophore sites: one hydrophobic point and two acceptor/lone-pair points (left). In the docking simulations, we deployed the "exclude points" to indicate the residues that exist in the VP35 binding site and prevent the larger ligands to interact with the binding site.

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we found four Indonesian natural product ligands, namely, multifloroside, myricetin 3-robinobioside, kaempferol 3-(6G-malonylneohesperidoside), and theasaponin, which have the $\Delta G_{binding}$ score lower than the STD01 ligand. The molecular structures of these ligands can be seen in **Figure 3**.

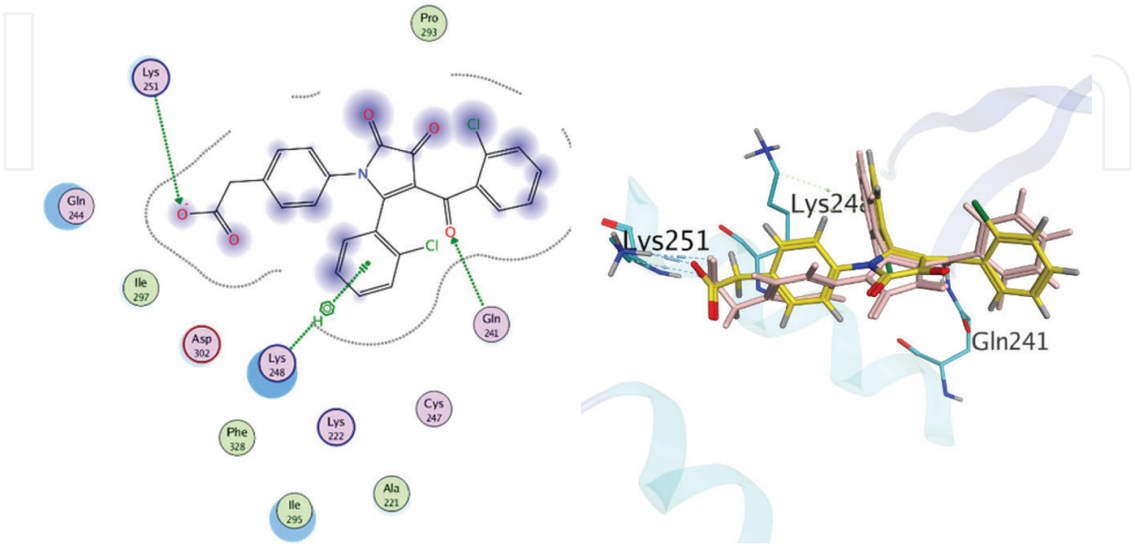

Figure 2. The binding interaction of the STD01 ligand and EBOV VP35 binding site. The 2D interaction after docking simulation is displayed in the left figure, while the right figure presents the difference between the initial pose (shown in yellow) and after the docking simulation was conducted (shown in pink).

