Employing an *in-silico* approach in the evaluation of *Aloe vera* metabolites as inhibitors of Omicron RBD and the ROS-dependent cellular signaling processes

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**ARTICLE INFO**

Received on: 18/08/2022  
Accepted on: 22/01/2023  
Available Online: 04/03/2023

**Key words:**  
*Aloe vera*, *in silico*, ROS, Omicron RBD, COVID-19, SARS-CoV-2.

**ABSTRACT**

The pathogenesis of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is related to increased reactive oxygen species (ROS) formation. This increasing ROS formation can mediate ROS-dependent cellular signaling processes inducing cytokines and inflammations that worsen the disease. The severity of coronavirus disease 2019 (COVID-19) can progress due to the self-sustaining cycle of ROS release, inflammatory mediators, and cellular damage. For the treatment, *Aloe vera* is a promising plant that has the potential to be used. In this study, therefore, we identified the metabolite composition of *A. vera* peel and gel using liquid chromatography-mass spectrometry (LC-MS). The metabolites were molecularly docked to Omicron receptor-binding domain (RBD) and ROS-producing enzymes to obtain medicinal compounds to inhibit these targets. The LC-MS analysis revealed the peel and gel compositions are distinct, in which 13 metabolites are identified in the peel and 12 in the gel. Furthermore, these metabolites might be promising inhibitors against Omicron variant SARS-CoV-2 RBD and ROS-producing enzymes based on the docking scores and the number of bonds formed. Thus, *A. vera* is one promising candidate for COVID-19 treatment due to its potential to alter the RBD function of forming a complex with ACE2 and inhibit the ROS-dependent cellular signaling processes related to COVID-19 pathogenesis and disease severity progression.

**INTRODUCTION**

The increased level of reactive oxygen species (ROS) appears to play a role in the pathogenesis of coronavirus disease 2019 (COVID-19) (Cecchini and Cecchini, 2020). ROS are small oxygen-derived molecules that can be in the form of oxidizing agents or also molecules that are easily converted into oxygen radicals (André-Lévigne *et al.*, 2017). Increased production of ROS can lead to oxidative stress-induced molecular damage. Recent methodological advances have identified the enzymes responsible for producing and elevating ROS levels. Elevated ROS levels have been linked to a variety of diseases, including cardiovascular, immune, and nervous system disorders, as well as aging and cancer. According to a recent study, selectively targeting specific ROS levels enables the development of a futuristic refined redox medicine (Sammar *et al.*, 2019; Sies and Jones, 2020). Production and elevation formation of ROS is correlated with almost every human disease. As a result, selectively targeting ROS-related proteins is an important step in the development of redox medicine. Network pharmacology and big data in drug discovery and development are modernized approaches in treating oxidative stress that has placed ROS as the basis of a number of diseases. However, studies on the regulation of ROS levels must be conducted in greater detail in order to elucidate the mechanism.
by which ROS causes disease and cellular damage (Casas et al., 2020).

Antioxidants have been extensively used as agents to lower ROS levels and treat oxidative stress. Antioxidant supplementation reduces COVID-19 severity due to its stress oxidative relieving effect (Delgado-Roche and Mesta, 2020; Pizzino et al., 2017).

The use of herbal medicine is becoming popular worldwide and well accepted and distributed as food composition and supplements that are safe as antioxidants (Ekor, 2014). Aloe vera is one of the plants known to exhibit great antioxidant activity. Hence, it has the potential to regulate ROS formation in the body. It contains a considerable number of metabolites, such as flavonoids, terpenoids, lectins, fatty acids, anthraquinones, pectins, hemicelluloses, glucomanann, tannins, campesterol, capreol, enzymes, salicylic acid, calcium, chromium, copper, iron, magnesium, manganese, potassium, phosphorus, sodium, zinc, retinol, ascorbic acid, alpha-tocopherol, beta-carotene, B1–B3, B6, choline, B12, and folic acid (Hês et al., 2019).

