ISSN 2744-1741
Defense and Security Studies
Original Research
Vol. 3, January 2022, pp.113-120
https://doi.org/10.37868/dss.v3.id217

Biodefense against Abrin and Ricin as bioterrorism agents - a virtual screening of Indonesian plant medicinal properties

Leni Tria Melati[1*], Timbul Siahaan[2], I Nengah Putra Apriyanto[3]
[1,2,3] Faculty of Defense Technology, Indonesian Defense University , Indonesia

*Corresponding author E-mail: leni.melati@tp.idu.ac.id[1]

Received Dec. 8., 2022
Revised Dec. 14., 2022
Accepted Dec. 21, 2022

Abstract

Because of their high lethality and easy way to produce, abrin and ricin are often used as bioweapons. Sources of raw materials for the abrin and ricin manufacture can be found in various regions, especially in tropical countries. Those make abrin and ricin have a greater chance to be used as bioterrorism agents. Furthermore, abrin and ricin have been classified as class B types of bioweapons used in bioterrorism.

In this study, a virtual screening (a bioinformatics method) will be carried out on 100 compounds derived from Indonesian Plants. Thus it can be known, the compounds that have the potential to be used as biodefense agents against abrin and ricin.

Finally, it is found that there are five compounds that have the best possibility as abrin inhibitors: procyanidin B4, ursolic acid, Corilagin, Vulgarin, and Gliberelic Acid. Whereas lanuginosine, xylonine, isovitexin, liriodenine, and procyanidin were found to have more potential as biodefense agents against ricin.

Keywords: Abrin; Biodefense; Bioinformatic; Bioweapon; Ricin

1. Introduction

Biological weapons are considered to be very detrimental and difficult to control so all forms of manufacture, development, and the usage of bioweapons in war have been prohibited since 1925 in Geneva Law [1]. Even so, there are still several cases of violation of these regulations by certain groups such as terrorists [2]. Even today, the use of technology such as genetic engineering provides opportunities for the development of biological weapons to a further level [3].

Bioweapons have actually been used for a long time in various forms of biological warfare. It is noted that the use of bioweapons has been started since medieval times. Where in 1340, the corpses of horses or other animals affected by the plague were being thrown using a launcher to the enemy groups, so it would cause a widespread of said disease within the enemy troops [1]. This shows how easy it is to develop and use bioweapons, even with only such simple equipment and materials.

The usage of biological weapons by terrorists is considered to result in high mortality rates as well as economic, social, and political losses [4]. This is because biological weapons are difficult to detect early on and their spread cannot be completely controlled (except for poisons), and these weapons may even attack the

user himself [1]. According to one of Indonesian military observer, bioweapons are widely used because they are cheaper and more effective [5]. One of the bioweapons that are widely used by terrorists is abrin and ricin. Abrin and ricin are ribosome-inactivating proteins (RIPs), which come from the *Abrus precatorius* plant that is easily found in tropical areas, especially in Southeast Asia [6]. In fact, due to the level of toxicity and ease of preparation, the two toxins are in category B as agents of bioterrorism [7]. In 2011, a militant group in Jakarta, Indonesia, was found to have attempted to attack the police using ricin. Followed in 2019, the Indonesian Police succeeded in seizing a bomb containing Abrin poison from a raid on the largest pro-Islamic State terrorist group in Indonesia [8]. In regards of these matters, it is necessary to have a strong defense against bioweapons (biodefense) within a country. As the United States (US) learned from the bitter experience, of the bioterrorism case that occurred in 2001, and increased research efforts in the field of biodefense in their country [9].

The drugs discovery process (in this case as biodefense agents) often takes a long time and requires a lot of money. The implementation of bioinformatics (in silico approach) provides a very significant influence in drug development. This approach provides more effective and cheaper process . Through an algorithmic simulation, accurate and fast research results can be obtained and only requires a small fee [10]. Furthermore, bioinformatic has also been widely applied and closely related to the biodefense [11], bioterrorism, and bioweapon research such as the bioweapon identification, detection (biosensors) development, as well as the development of vaccines and drugs from bioweapons [12].

