Molecular Docking Study of Aloesin and Its Derivatives as Potential Antiaging Agents

Sri Handayani[1](✉), Dwi Ni'maturrohmah[1], Anastasia W. Indrianingsih[1], Khoirun Nisa[1], Anjar Windarsih[1], Cici Darsih[1], Ardiba R. Sefrienda[1], Ade E. Suryani[1], and Sari Haryanti[2]

[1] Research Center for Food Technology and Processing, National Research and Innovation Agency (BRIN), Yogyakarta, Indonesia
srih012@brin.go.id

[2] Research Center for Pharmaceutical Ingredients and Traditional Medicine, National Research and Innovation Agency (BRIN), Tawangmangu, Indonesia

Abstract. Aloe vera is a beneficial plant used for food supplements, cosmetics, and medicine for years. Currently, the popularity of herbal, cosmetic, foods, and beverages products containing aloe gel induce a big demand in the global market for aloe vera. Aloesin and its derivatives are chromone compounds found in aloe vera. This study aims to identify the binding affinity of aloesin, isoaloeresin D, and 7 methyl ether 2′ feruloylaloesin to show their anti-collagenase and anti-elastase activities. Bioinformatic studies to show the interaction of aloesin compounds with collagenase protein (PDB ID: 2Y6I) and elastase protein (PDB ID: 1BRU) were done using YASARA and PLANTS docking tools and visualized by Ligplot^{+} software. The acute toxicity of compounds was predicted using ProTox-II webserver. The result of this study revealed that interaction of collagenase protein with isoaloeresin D performed the lowest docking score (-87.3 ± 4.6). Meanwhile, the interaction of elastase protein with aloesin, isoaloeresin D, and 7 methyl ether 2′ feruloylaloesin shows similar docking scores i.e. -80.4 ± 2.2, -80.6 ± 1.7, and -81.2 ± 1.6 respectively. The affinity of isoaloeresin D and 7 methyl ether 2′ feruloylaloesin with elastase protein were higher compared to the native ligand, but not aloesin. While the affinity of all the aloesin derivatives used in this study with elastase protein were higher compared to the native ligand. The acute toxicity of aloesin and its derivatives were predicted at a similar level. Aloesin and its derivatives are potential to be used as active materials from aloe vera with antiaging effects, especially for anti-collagenase and anti-elastase activities.

Keywords: aloe gel extract · antiaging · collagenase · elastase · molecular docking · aloesin

1 Introduction

Recently, the growing popularity of herbal, cosmetic, foods, and beverages containing aloe gel create a big demand within the global market for aloe vera [1]. The aloe genus is belonging to Xanthorrhoeaceae family. Aloe genus has several species including *Aloe*

I. Nurlaila et al. (Eds.): ICHR 2022, AHSR 56, pp. 288–299, 2023.
https://doi.org/10.2991/978-94-6463-112-8_28

vera (*Aloe barbadensis* Miller), *Aloe arborescens, Aloe vera* L. *var. Chinensis* (Haw.) Berg., *Aloe ferox, Aloe indica, Aloe littoralis,* and *Aloe perfoliata* [2]. This perennial green herb is native to southern and eastern Africa and expanded to warm climatic areas of Asia, Europe, and America [3, 4]. Aloe vera is a beneficial plant used for food supplements, cosmetics, and medicine [5–7]. Studies of the aloe plant for cosmetics started a long time ago. In vivo study reported that aloe gel significantly inhibits wrinkles and improves elasticity in photoaged human skin through increasing collagen production and decreasing collagen-degrading MMP-1 gene expression [8]. Aloe treatment makes skin more elastic and less wrinkled through its stimulation of fibroblast which produces collagen and elastin fibers [9]. The activation of mitochondria and inhibition of the photo-aging of UV_b-irradiated skin fibroblasts was increased after treatment with the fermentation of the skin of aloe leaves [10].