The variety of metabolite makeup of a plant depends on several aspects, such as the type and conditions of cultivation, climate condition, harvesting time, position of the harvested leaves, species, variety, and method used for harvesting leaves (Giannakoudakis et al., 2018). We hypothesized that A. vera grown in North Sulawesi, Indonesia, contains different metabolites than those grown in other regions, owing to the different cultivation conditions and climates. Information on metabolite classes has been published. Specific metabolites from the peel and gel of A. vera collected from the Indonesian island of North Sulawesi, on the other hand, have never been previously reported.

There is a dearth of information regarding the composition of A. vera metabolites from North Sulawesi and their effect on ROS levels and COVID-19 patients. Hence, we used liquid chromatography-mass spectrometry (LC-MS) to determine the metabolite composition of the peel and gel of A. vera collected in North Sulawesi, Indonesia. Further docking of the compounds to the ROS-producing enzymes was performed to identify compounds that have the potential to inhibit the proteins’ function and stop their biological processes. In addition, to look at the direct inhibition potential against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), we also evaluated the inhibition potential of the metabolites against the receptor-binding domain (RBD) of the Omicron variant using the same method as against the ROS-producing enzymes. Since RBD plays an essential role in viral entry into human cells, targeting this domain can illustrate the role of the metabolites in halting the virus’s life cycle.

**METHODS**

**Metabolite identification**

The identification of the metabolites was carried out using an LC-MS instrument in accordance with the protocol described by Nur et al. (2021), with slight modifications to the run time, specifically for 25 minutes.

**Molecular docking protocol**

The identified metabolites were used as ligands. The structures were obtained from PubChem, their energy was minimized, and then PyRx 8.0 was used to dock them to the ROS-producing enzymes. The targets used in this protocol are RBD of SARS-CoV-2 Omicron variant (PDB code: 7T9L), inducible nitric oxide synthase (NOS) (PDB code: 4U6X), endothelial nitric oxide synthase (eNOS) (PDB code: 6CIF), neuronal nitric oxide synthase (nNOS) (PDB code: 6PMY), cyclooxygenase-1 (PDB code: 5U6X), cyclooxygenase-2 (PDB code: 6V3R), and 5-lipoxygenase (5-LOX) (PDB code: 6N2W). Vitamin C was used as a benchmark because it is an extremely powerful antioxidant. The docking result was ranked based on the docking scores (kcal/mol) and the number of hydrogen bonds due to the evaluation of the two of these aspects is important and may enhance the linearity with experimental data (Cheshire et al., 2011; Cinelli et al., 2020; Cingolani et al., 2017; Dallakyan and Olson, 2015; Gilbert et al., 2020; Li et al., 2018; Mousavi et al., 2021; Uddin et al., 2020; Tallei et al., 2020). The top-ranked ligands were further visualized using the BIOVIA Discovery Studio Visualizer.

**RESULTS AND DISCUSSION**

**Metabolite identification**

The previous study lacked data on the metabolites found in A. vera peel. Only the peel metabolites classes had been extensively identified, such as steroids, tannins, terpenoids, catechin, carotenoids, and anthraquinones (Bista et al., 2020; Dharajiya et al., 2017; Muthukumaran et al., 2018; Nalimu et al., 2021). However, the majority of the available research data did not differentiate between the peel and gel metabolite classes, so the localized major metabolites in each component of the plant are poorly known (Raad et al., 2020; Sánchez et al., 2020). We found that there are differences in the metabolites in the peel and gel of A. vera. The only available data on the metabolites in A. vera peel was the result of the study conducted by López et al. (2013), using samples collected from the Canary Islands of Spain. They identified sinapic acid, quercitroside, kaempferol, chamomile, protocatechuic, catechin, vanillic acid, epicatechin, cedric acid, 3-O-caffeylquinic acid, gentisic acid, 3,4-dihydroxycinnamic acid, coumaric acid, 4-hydroxy-3-methoxycinnamic acid, rutin, myricetin, and quercetin. Our finding showed that A. vera collected from North Sulawesi of Indonesia contained different types of metabolites. As a result, it can be justified that plants’ metabolite content will vary according to their cultivation location (Kumar et al., 2017).