This research aimed to identify plants derived compounds that commonly found in Indonesia as ricin and abrin inhibitors. The identification process is carried out by virtual screening method (bioinformatics procedures) based on molecular docking virtual simulations [12].

2. Research method

2.1. Receptor preparation

Target receptors, namely Ricin [13] and Abrin [14], were downloaded from http://www.rscb.org/pdb. The target receptor preparation process was carried out using the PyMOL 2.5 software by Schrödinger. The geometry optimization process uses PyMOL software by . The initial step is the removal of water molecules (H_2O) around the receptor, hetero atoms, and natural ligands. Next is the addition of Gasteiger and Hydrogen charges. The receptor and its natural ligand are separated (extract object) and each is stored in PDBQT format.

2.2. Inhibitor preparation

A list of 100 compounds derived from plants in Indonesia can be obtained on following link: http://herbaldb.farmasi.ui.ac.id/v3/. These compounds (inhibitors) can be downloaded from the https://pubchem.ncbi.nlm.nih.gov/ page. The inhibitor compounds were then optimized using OpenBable GUI 3.1.1 software and saved in PDBQT format. So that the ligands can then be used in the virtual screening process.

2.3. Virtual Screening

The virtual screening process is carried out through a simulation process of combining receptors and their inhibitors using the AutoDock Vina software [15]. As a result, the value of binding affinity or the tendency for binding between the ligand and the receptor protein will be obtained. Thus it can be determined which compound has the best binding affinity at Root Mean Square Deviation (RMSD) 0.0. Of all the compounds, 5 compounds that give the best results will be taken to be further visualized and analyzed.

2.4. Visualization

The virtual screening results were visualized in 3D graphics using PyMOL 2.5 (by Schrödinger) and 2D graphics using LigPlot+ version 2.2 (by EMBL's European Bioinformatics Institute). Furthermore, the results of the visualization can be used for the process of analyzing the inhibitory abilities of the selected compounds.

To assist the analysis process, a binding site prediction of the receptor will be carried out on https://prankweb.cz/. Thus, based on the results of 2D visualization, comparisons can be made to find out whether the selected compound binds to the target binding site so that it can be a good competitive inhibitor and thus can be used as abrin or ricin antitoxin.

3. Results and discussion

Indonesia has an abundant amount of natural resources, particularly in the form of plants. Some plants can produce secondary metabolomic compounds that have the opportunity to be developed as drugs. The screening process is carried out to filter metabolomic compounds from the database, which have the opportunity to become drug materials based on the virtual computing method [12]. The docking process is based on the binding activity of certain compounds (ligands/inhibitors) on the target protein. Protein itself is the basic structure of all living things. Knowledge of the proteins that make up an organism and understanding their functions is the basic foundation of molecular biology [16]. For this reason, protein is often used as the main target in drug or therapy development processes.