Aloe plants contain several active compounds that have a role in nutraceutical effects such as polysaccharides, phenolic compounds, vitamins, minerals, enzymes, and organic acids [5]. Quispe et al. (2018) revealed several compounds inside the aloe vera plant including aloesin derivatives that we choose in this study (aloesin, isoaloeresin D, and 7 methyl ether 2′ feruloylaloesin) [11]. Aloesin and its derivatives are chromone compounds specifically found in aloe plants. Chromone structure is the core structure of flavonoids, flavone, and isoflavone [12]. Chromone, polyphenol, and flavonoid are widely studied as antiaging agents. The mechanism for hyperpigmentation of epigallocatechin gallate, quercetin, aloesin, hydroxystilbene derivates, and licorice extracts is because of their ability to remove ROS and to chelate metals ions at the active sites of metalloenzymes [13]. In vitro and bioinformatic studies reported that aloesin inhibits tyrosinase with IC_{50} value 31.5 μM and binds to the active site of tyrosinase enzyme [14]. Aloesin treatment also suppressed pigmentation in human skin after UV radiation by 34% [15]. Nevertheless, anti-collagenase and anti-elastase activities of aloesin, isoaloeresin D, and 7 methyl ether 2′ feruloylaloesin especially in silico study was never reported yet. Then, this study aims to identify the in silico binding affinity of aloesin, isoaloeresin D, and 7-methyl ether 2′ feruloylaloesin to show their anti-collagenase and anti-elastase activities.

2 Materials and Methods

2.1 Preparation of Ligands and Receptors

The Two-dimensional (2D) and three-dimensional (3D) structures of test compounds and reference compounds were visualized using MarvinSketch software (https://chemaxon.com/products/marvin) for molecular docking studies with the selected enzymes. Meanwhile, the 3D structure of receptors i.e. collagenase (PDB ID 2Y6I), and elastase (PDB ID 1BRU) were downloaded from RCSB protein database (http://www.rcsb.org/pdb/home/home.do). Preparation of protein receptors and ligands were done using YASARA 20.8.23 software. Molecular docking was generated using Protein-Ligand Ant System (PLANTS) docking tool. Docking interaction was visualized by Ligplot$^+$ v.2.2 software.

2.2 Docking Protocol Validation

The validation of docking protocol was done by calculating the Root Mean Square Deviation (RMSD) values between the native ligand isolated from PDB file with its conformation of the docked ligand. The RMSD value was calculated using YASARA software. The docking protocol can be used for the further docking process if the RMSD has a value of less than 2.5 Å [16].

2.3 Molecular Docking

Interaction of protein-ligand was done with a standard procedure of molecular docking using PLANTS [17]. The docking scores shows the energy of the ligand in binding to the target protein. The affinity of the ligand binding to the protein is stronger when the docking score is more negative. Afterward, the 2D docking interaction of protein receptor and ligand was visualized by Ligplot^{+} software.

2.4 The Acute Toxicity Prediction

The acute toxicity of aloesin, isoaloeresin D, and 7 methyl ether 2′ feruloylaloesin were predicted using ProTox-II [18]. The toxicity level was shown in LD_{50}, mg/kg unit.

3 Results

3.1 The Affinity of Protein-Ligand

Validation of docking protocol was done using RMSD parameter. The docking protocol validation in this study shows that both collagenase (2Y6I) and elastase (1BRU) used in this study meet the required RMSD value ($\leq$2.5 Å). The affinity of protein-ligand can be shown from the interaction of the ligand in the active site of the protein. The 3D molecular docking cavities of native ligands with collagenase (2Y6I) and elastase (1BRU) is shown in Fig. 1. Interaction of protein-ligand was measured with docking scores parameter. Then, we confirmed the activity of aloesin and its derivatives (Fig. 2) against collagenase and elastase using this protocol. We used ursolic acid as a reference standard inhibitor against collagenase and elastase enzymes [19]. The result revealed that isoaloeresin D, and 7 methyl ether 2′ feruloylaloesin show interaction with collagenase enzyme with docking scores i.e. -87.3 $\pm$ 4.6, and -83.6 $\pm$ 2.2 respectively. The docking score of both compounds were lower than the native ligand (-79.1 $\pm$ 2.8) and ursolic acid (-63.4 $\pm$ 3.5). While aloesin shows interaction with collagenase enzyme with docking score -75.1 $\pm$ 2.0. Aloesin has a lower docking score compared to ursolic acid, but has a higher docking score compared to isoaloeresin D, 7 methyl ether 2′ feruloylaloesin, and the native ligand (Table 1). On the other hand, the interaction of aloesin, isoaloeresin D, and 7 methyl ether 2′ feruloylaloesin with elastase protein shows similar docking scores i.e. -80.4 $\pm$ 2.2, -80.6 $\pm$ 1.7, and -81.2 $\pm$ 1.6 respectively. The interaction of all the aloesin derivatives with elastase protein was also higher compared to the ursolic acid (-56.7 $\pm$ 5.0) and the native ligand (-63.5 $\pm$ 0.9) (Table 2). Nevertheless, the aloesin, isoaloeresin D, and 7 methyl ether 2′ feruloylaloesin has good affinities with both collagenase and elastase enzymes compared to ursolic acid as a reference standard.