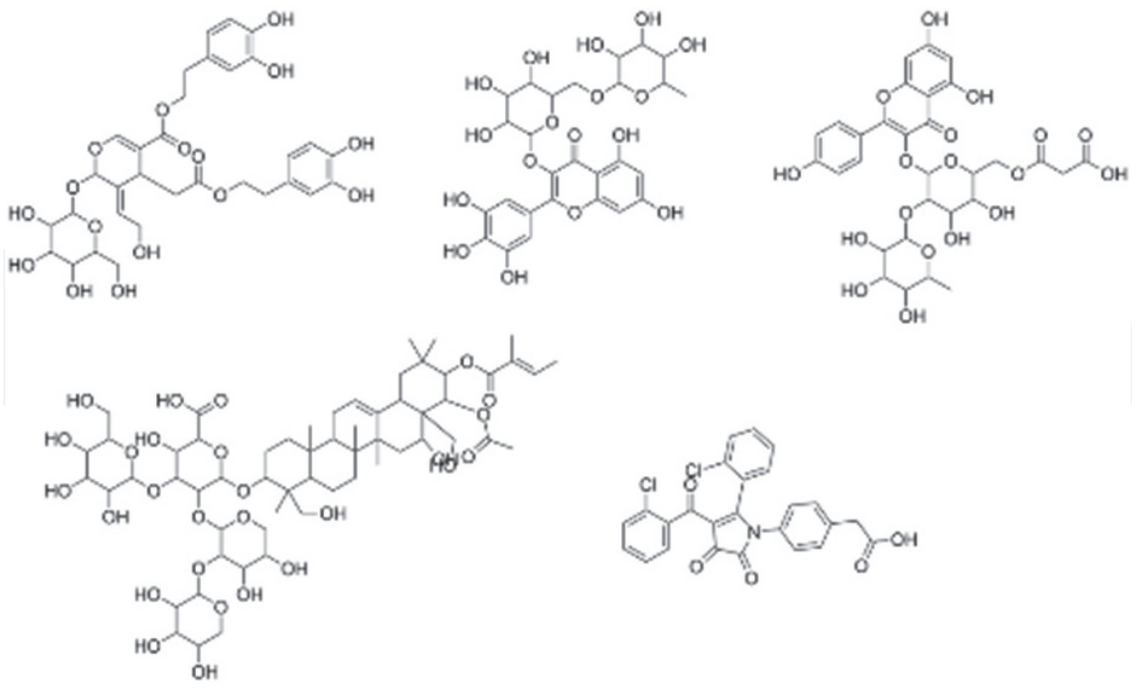

Figure 3. The molecular structure of multifloroside (top left), myricetin 3-robinobioside (top middle), kaempferol 3-(6G-malonylneohesperidoside) (top right), theasaponin (bottom left), and 2-(4-(4-(2-chlorobenzoyl)-5-(2-chlorophenyl)-2,3-dioxo-2,3-dihydro-1H-pyrrol-1-yl)phenyl)acetic acid (bottom right).

Molecule name	$\Delta G_{binding}$ (RMSD)	H-bond interaction residues
Multifloroside	−10.8405 kcal/mol (3.2691)	Arg225, Tyr229, Lys 248, and Lys251
Myricetin 3-robinobioside	−10.0897 kcal/mol (1.2275)	Lys222, Arg225, Gln241, and Lys251
Kaempferol 3-(6G-malonylneohesperidoside)	−9.8721 kcal/mol (1.0311)	Gln241, Gln244, Lys251, and His296
Theasaponin	−9.0175 kcal/mol (0.4352)	Arg225, Gln241, and Lys251
STD01 ligand (*standard*)	−8.4579 kcal/mol (0.7747)	Gln241, Lys248, and Lys251

Table 1. The results of molecular docking simulation.

After the first docking simulation had been performed, the second docking simulation (Induced-Fit protocol) was utilized against these four proteins to revalidate the docking pose that was produced in the previous simulations. If the RMSD difference was lower than 2.0 Å, it means that the docking pose is good enough and may be reproduced in the actual simulation [73]. In this simulation, we found that multifloroside ligand has the lowest $\Delta G_{binding}$ score of −10.8405 kcal/mol, followed by myricetin 3-robinobioside (−10.0897 kcal/mol), kaempferol 3-(6G-malonylneohesperidoside) (−9.8721 kcal/mol), and theasaponin (−9.0175 kcal/mol). These results were significantly lower than the STD01 ligand, which sits in −9.0175 kcal/mol. However, we must take into account that the RMSD value of multifloroside ligand was 3.2691 Å, higher than 2.0 Å; it means that the docking pose that was generated during the docking simulation was not acceptable. Meanwhile,