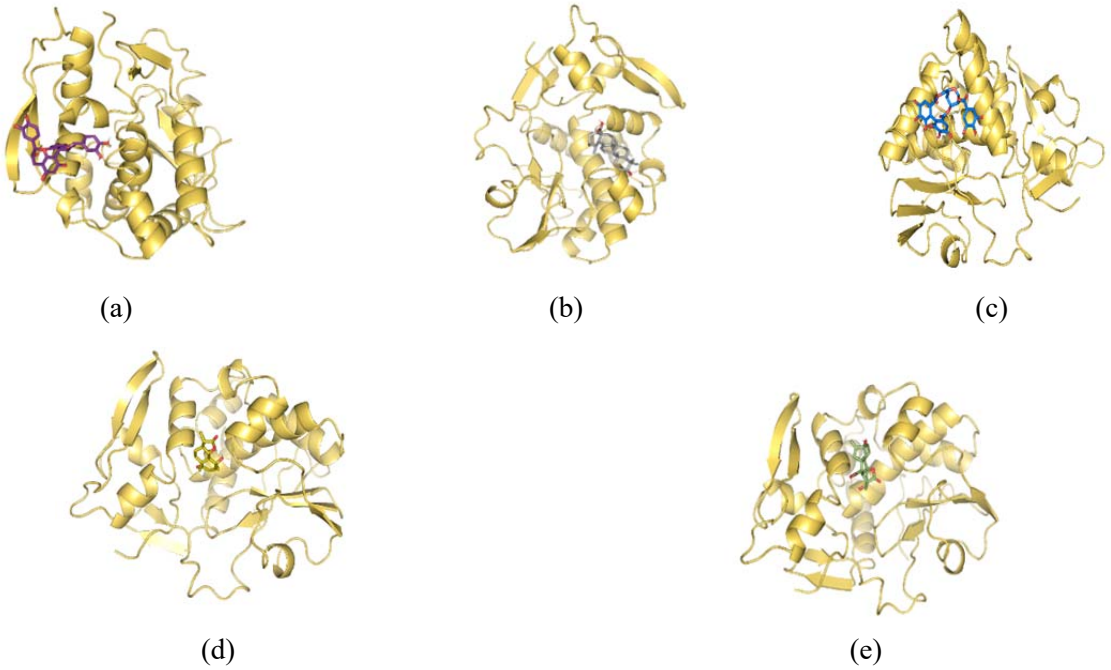
Based on the virtual screening results, out of 100 compounds, there are 5 compounds that gave the best binding affinity value at RMSD 0.0. The list of these compounds can be seen in Table 1. The binding of each selected ligand to the target protein (ricin and abrin) was then visualized in 2D and 3D using LigPlot and PyMOL software. Previously, binding site predictions from ricin and abrin had been carried out, as shown in Table 1. Based on these predictions, it was possible to compare the 2D visualization results from virtual screening results for further analysis.

Table 1. Binding site prediction results and five compounds that have the best potential as abrin and ricin inhibitors

Receptor	Binding site (residue)	Ligan / inhibitor	
		PubChem ID	Molecule
Abrin	**Pocket 1** (A_111 A_112 A_113 A_159 A_163 A_164 A_167 A_195 A_196 A_198 A_199 A_244 A_71 A_72 A_74 A_75 A_87 A_89 A_90 A_91) **Pocket 2** (A_140 A_141 A_144 A_145 A_146 A_151 A_78 A_80 A_83 A_85) **Pocket 3** (A_170 A_190 A_194 A_219 A_220 A_221 A_236 A_239)	147299 [17] 64945 [18] 73568 [19] 94253 [20] 6466 [21]	Procyanidin B4 Ursolic Acid Corilagin Vulgarin Gibberellic Acid
Ricin	**Pocket 1** (A_100 A_104 A_120 A_121 A_122 A_123 A_172 A_177 A_180 A_208 A_209 A_211 A_212 A_256 A_258 A_48 A_75 A_78 A_80 A_81 A_82 A_93 A_94 A_95 A_96 A_97) **Pocket 2** (A_183 A_203 A_207 A_233 A_234 A_235 A_240 A_251)	97622 [22] 160503 [23] 162350 [24] 10144 [25] 122738 [26]	Lanuginosine Xylopine Isovitexin Liriodenine Procyanidine