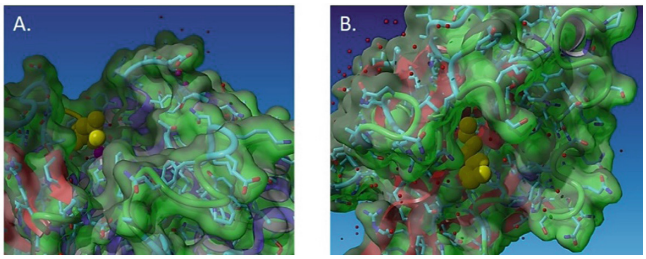

Fig. 1. The 3D molecular docking cavities of native ligands with A. collagenase, and B. elastase. Native ligands are shown in yellow.

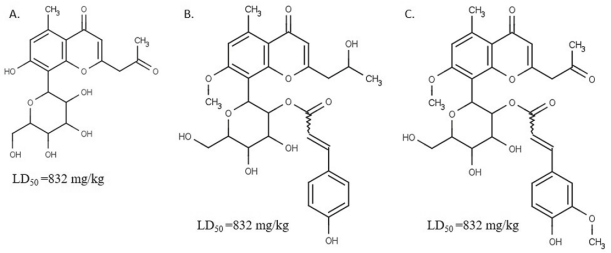

Fig. 2. Chemical structures and prediction of acute toxicity level (LD_{50}) of A. Aloesin, B. Isoaloesin D, C. 7 methyl ether 2′ feruloylaloesin.

3.2 Protein-Ligand Interaction

The 2D visualization of collagenase-ligand interaction using Ligplot^{+} shows that the native ligand of 2Y6I (Ipi1-gly-pro-ala) has a hydrogen bond with Zn cation of this enzyme (2.56 Å). The native ligand also shows hydrophobic bonds with Glu524, Phe515, Leu520, Gly493, Gly494, and Ser513. The reference ligand ursolic acid also shows a hydrogen bond with catalytic zinc ion (2.29 Å). Moreover, ursolic acid also has hydrogen bonds with His527 (2.25 Å), Glu555 (3.15 Å), and Tyr506 (3.33 Å) residues. Ursolic acid shows hydrophobic bonds with Glu524, Phe515, Leu520, Leu495, Gly494, Ser513, and Asn492 residues. This reference ligand also has one external bond with each Zn, and Gly493 residues. Aloesin shows hydrogen bonds with Zn (3.13 Å), Glu524 (3.25 Å), and Tyr506 (3.21 Å) residues. Hydrophobic bonds with Glu559, Leu520, Leu495, Asn492, and Tyr607 residues and three external bonds with Gly494 residue were shown in the interaction of aloesin with collagenase enzyme. Isoaloeresin D has only one hydrogen bond with Zn (2.34 Å). Nevertheless, this compound has hydrophobic bonds with Glu524, Phe515, Gly494, Asn492, and Asp554 residues, 7 external bonds with Glu555 residue, and 1 external bond with Gly493 residue. On the other hand, 7 methyl ether 2′ feruloylaloesin did not have any hydrogen bonds. However, this compound has hydrophobic bonds with Glu524, His523, His527, Asn492, Thr505, Asp554, and Ile514 residues, and external bonds with Glu555 (5), Leu520 (4), Gly493 (1), and Gly494 (1) residues (Table 1, Fig. 3).

Table 1. Protein-ligand interaction of collagenase protein (2Y6I) with aloesin, isoaloeresin D, and 7 methyl ether 2′ feruloylaloesin.