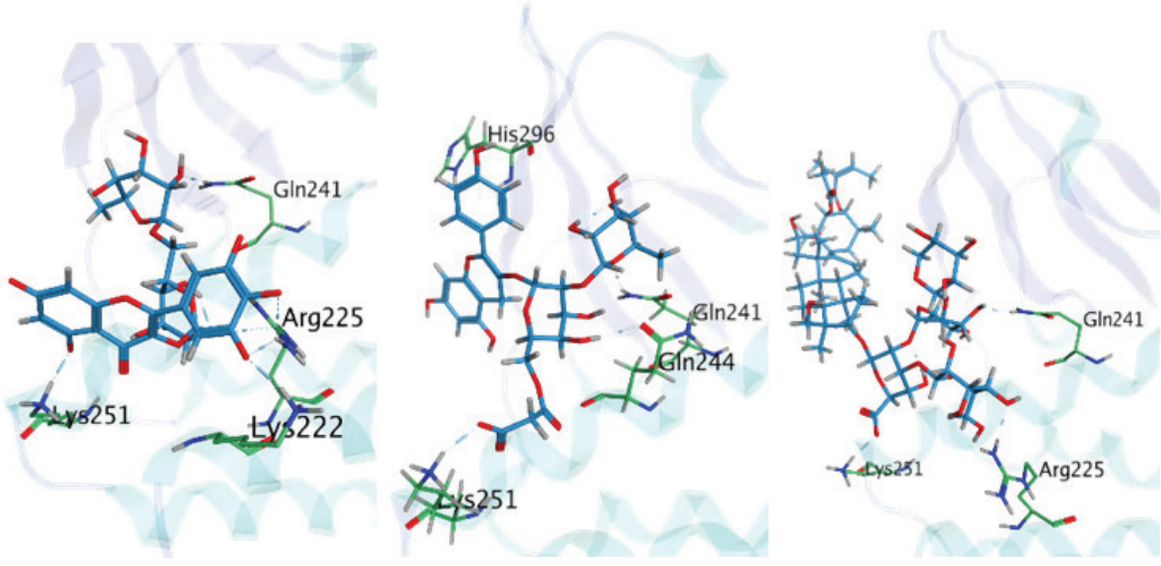

Figure 4. The interacting residues of EBOV VP35 protein with myricetin 3-robinobioside (left), kaempferol 3-(6G-malonylneohesperidoside) (middle), and theasaponin (right).

the other three ligands possessed the tolerable RMSD value (1.2275, 1.0311, and 0.4352 Å for myricetin 3-robinobioside, kaempferol 3-(6G-malonylneohesperidoside), and theasaponin, respectively). Furthermore, we also observed the interactions between the ligands and the binding site of EBOV VP35. From the docking simulation, we figured out that all three remaining ligands made interactions with Gln241 and Lys251, which are important in suppressing the EBOV VP35 activity. The full results of molecular docking simulations can be seen in **Table 1** and **Figure 4**.

3. Conclusions

Without no doubt, the drug developments of Ebola are desperately needed due to high pathogenicity and mortality rate that emitted by this disease. Through this chapter, we present that bioinformatics and CADDD, especially the pharmacophore-based drug design, may be the solution to significantly increase the viability of the newly discovered lead compounds that can be introduced as the drug candidate of Ebola virus, which can be supported later through in vitro study to validate the results that we found in previous research. The dry lab experiments should play a significant role in the development of drugs, not only Ebola but also for all diseases due to low cost and not a time-consuming process. Therefore, the improvements and developments of bioinformatics and CADDD should also speed up the time that we needed to obtain the drug candidates for our health problems.

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Conflict of interest

None are declared.

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References

[1] Rajak H, Jain DK, Singh A, et al. Ebola virus disease: past, present and future. Asian Pacific Journal of Tropical Biomedicine. 2015;**5**:337-343

[2] Pourrut X, Kumulungui B, Wittmann T, et al. The natural history of Ebola virus in Africa. Microbes and Infection. 2005;**7**:1005-1014

[3] Ansari AA. Clinical features and pathobiology of Ebolavirus infection. Journal of Autoimmunity. 2015;**55**:1-9

[4] Bausch DG, Towner JS, Dowell SF, et al. Assessment of the risk of Ebola virus transmission from bodily fluids and fomites. The Journal of Infectious Diseases. 2007;**2699**:2-7

[5] Gatherer D. The 2014 Ebola virus disease outbreak in West Africa. The Journal of General Virology. 2014;**95**:1619-1624

[6] Smith I, Wang LF. Bats and their virome: An important source of emerging viruses capable of infecting humans. Current Opinion in Virology. 2013;**3**:84-91

[7] Feldmann H, Geisbert TW. Ebola haemorrhagic fever. Lancet. 2011;**377**:849-862

[8] Bishop BM. Potential and emerging treatment options for Ebola virus disease. The Annals of Pharmacotherapy. 2015;**49**:196-206

[9] Lawrence P, Danet N, Reynard O, et al. Human transmission of Ebola virus. Current Opinion in Virology. 2017;**22**:51-58

[10] Kuhn JH, Bao Y, Bavari S, et al. Virus nomenclature below the species level: A standardized nomenclature for laboratory animal-adapted strains and variants of viruses assigned to the family Filoviridae. Archives of Virology. 2013;**158**:1425-1432

[11] Lefebvre A, Fiet C, Belpois-Duchamp C, et al. Case fatality rates of Ebola virus diseases: A meta-analysis of World Health Organization data. Médecine et Maladies Infectieuses. 2014;**44**:8-12