3.1. Virtual screening against abrin

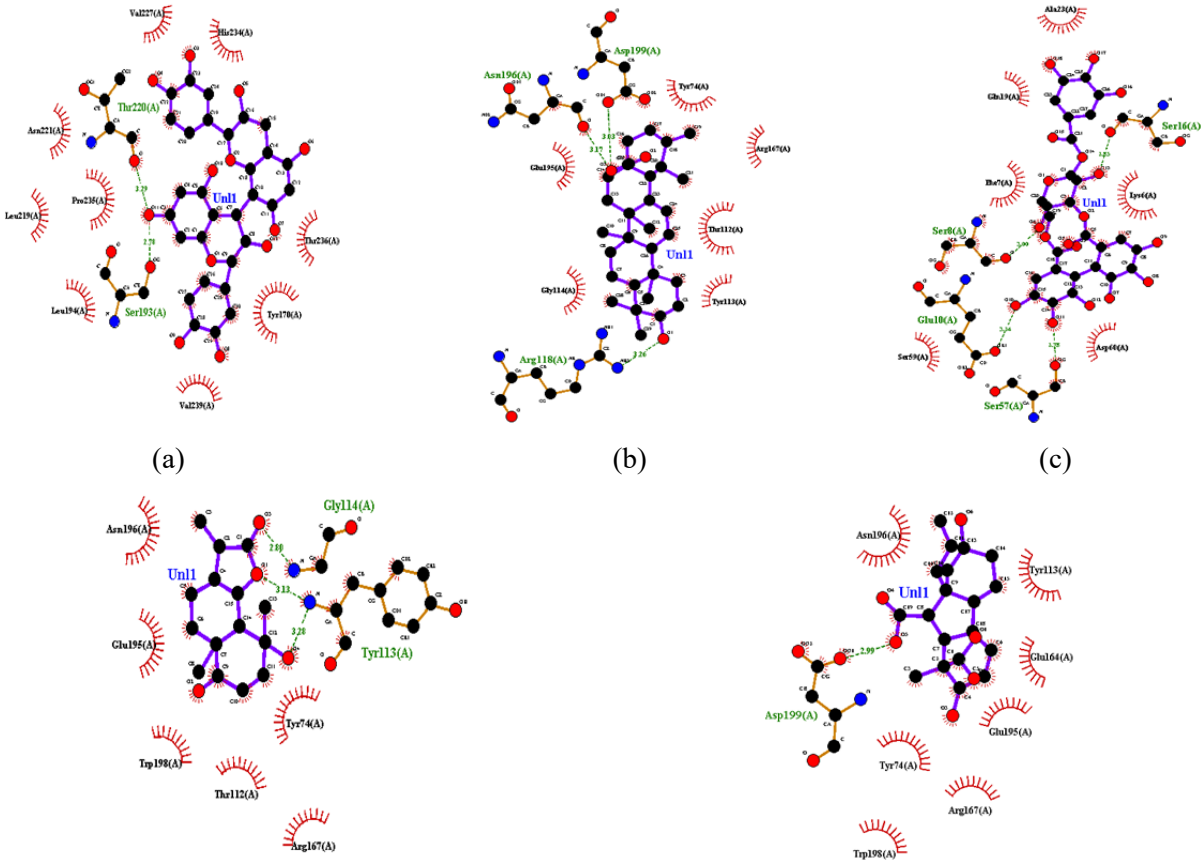
Based on the results of abrin virtual screening, it is known that procyanidin B4, ursolic acid, Corilagin, Vulgarin, and Gliberelic Acid have the best potential as lnhibitor. Figure 1 shows the results of the abrin virtual screening 3D visualization in PyMOL 2.5 (by Schrödinger). This results then further processed LigPlot + 2.2 (by EMBL's European Bioinformatics Institute) to obtain 2D visualization of the virtual screening process that are shown in Figure 2. This way we can understand the bonds and interactions that formed between each compound against abrin.



(a) (b) (c)

(d) (e)

Figure 1. 3D visualization in PyMOL 2.5 (by Schrödinger) to show the interaction between abrin and (a) procyanidin B4; (b) ursolic acid; (c) corilagin; (d) vulgarine; and (e) glibberellic acid as virtual screening result using AutoDock Vina.



(a) (b) (c)

(d) (e)

Figure 2. 2D visualization in LigPlot + 2.2 (by EMBL's European Bioinformatics Institute) to show the interaction between abrin and (a) procyanidin B4; (b) ursolic acid; (c) corilagin; (d) vulgarine; and (e) glibberellic acid as virtual screening result using AutoDock Vina.