No	Compound	Docking Scores	Hydrogen bond		Hydrophobic Bond	External Bond (Total Bonds)
			residue	distance (Å)		
1	Ipi1-gly-pro-ala (native ligand)	-79.1 ± 2.8	Zn	2.46 (O2)	Glu524, Phe515, Leu520, Gly493, Gly494, Ser513	
2	Ursolic acid	-63.4 ± 3.5	Zn	2.29 (O1)	Glu524, Phe515, Leu520, Leu495, Gly494, Ser513, Asn492	Zn (1), Gly493 (1)
			His527	2.25 (O1)		
			Glu555	3.15 (O1)		
			Tyr506	3.33 (O3)		
3	Aloesin	-75.1 ± 2.0	Glu524	3.25 (O8)	Glu559, Leu520, Leu495, Asn492, Tyr607	Gly494 (3)
			Zn	3.13 (O8)		
			Tyr506	3.21 (O1)		
4	Isoaloeresin D	-87.3 ± 4.6	Zn	2.34 (O3)	Glu524, Phe515, Gly494, Asn492, Asp554	Glu555 (7), Gly493 (1)
5	7 methyl ether 2′ feruloylaloesin	-83.6 ± 2.2			Glu524, His523, His527, Asn492, Thr505, Asp554, Ile514	Glu555 (5), Leu520 (4), Gly493 (1), Gly494 (1)

The elastase-ligand interaction shows that the native ligand of 1BRU (GR143783) has hydrogen bonding with Ser195 (2.51 Å) and Gly193 (2.79 Å). The native ligand also shows hydrophobic bonds with Gly216, Ser190, Ser214, Val213, Asn192, and Asp194 residues. On the other hand, none of the hydrogen bonds were shown in the interaction of 1BRU with reference ligand ursolic acid. Nevertheless, ursolic acid shows hydrophobic-bond with Gly216, Ser190, Ser214, Phe215, Gly193, Asp194, Cys42, and Cys58 residues and has external bonds with His57 (5), Ser195 (1), Cys191 (1), Cys220 (1) residues. Aloesin shows hydrogen bonds with Gly216 (2.11 Å) and Cys58 (2.43 Å) residues. Moreover, hydrophobic bonds with Ser190, Ser214, Val213, Gly196, Asp194, Arg143, Ala55, and Cys42 residues were shown in the interaction of aloesin with elastase enzyme 1BRU. Isoaloeresin D has only one hydrogen bond with Cys191 (3.16 Å). This compound also has hydrophobic bonds with Gly216, Ser190, Phe215, Gly193, Arg143, Cys42, Cys58, Cys220, and Ile59 residues and external bonds with Ser214 (2), Val213

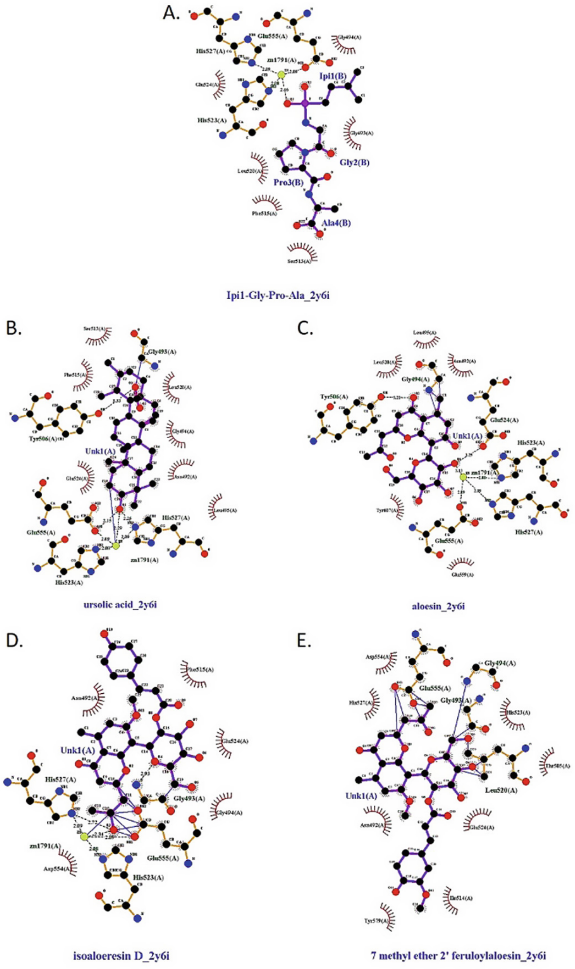

Fig. 3. 2D molecular docking poses predicted for collagenase (2Y6I). A. Native ligand Ipi1-Gly-Pro-Ala, B. Ursolic acid, C. Aloesin, D. Isoaloesin D, E. 7 methyl ether 2′ feruloylaloesin exhibiting different types of intermolecular interactions i.e. hydrogen bond (green lines), external bond (light purple lines), hydrophobic interactions (red bricks).