[12] Formenty P. Ebola virus disease. In: Emerging Infectious Diseases: Clinical Case Studies. London: Elsevier, 2014. Epub ahead of print 2014. DOI: 10.1016/B978-0-12-416975-3.00009-1

[13] Kortepeter MG, Bausch DG, Bray M. Basic clinical and laboratory features of filoviral hemorrhagic fever. The Journal of Infectious Diseases. 2011;**204**(Suppl):S810-S816

[14] Ikegami T, Calaor AB, Miranda ME, et al. Genome structure of Ebola virus subtype Reston: Differences among Ebola subtypes. Archives of Virology. 2001;**146**:2021-2027

[15] Bray M, Paragas J. Experimental therapy of filovirus infections. Antiviral Research. 2002;**54**:1-17

[16] Balmith M, Soliman MES. Potential Ebola drug targets – Filling the gap: A critical step forward towards the design and discovery of potential drugs. Biologia (Bratisl). 2017;**72**:1-13

[17] Takada A, Kawaoka Y. The pathogenesis of Ebola hemorrhagic fever. Trends in Microbiology. 2001;**9**:506-511

[18] Volchkov VE, Volchkova VA, Chepurnov AA, et al. Characterization of the L gene and 5 h trailer region of Ebola virus. The Journal of General Virology. 1999;**80**:355-362

[19] Colebunders R, Borchert M. Ebola haemorrhagic fever – A review. The Journal of Infection. 2000;**40**:16-20

[20] Fauci AS. Ebola — Underscoring the global disparities in health care resources. The New England Journal of Medicine. 2014;**371**:1084-1086

[21] Calain P, Bwaka MAA, Colebunders R, et al. Ebola Hemorrhagic fever in Kikwit, Democratic Republic of the Congo: Clinical observations in 103 patients. The Journal of Infectious Diseases. 1999;**179**:S1-S7

[22] World Health Organization (WHO). Ebola Data and Statistic. 2016. Available from: http://apps.who.int/gho/data/view.ebola-sitrep.ebola-summary-latest?lang=en [Accessed: May 11, 2016]

[23] Report of a WHO/International Study team. Ebola haemorrhagic fever in Sudan, 1976. Bulletin of the World Health Organization. 1978;**56**:247-270

[24] Team WHOER. Ebola virus disease in West Africa – The first 9 months of the epidemic and forward projections. The New England Journal of Medicine. 2014;**371**:1481-1495

[25] Stein RA. What is Ebola? International Journal of Clinical Practice. 2014;**69**:49-58

[26] Briand S, Bertherat E, Cox P, et al. The international Ebola emergency. The New England Journal of Medicine. 2014;**371**:1180-1183

[27] World Health Organization (WHO). Ebola Virus Disease. 2016. Available from: http://www.who.int/mediacentre/factsheets/fs103/en/

[28] Centers for Disease Control and Prevention. 2014-2016 Ebola Outbreak in West Africa. U.S. Department of Health and Human Services. 2016. Available from: https://www.cdc.gov/vhf/ebola/outbreaks/2014-west-africa/index.html

[29] MacNeil A, Rollin PE. Ebola and Marburg Hemorrhagic fevers: Neglected tropical diseases? PLoS Neglected Tropical Diseases. 2012;**6**:e1546

[30] Trouiller P, Olliaro P, Torreele E, et al. Drug development for neglected diseases: A deficient market and a public-health policy failure. Lancet (London, England). 2002; **359**:2188-2194

[31] Qiu X, Wong G, Audet J, et al. Reversion of advanced Ebola virus disease in nonhuman primates with ZMapp. Nature. Epub ahead of print August 2014. DOI: 10.1038/nature13777

[32] La Torre G, Nicosia V, Cardi M, et al. Ebola: A review on the state of the art on prevention and treatment. Asian Pacific Journal of Tropical Biomedicine. 2014;**4**:925-927

[33] Warren TK, Wells J, Panchal RG, et al. Protection against filovirus diseases by a novel broad-spectrum nucleoside analogue BCX4430. Nature. 2014;**508**:402-405

[34] Sweiti H, Ekwunife O, Jaschinski T, et al. Repurposed therapeutic agents targeting the Ebola virus: A systematic review. Current Therapeutic Research. 2017;**84**:10-21