From previous figures, the procyanidin B4 compound appears to be bound to pocket 3 of the abrin binding site. There are amino acid residues (A) numbered 170, 194, 219, 220, 221, 236, and 239 which are around the procyanidin B4 compound. Visualization results also show the formation of hydrogen bonds (green dash) and hydrophobic interactions between the inhibitor and the target receptor (Figure 2a). Meanwhile, ursolic acid, vulgarin, and gibberellic acid compounds were seen to interact with several amino acids in pocket 1 of the abrin binding site, through hydrophobic interactions and hydrogen bonds (Figures 2b, 2d, and 2e). Meanwhile, the Corilagin compound does not seem to bind to one of the pockets on the binding site of abrin, so there are two possibilities, whether the compound is a non-competitive inhibitor or does not even have the ability to inhibit abrin.

3.2. Virtual screening against ricin

Based on the virtual screening results for ricin, five compounds that had the best potential to be used as ricin inhibitors were obtained. These compounds are lanuginosine, xylonine, isovitexin, liriodenine, and procyanidin. The 3D visualization results (visualized in PyMOL) of the five compounds with ricin can be seen in Figure 3. Then the visualization process is continued with LigPlot to obtain 2D visualization.

In 2D visualization, it was observed that there was a hydrophobic interaction between lanuginosine and ricin compounds. The interaction occurs in pocket 1 of the ricin binding site. It can be observed that several amino acid residues (A) are involved in this interaction, including numbers 80, 81, 82, 93, 94, 121, 122, and 172. Likewise with the other four compounds which also interact with ricin in pocket 1 (Fig. 4). Even the compounds isovitexin and procyanidin, managed to form hydrogen bonds (green dash) with some of the amino acid residues that make up pocket 1 (Figures 4c and 4e).

The interactions that occur at the pocket binding site on the protein receptors (ricin and abrin) indicate that these compounds have the opportunity to become competitive inhibitors for abrin and ricin. Thus these compounds have the opportunity to be used as raw materials and further researched for the development of antitoxins for ricin and abrin, as a form of biodefense effort.