(2), Asp194 (1) residues. The 7 methyl ether 2′ feruloylaloesin did not have any hydrogen bonds. However, this compound has hydrophobic bonds with Gly216, Ser190, Gly193, Asp194, Cys42, Cys58, Cys191, Thr41, and Ile59 residues, and external bonds with Ser195 (8), Asn192 (5) residues (Table 2; Fig. 4).

3.3 The Acute Toxicity Prediction

Somehow, the toxicity of chemicals should be known to avoid unwanted events. The acute toxicity prediction of aloesin and its derivatives in this study were done using ProTox-II webserver [18]. The result shows that aloesin, isoaloeresin D, and 7 methyl

Table 2. Protein-ligand interaction of elastase protein (1BRU) with aloesin, isoaloeresin D, and 7 methyl ether 2′ feruloylaloesin.

No	Compound	Docking Scores	Hydrogen bond		Hydrophobic Bond	External Bond (Total Bonds)	Other Information
			residue	distance (Å)			
1	GR143783 (native ligand)	-63.5 ± 0.9	Ser195	2.51 (O5)	Gly216, Ser190, Ser214, Val213, Asn192, Asp194		H-bond Asp102 with His57 is detected recognized at 2.71 Å and 3.1 Å
			Gly193	2.79 (O5)			
2	Ursolic acid	-56.7 ± 5.0			Gly216, Ser190, Ser214, Phe215, Gly193, Asp194, Cys42, Cys58	His57 (5), Ser195 (1), Cys191 (1), Cys220 (1)	H-bond Asp102 with His57 is detected at 2.71 Å and 3.1 Å
3	Aloesin	-80.4 ± 2.2	Gly216	2.11 (O4)	Ser190, Ser214, Val213, Gly196, Asp194, Arg143, Ala55, Cys42		H-bond Asp102 with His57 is detected at 2.71 Å and 3.1 Å
			Cys58	2.43 (O5)			
4	Isoaloeresin D	-80.6 ± 1.7	Cys191	3.16 (O3)	Gly216, Ser190, Phe215, Gly193, Arg143, Cys42, Cys58, Cys220, Ile59	Ser214 (2), Val213 (2), Asp194 (1)	
5	7 methyl ether 2′ feruloylaloesin	-81.2 ± 1.6			Gly216, Ser190, Gly193, Asp194, Cys42, Cys58, Cys191, Thr41, Ile59	Ser195 (8), Asn192 (5)	