[35] Johansen LM, Dewald LE, Shoemaker CJ, et al. A screen of approved drugs and molecular probes identifies therapeutics with anti – Ebola virus activity. Science Translational Medicine. 2015;**7**:1-14

[36] Madrid PB, Chopra S, Manger ID, et al. A systematic screen of FDA-approved drugs for inhibitors of biological threat agents. PLoS One. 2013;**8**. Epub ahead of print. DOI: 10.1371/journal.pone.0060579

[37] Piccoli E, Nadai M, Caretta CM, et al. Amiodarone impairs trafficking through late endosomes inducing a Niemann-pick C-like phenotype. Biochemical Pharmacology. 2011;**82**:1234-1249

[38] Stanley DA, Honko AN, Asiedu C, et al. Chimpanzee adenovirus vaccine generates acute and durable protective immunity against Ebolavirus challenge. Nature Medicine. 2014;**20**:1126

[39] Henao-Restrepo AM, Camacho A, Longini IM, et al. Efficacy and effectiveness of an rVSV-vectored vaccine in preventing Ebola virus disease: final results from the Guinea ring vaccination, open-label, cluster-randomised trial (Ebola Ça Suffit!). Lancet. December 2016. Epub ahead of print. DOI: 10.1016/S0140-6736(16)32621-6

[40] Federal Select Agent Program. Select Agents & Toxins FAQ's. U.S. Department of Health and Human Services 2017. Available from: https://www.selectagents.gov/faq-general.html

[41] Federal Select Agent Program. APHIS/CDC Form 1 – Registration for Possession, Use, and Transfer of Select Agents and Toxins. U.S. Department of Health and Human Services 2017. Available from: https://www.selectagents.gov/form1.html

[42] Center for Disease Control and Prevention. Interim Guidance Regarding Compliance with Select Agent Regulations for Laboratories Handling Patient Specimens Under Investigation or Confirmed for Ebola Virus Disease (EVD)

[43] Kapetanovic IM. Computer-aided drug discovery and development (CADDD): In silico-chemico-biological approach. Chemico-Biological Interactions. 2008;**171**:165-176

[44] Scott DE, Coyne AG, Hudson SA, et al. Fragment based approaches in drug discovery and chemical biology. Biochemistry. 2012;**51**:4990-5003

[45] Halperin I, Ma B, Wolfson H, et al. Principles of docking : An overview of search algorithms and a guide to scoring functions. Proteins: Structure, Function, and Genetics. 2002;**443**:409-443

[46] Hassan Baig M, Ahmad K, Roy S, et al. Computer aided drug design: Success and limitations. Current Pharmaceutical Design. 2016;**22**:572-581

[47] Talele T, Khedkar S, Rigby A. Successful applications of computer aided drug discovery: Moving drugs from concept to the clinic. Current Topics in Medicinal Chemistry. 2010;**10**:127-141

[48] Leung DW, Borek D, Luthra P, et al. An intrinsically disordered peptide from Ebola virus VP35 controls viral RNA synthesis by modulating nucleoprotein-RNA interactions. Cell Reports. 2015;**11**:376-389

[49] Haasnoot J, De Vries W, Geutjes EJ, et al. The ebola virus VP35 protein is a suppressor of RNA silencing. PLoS Pathogens. 2007;**3**:0794-0803

[50] Brown CS, Lee MS, Leung DW, et al. In silico derived small molecules bind the filovirus VP35 protein and inhibit its polymerase cofactor activity. Journal of Molecular Biology. 2014;**426**:2045-2058

[51] Médail F, Quézel P. Biodiversity hotspots in the Mediterranean Basin: Setting global conservation priorities. Conservation Biology. 1999;**13**:1510-1513

[52] Arifin HS, Nakagoshi N. Landscape ecology and urban biodiversity in tropical Indonesian cities. Landscape and Ecological Engineering. 2011;**7**:33-43

[53] Yanuar A, Mun'im A, Lagho ABA, et al. Medicinal plants database and three dimensional structure of the chemical compounds from medicinal plants in Indonesia. International Journal of Computer Science 2011;**8**:180-183

[54] Allin SM, Duffy LJ, Page PCB, et al. Towards a total synthesis of the manadomanzamine alkaloids: The first asymmetric construction of the pentacyclic indole core. Tetrahedron Letters. 2007;**48**:4711-4714

[55] Jadulco R, Brauers G, Edrada RA, et al. New metabolites from sponge-derived fungi *Curvularia lunata* and *Cladosporium herbarum*. Journal of Natural Products. 2002;**65**:730-733