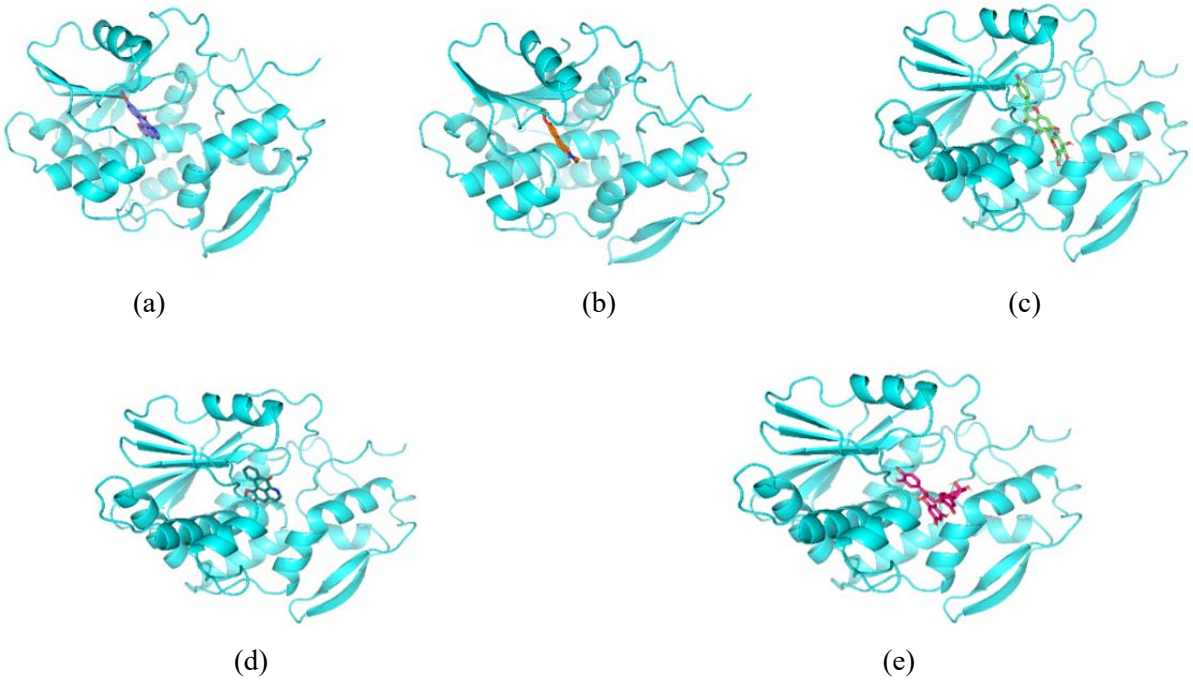


Figure 3. 3D visualization in PyMOL 2.5 (by Schrödinger) to show the interaction between ricin and (a) lanuginosine; (b) xylopine; (c) isovitexin; (d) liriodenine; and (e) procyanidine, as virtual screening result using AutoDock Vina.