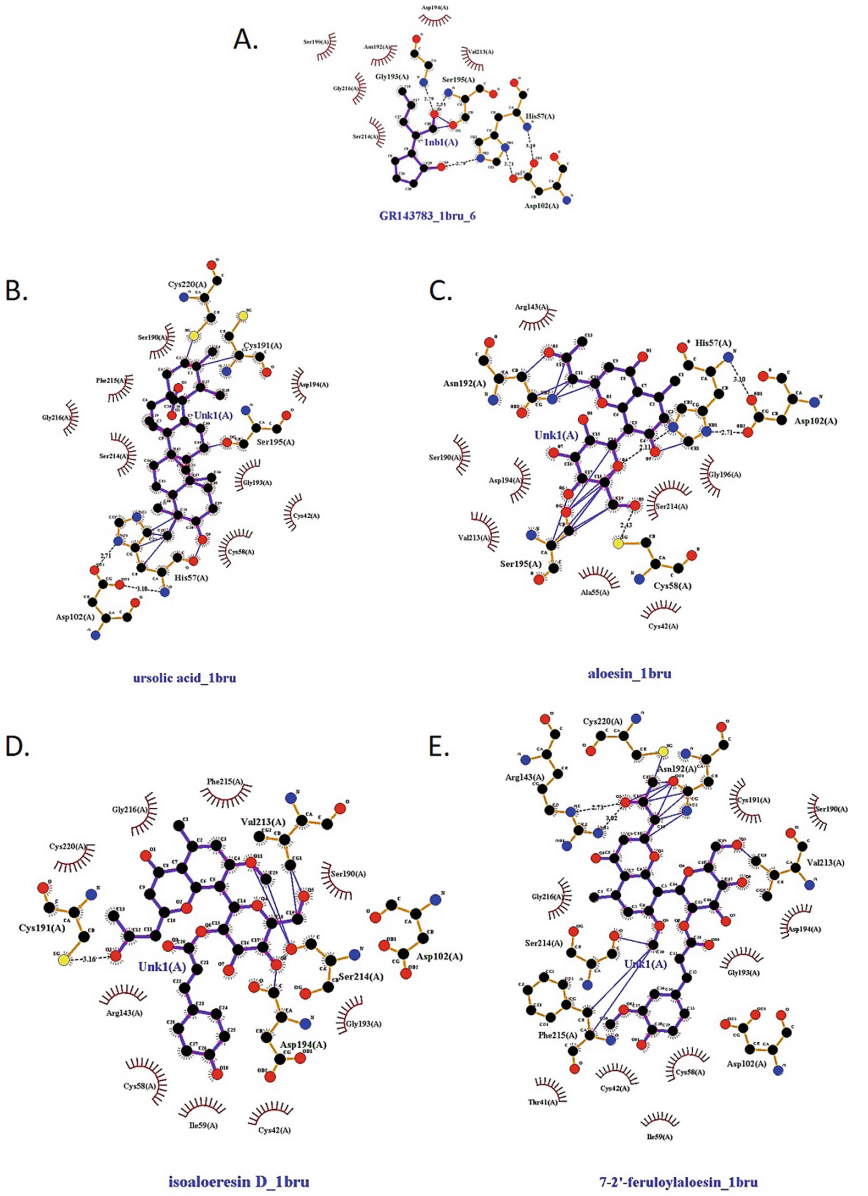

Fig. 4. 2D molecular docking poses predicted for elastase (1BRU). A. Native ligand GR143783, B. Ursolic acid, C. Aloesin, D. Isoaloesin D, E. 7 methyl ether 2′ feruloylaloesin exhibiting different types of intermolecular interactions i.e. hydrogen bond (green lines), external bond (light purple lines), hydrophobic interactions (red bricks).

ether 2′ feruloylaloesin has similar LD_{50} i.e. 832 mg/kg (Fig. 2). The result of the toxicity level was predicted similar probably because the three compounds have almost similar structures. This toxicity level was categorized as toxicity class number 4 from 6 classes and turned to moderate toxicity especially for oral consumption.

4 Discussion

Since molecular docking is one of the effective methods to prove the antiaging activity of compounds [19], we confirmed the activity of aloesin and its derivatives against collagenase and elastase using this method. The Glu524 is the active site for collagenase activity. Meanwhile, His523, His527, and Glu555 residues were zinc metal-binding sites of the collagenase enzyme [19]. The Zn cation is the catalytic site of the collagenase enzyme, interaction of the native ligand with this catalytic zinc ion indicated inhibition of the activity of this enzyme [20, 21]. Nevertheless, this study revealed that external interaction of ligands with His523, His527, and/or Glu555 residues indicated higher affinity with collagenase enzyme 2Y6I. Ursolic acid has the weakest affinity toward collagenase enzyme possibly because this compound only interacted with Glu524, Zn, His527, and Glu555 by hydrogen bond and hydrophobic bond. The seven external bonds of isoaloeresin D with collagenase enzyme at Glu555 residue possibly made isoaloeresin D get the lowest docking interaction with this enzyme. Besides that, other interactions with Glu524, and Zn residues made the affinity stronger. Although 7 methyl ether 2′ feruloylaloesin did not interact with Zn, this compound still has the second-lowest energy against collagenase enzyme because of its interaction with 5 external bonds at Glu555 residue. At the same time, other interactions with Glu524, His523, His527 residues were strengthen the affinity.