[56] Liu H, Pratasik SB, Nishikawa T, et al. Lissoclibadin 1, a novel trimeric sulfur-bridged dopamine derivative, from the tropical ascidian Lissoclinum cf. badium. Tetrahedron Letters. 2004;**45**:7015-7017

[57] Cencic R, Carrier M, Galicia-Vázquez G, et al. Antitumor activity and mechanism of action of the cyclopenta[b]benzofuran, silvestrol. PLoS One. 2009;**4**:e5223

[58] Litaudon M, Trigalo F, Martin M-T, et al. Lissoclinotoxins: Antibiotic polysulfur derivatives from the tunicate Lissoclinum perforatum. Revised structure of lissoclinotoxin A. Tetrahedron. 1994;**50**:5323-5334

[59] Litaudon M, Guyot M. Lissoclinotoxin a, an antibiotic 1,2,3-trithiane derivative from the tunicate Lissoclinum perforatum. Tetrahedron Letters. 1991;**32**:911-914

[60] Liu H, Fujiwara T, Nishikawa T, et al. Lissoclibadins 1-3, three new polysulfur alkaloids, from the ascidian Lissoclinum cf. badium. Tetrahedron. 2005;**61**:8611-8615

[61] Wang W, Takahashi O, Oda T, et al. Lissoclibadins 8-14, polysulfur dopamine-derived alkaloids from the colonial ascidian Lissoclinum cf. badium. Tetrahedron. 2009; **65**:9598-9603

[62] Nakazawa T, Xu J, Nishikawa T, et al. Lissoclibadins 4-7, polysulfur aromatic alkaloids from the Indonesian ascidian Lissoclinum cf. badium. Journal of Natural Products. 2007;**70**:439-442

[63] Oda T, Kamoshita K, Maruyama S, et al. Cytotoxicity of Lissoclibadins and Lissoclinotoxins, isolated from a Tropical Ascidian Lissoclinum cf. badium, against human solid-tumor-derived cell lines. Biological and Pharmaceutical Bulletin. 2007;**30**:385-387

[64] Pieterse CMJ, van Loon LC. Salicylic acid-independent plant defence pathways. Trends in Plant Science 1999;**4**:52-58

[65] Yan L-H, Nuhant P, Sinigaglia I, et al. Synthesis of the Indolic Pentacyclic Core of Manadomanzamine A following biogenetically based strategies. European Journal of Organic Chemistry. 2012;**2012**:1147-1157

[66] Oshimi S, Zaima K, Matsuno Y, et al. Studies on the constituents from the fruits of Phaleria macrocarpa. Journal of Natural Medicines. 2008;**62**:207-210

[67] Calderon-Montano MJ, Burgos-Moron E, Perez-Guerrero C, et al. A review on the dietary flavonoid Kaempferol Mini-Reviews Medicinal Chemistry 2011;**11**:298-344

[68] Liu T, Nair SJ, Lescarbeau A, et al. Synthetic silvestrol analogues as potent and selective protein synthesis inhibitors. Journal of Medicinal Chemistry. 2012;**55**:8859-8878

[69] Kamiya K, Tanaka Y, Endang H, et al. New Anthraquinone and Iridoid from the fruits of Morinda Citrifolia. Chemical & Pharmaceutical Bulletin. 2005;**53**:1597-1599

[70] Chemical Computing Group. Molecular Operating Environment (MOE) 2014.09. 2015. Chemical Computing Group Inc. Available from: https://www.chemcomp.com/MOE2014.htm

[71] Tambunan USF, Parikesit AA, Nasution MAF, et al. Exposing the molecular screening method of Indonesian natural products derivate as drug candidates for cervical cancer. Iranian Journal of Pharmaceutical Research. 2017;**16**:1115-1129

[72] Tambunan USF, Alkaff AH, Nasution MAF, et al. Screening of commercial cyclic peptide conjugated to HIV-1 tat peptide as inhibitor of N-terminal heptad repeat glycoprotein-2 ectodomain Ebola virus through in silico analysis. Journal of Molecular Graphics & Modelling. 2017;**74**:366-378

[73] Liebeschuetz JW, Cole JC, Korb O. Pose prediction and virtual screening performance of GOLD scoring functions in a standardized test. Journal of Computer-Aided Molecular Design. 2012;**26**:737-748