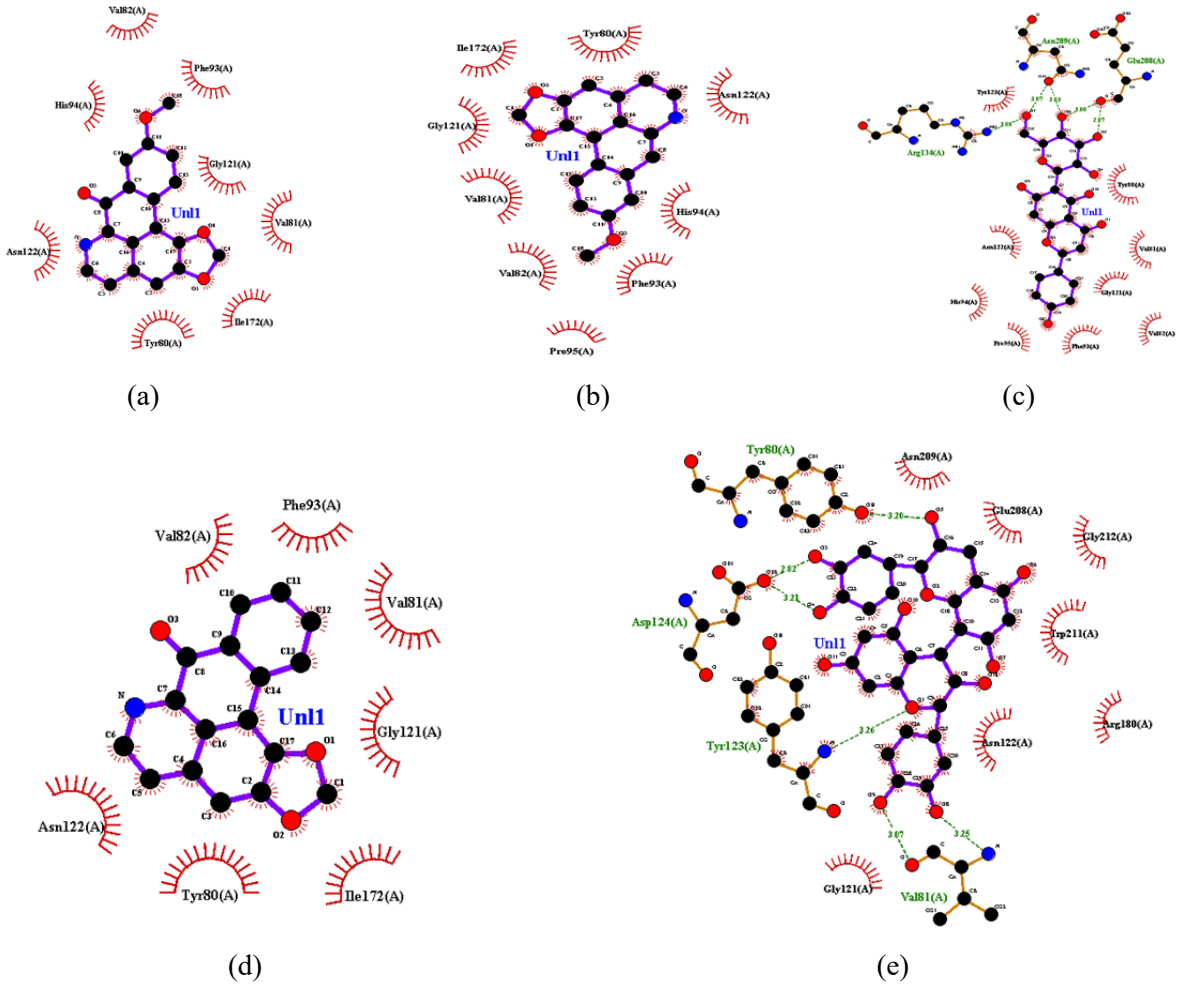


Figure 4. 2D visualization in LigPlot + 2.2 (by EMBL's European Bioinformatics Institute) to show the interaction between ricin and (a) lanuginosine; (b) xylopine; (c) isovitexin; (d) liriodenine; and (e) procyanidine, as virtual screening result using AutoDock Vina.

4. Conclusions

Abrin and ricin are toxic compounds with high level of lethality that can be easily produced, therefore have a great chance to be used as bioweapons in some bioterrorism cases. This study conducted a virtual screening of 100 compounds derived from several plants in Indonesia. From this virtual screening process five compound has been found to have the best potential for further research as raw materials for antitoxins against abrin and and another five for ricin. Compounds that have the potential as biodefense agents against abrin are procyanidin B4, ursolic acid, Corilagin, Vulgarin, and Gliberelic Acid. While the potential compounds to become biodefense agents against ricin include lanuginosine, xylonine, isovitexin, liriodenine, and procyanidin. This research is based on the results of virtual simulations, so it is necessary to carry out further research using other tests to determine the effectiveness of these compounds as biodefense agents against abrin and ricin.

Declaration of competing interest

The authors declare that they have no any known financial or non-financial competing interests in any material discussed in this paper.

Funding information

No funding was received from any financial organization to conduct this research.

References and citations

[1] V. S. Georgiev, In National Institute of Allergy and Infectious Diseases, NIH Volume 2, Humana Press: Springer Nature Switzerland AG, 2009.

[2] R. F. Lyon, "The covid-19 response has uncovered and increased our vulnerability to biological warfare", *Military Medicine,* vol. 180, no. 7-8, pp. 193-196, 2021.

[3] P. K. Yadav, "Evolving dimension of security: bioterrorism", *Unity Journal,* vol. 3, no. 01, pp. 266-277, 2022.

[4] T. Chugh, "Bioterrorism: Clinical and public health aspects of anthrax", *Current Medicine Research and Practice*, vol. 9, no. 3, pp. 110-111, 2019.

[5] Y. Elyasa, "Bioterrorism: the development and its regulations according to the international law", *Lampung Journal of International Law,* vol. 3, no. 1, pp. 253-256, 2021.

[6] A. Godal, S. Olnes, A. Pihi, "Radioimmunoassays of abrin and ricin in blood", *Journal of Toxicology and Environmental Health,* vol. 8, no. 3, 409-417, 2015.

[7] X. Bai, C. Hu, L. Chen et al, "A self-driven microfluidic chip for ricin and abrin detection", *Sensors,* vol.22, no. 3461, pp. 1-14, 2022.

[8] Global Biodefense Staff, "Biological terrorism in Indonesia", Global Biodefense Website : https://globalbiodefense.com/headlines/biological-terrorism-in-indonesia/, 2019.

[9] G. A. Petsko, "Biodefense versus bioterrorism", *Genome Biol* vol. 9 no. 108, https://doi.org/10.1186/gb-2008-9-8-108, 2008.