Enzymatic activity of elastase enzyme came from the presence of a catalytic triad formed by three amino acid residues, Asp102, His57, and Ser195 [22]. On the other hand, Gly216 residue was confirmed as an active site of inhibitory activity in the elastase enzyme [19]. The His57 and Ser195 residues were identified as active sites of caffeine in the interaction against elastase 1BRU. Meanwhile, Val213 and Phe215 residues were observed for disulfide bond formation to stabilize the interaction [19]. The hydrogen bond of Asp102 residue with His57 in elastase enzyme 1BRU was recognized at 2.71 Å and 3.1 Å (Table 2). The hydrogen bond of Asp102 and His57 did not show in the interaction of 1BRU with isoaloesin D and 7 methyl ether 2′ feruloylaloesin. The Asp102 residue that was seen without any interaction possibly was identified as an inactivation mechanism of isoaloesin D and 7 methyl ether 2′ feruloylaloesin against elastase enzyme. The 7 methyl ether 2′ feruloylaloesin has the lowest docking score possibly because this ligand also has a hydrophobic bond with Gly216 residue and two external bonds with Ser195 residue. The interaction of 1BRU with isoaloesin D was strengthened by its hydrophobic bond with Gly216, and Phe215 residue and two external bonds with Val213 residue. Meanwhile, the hydrogen bond of Asp102 residue with His57 residue in elastase enzyme 1BRU was still exist after the interaction of this enzyme with native ligand, reference ligand (ursolic acid) and aloesin. However, docking score of aloesin was almost similar compared to the other aloesin derivatives probably because the existence of hydrogen bond with Gly216 residue, and hydrophobic bond with Val213 residue.

After the ability to breakdown hydrogen bonding of Asp102 and His57, the hydrogen bond with Gly216 residue also made possible reason to strengthen the affinity of aloesin derivatives with elastase enzyme. In vitro and in vivo studies need to be done in the future studies to strengthen the result of antiaging activity of aloesin derivatives of this in silico study. Then, aloesin, isoaloesin D, and 7 methyl ether 2′ feruloylaloesin can be developed as antiaging agents.

5 Conclusion

The interaction of collagenase enzyme (2Y6I) with isoaloeresin D showed the highest affinity. While the interaction of elastase protein (1BRU) with 7 methyl ether 2′ feruloylaloesin showed the highest affinity. In general, aloesin, isoaloesin D, and 7 methyl ether 2′ feruloylaloesin can be developed as antiaging agents. Nevertheless, in vitro and in vivo studies need to be done in the future studies to strengthen the result of antiaging activity in this in silico study.

Acknowledgement. The authors would like to thank the Research and Innovation Program for Advanced Indonesia grant by the National Research and Innovation Agency (BRIN) (Grant No. 60/II/HK/2022) and Dr. Hari Purnomo, Faculty of Pharmacy, Gadjah Mada University, for his guidance to use docking software.

References

1. Transparency Market Research. Aloe Vera Gel Market - Global Industry Analysis 2026. Published 2021. Accessed May 28, 2021. https://www.transparencymarketresearch.com/aloe-vera-gel-market.html
2. Adlakha K, Koul B, Kumar A. Value-added products of Aloe species: Panacea to several maladies. *South Afr J Bot*. Published online February 3, 2021. doi: https://doi.org/10.1016/j.sajb.2020.12.025
3. Ahlawat KS, Khatkar BS. Processing, food applications and safety of aloe vera products: a review. *J Food Sci Technol*. 2011; 48(5): 525–533. doi: https://doi.org/10.1007/s13197-011-0229-z
4. Sánchez M, González-Burgos E, Iglesias I, Gómez-Serranillos MP. Pharmacological Update Properties of Aloe Vera and its Major Active Constituents. *Mol Basel Switz*. 2020; 25(6). doi: https://doi.org/10.3390/molecules25061324
5. Hamman JH. Composition and applications of Aloe vera leaf gel. *Mol Basel Switz*. 2008; 13(8): 1599–1616. doi: https://doi.org/10.3390/molecules13081599
6. Kumar R, Singh AK, Gupta A, Bishayee A, Pandey AK. Therapeutic potential of Aloe vera-A miracle gift of nature. *Phytomedicine Int J Phytother Phytopharm*. 2019; 60: 152996. doi: https://doi.org/10.1016/j.phymed.2019.152996
7. Handayani S, Aprilia D, Nisa K, et al. A Mini-Review: Possible Mechanisms of Hepatoprotective Effect of Aloe Vera Gel. *Indones J Cancer Chemoprevention*. 2021; 12(3): 170–179. doi: https://doi.org/10.14499/indonesianjcanchemoprev12iss3pp170-179
8. Cho S, Lee S, Lee MJ, et al. Dietary Aloe Vera Supplementation Improves Facial Wrinkles and Elasticity and It Increases the Type I Procollagen Gene Expression in Human Skin in vivo. *Ann Dermatol*. 2009; 21(1): 6–11. doi: https://doi.org/10.5021/ad.2009.21.1.6

9. Surjushe A, Vasani R, Saple DG. ALOE VERA: A SHORT REVIEW. *Indian J Dermatol*. 2008; 53(4): 163–166. doi: https://doi.org/10.4103/0019-5154.44785
10. Lee H, Choi W, Ro H, Kim G, Lee H. Skin Antiaging Effects of the Fermented Outer Layers of Leaf Skin of Aloe barbadensis Miller Associated with the Enhancement of Mitochondrial Activities of UVb-Irradiated Human Skin Fibroblasts. *Appl Sci*. 2021; 11(12): 5660. doi: https://doi.org/10.3390/app11125660
11. Quispe C, Villalobos M, Bórquez J, Simirgiotis M. Chemical Composition and Antioxidant Activity of Aloe vera from the Pica Oasis (Tarapacá, Chile) by UHPLC-Q/Orbitrap/MS/MS. *J Chem*. 2018; 2018: e6123850. doi: https://doi.org/10.1155/2018/6123850
12. Wang T yang, Li Q, Bi K shun. Bioactive flavonoids in medicinal plants: Structure, activity and biological fate. *Asian J Pharm Sci*. 2018; 13(1): 12–23. doi: https://doi.org/10.1016/j.ajps.2017.08.004
13. Qian W, Liu W, Zhu D, et al. Natural skin-whitening compounds for the treatment of melanogenesis (Review). *Exp Ther Med*. 2020; 20(1): 173–185. doi: https://doi.org/10.3892/etm.2020.8687
14. Mikayoulou M, Mayr F, Temml V, et al. Anti-tyrosinase activity of South African Aloe species and isolated compounds plicataloside and aloesin. *Fitoterapia*. 2021; 150: 104828. doi: https://doi.org/10.1016/j.fitote.2021.104828
15. Choi S, Lee SK, Kim JE, Chung MH, Park YI. Aloesin inhibits hyperpigmentation induced by UV radiation. *Clin Exp Dermatol*. 2002; 27(6): 513–515. doi: https://doi.org/10.1046/j.1365-2230.2002.01120.x
16. Mumpuni E, Mulatsari E. Molecular Docking and Toxicity Test of Apigenin Derivative Compounds as an Anti-Aging Agent. *J Appl Chem Sci*. 2018; 5(1): 409–413. doi: https://doi.org/10.35508/jacs.v5i1.1747
17. Meiyanto E, Putri DDP, Susidarti RA, et al. Curcumin and its Analogues (PGV-0 and PGV-1) Enhance Sensitivity of Resistant MCF-7 Cells to Doxorubicin through Inhibition of HER2 and NF-kB Activation. *Asian Pac J Cancer Prev*. 2014; 15(1): 179–184. doi: https://doi.org/10.7314/APJCP.2014.15.1.179
18. Banerjee P, Eckert AO, Schrey AK, Preissner R. ProTox-II: a webserver for the prediction of toxicity of chemicals. *Nucleic Acids Res*. 2018; 46(W1): W257–W263. doi: https://doi.org/10.1093/nar/gky318
19. Lee KE, Bharadwaj S, Yadava U, Kang SG. Evaluation of caffeine as inhibitor against collagenase, elastase and tyrosinase using in silico and in vitro approach. *J Enzyme Inhib Med Chem*. 2019; 34(1): 927–936. doi: https://doi.org/10.1080/14756366.2019.1596904
20. Eckhard U, Schönauer E, Brandstetter H. Structural basis for activity regulation and substrate preference of clostridial collagenases G, H, and T. *J Biol Chem*. 2013; 288(28): 20184–20194. doi: https://doi.org/10.1074/jbc.M112.448548
21. Schönauer E, Kany AM, Haupenthal J, et al. Discovery of a Potent Inhibitor Class with High Selectivity toward Clostridial Collagenases. *J Am Chem Soc*. 2017; 139(36): 12696–12703. doi: https://doi.org/10.1021/jacs.7b06935
22. Donarska B, Z Łączkowski K. Recent advances in the development of elastase inhibitors. *Future Med Chem*. 2020; 12(20): 1809–1813. doi: https://doi.org/10.4155/fmc-2020-0163