[10] S. Brogi, T. C. Ramalho, K. Kuca, J. L. Medina-Franco, M Valk, "In silico methods for drug design and discovery", *Frontiers in chemistry*, vol. 8, no. 612, 2020

[11] J. S. Meisel, D. J. Nasko, B. Brubach, V. Cepeda-Espinoza, J. Chopyk, H. Corrada-Bravo, et al., "Current progress and future opportunities in applications of bioinformatics for biodefense and pathogen detection: report from the Winter Mid-Atlantic Microbiome Meet-up, College Park, MD, on January 10 2018". *Microbiome*, vol. 6, no. 1, 1-10, 2018.

[12] R. K. Mondal, A. Dey, "Bioinformatics: past, present and future", *International Research Journal of Modernization in Engineering Technologyy and Science,* vol. 04, no. 07, pp. 442-454, 2018.

[13] J.H. Carra. et al., "Fragment-based identification of determinants of conformational and spectroscopic change at the ricin active site", *BMC structural biology,* vol. 7, no. 1, pp. 1-11, 2007.

[14] H. Bansia, S. Bagaria, A. A. Karande, S. Ramakumar, "Structural basis for neutralization of cytotoxic abrin by monoclonal antibody D6F10." *The FEBS Journal*, vol. 286, no. 5, pp. 1003-1029, 2019.

[15] O. Trott, A. J. Olson, "AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading", *Journal of Computational Chemistry*, vol. 31, pp. 455-461, 2010.

[16] C. M. V.Benítez, H. S. Lopes, " Molecular dynamics for simulating the protein folding process using the 3D AB off-lattice model" In *Brazilian Symposium on Bioinformatics,* pp. 61-72, August 2012.

[17] National Center for Biotechnology Information. "PubChem Compound Summary for CID 147299, Procyanidin B4", *PubChem*, https://pubchem.ncbi.nlm.nih.gov/compound/Procyanidin-B4, Accessed 10 November, 2022.

[18] National Center for Biotechnology Information. "PubChem Compound Summary for CID 64945, Ursolic acid", *PubChem*, https://pubchem.ncbi.nlm.nih.gov/compound/Ursolic-acid, Accessed 10 November, 2022.

[19] National Center for Biotechnology Information. "PubChem Compound Summary for CID 73568, Corilagin", *PubChem*, https://pubchem.ncbi.nlm.nih.gov/compound/Corilagin, Accessed 10 November, 2022.

[20] National Center for Biotechnology Information. "PubChem Compound Summary for CID 94253, Vulgarin", *PubChem*, https://pubchem.ncbi.nlm.nih.gov/compound/Vulgarin, Accessed 10 November, 2022.

[21] National Center for Biotechnology Information. "PubChem Compound Summary for CID 6466, Gibberellic acid", *PubChem*, https://pubchem.ncbi.nlm.nih.gov/compound/Gibberellic-acid, Accessed 10 November, 2022.

[22] National Center for Biotechnology Information. "PubChem Compound Summary for CID 97622, Lanuginosine", *PubChem*, https://pubchem.ncbi.nlm.nih.gov/compound/Lanuginosine, Accessed 10 November, 2022.

[23] National Center for Biotechnology Information. "PubChem Compound Summary for CID 160503, Xylopine", *PubChem*, https://pubchem.ncbi.nlm.nih.gov/compound/Xylopine, Accessed 10 November, 2022.

[24] National Center for Biotechnology Information. "PubChem Compound Summary for CID 162350, Isovitexin", *PubChem*, https://pubchem.ncbi.nlm.nih.gov/compound/Isovitexin, Accessed 10 November, 2022.

[25] National Center for Biotechnology Information. "PubChem Compound Summary for CID 10144, Liriodenine", *PubChem*, https://pubchem.ncbi.nlm.nih.gov/compound/Liriodenine, Accessed 10 November, 2022.

[26] National Center for Biotechnology Information. "PubChem Compound Summary for CID 122738, Procyanidin B2", *PubChem*, https://pubchem.ncbi.nlm.nih.gov/compound/Procyanidin-B2. 10 November, 2022,