There are 13 metabolites identified in the peel and 12 metabolites in the gel of A. vera. Figures 1 and 2 depict the LC-MS chromatogram. The result showed that there are differences in metabolites in the peel and gel of A. vera. The metabolites detected in the peel include triacetetyl-bromo-emodin, 6-O-(alpha-L-rhamnopyranosyl)-(1->6)-beta-D-glucopyranosyl)emodin, 8-demethyl-8-(alpha-L-rhamnosyl)tetracenomycinC,(10R)-1,8,10alpaha-triacetoxy-3-acetoxymethyl-10-(2-O,3-O,4-O,6-O-tetraacetytl-beta-D-glucopyranosyl)anthrone, aloe-emodin dianthrone diglucoside, (9R)-9-[(9S)-2-carboxy-5-[(2S,3R,4S,5S,6R)-
Figure 1. LC-MS result of *A. vera* peel.

Figure 2. LC-MS result of *A. vera* gel.
4,5-dihydroxy-6-(hydroxymethyl)-3-oxalooxyoxan-2-yl)oxy-4-hydroxy-10-oxo-9H-anthracen-9-yl]-4-hydroxy-10-oxo-5-[2(S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-9H-anthracene-2-carboxylic acid, landomycin X, emodin-8-0-beta-gentiobioside, L-rhodinose-2-deoxy-L-fucose-2-deoxy-L-fucose-10-decarboxymethoxy-episilin-rhodocymicine none, adaxanthomycin A, landomycin B, and landomycin W. The metabolites found in gel include aloin 8-O-alpha-D-glucopyranoside, aloe-emodin-d5, 1-hydroxyanthrone, 6-O-(alpha-L-rhamnopyranosyl)-(1→6)-beta-D-glucopyranosyl) emodin, 8-demethyl-8-(alpha-L-rhamnopynosyl)tetracenominycin C, 12-demethyl-elloramicyn, (10R)-l,8,10alpha-triacetoxy-3-acetoxymethyl-10-(2-O,3-O,4-O,6-O-tetraacetyl-beta-Dglucopyranosyl)anthrone, aloe-emodin dianthrone diglucoside, (9R)-9-{[(9S)-2-carboxy-5-{(2S,3R,4S,5S,6R)-4,5-dihydroxy-6-(hydroxymethyl)-3-oxalooxyoxan-2-yl]oxy-4-hydroxy-10-oxo-9H-anthracen-9-yl]-4-hydroxy-10-oxo-5-{[2(S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-9H-anthracene-2-carboxylic acid, landomycin X, emodin-8-0-beta-gentiobioside, landomycin A, and adaxanthomycin A (Table 1). The Pubchem CIDs of the identified metabolites are listed in Supplementary Tables S1-S2.

**Molecular docking**

The docking scores of vitamin C and A. vera metabolites docked to target the enzymes are shown in Table 2. The docking score, measured in kcal/mol, which is an energy per molecule unit, can represent the binding energy of a ligand against a particular protein target. The lower this score is, the higher the affinity is or the better the binding of the ligand-target complex is (Tallei et al., 2020). Due to all of the metabolites found in A. vera’s peel and gel having lower docking scores than vitamin C, they would highly likely form a better binding complex with the enzymes.

**Figure 3** shows vitamin C interactions with all the target proteins, while Figures 4–10 show the visualization of the binding interactions. Numerous bond formations occur in the complexes of the compounds and all of the targeted enzymes, such as conventional hydrogen bond (dark green), carbon-hydrogen bond (light green), alkyl/pi-alkyl bond (light pink), pi-pi stacked bond (dark pink), pi-cation/anion bond (dark orange), pi-sulfur bond (light orange), and pi-sigma bond (purple), with hydrogen and alkyl bonds dominating the interaction.

In addition, the ligands also interact with the enzymes’ amino acids through van der Waals interactions. The interactions are also evaluated according to which amino acids in the metabolites bind to because each protein might have certain amino acids that are critical in the inhibition process (Kumar et al., 2021).

**SARS-CoV-2 Omicron RBD inhibitor**

There are 30 mutations that occur in the viral spike of the Omicron variant, among which eight mutations are situated in the spike RBD. These mutations invite the most interest from science and medical researchers due to their significant role as host recognition sites. D339, L371, P373, F375, N471, K440, S446, N477, K478, A484, R493, S496, R498, Y501, and H505 were the output residues of the Omicron variants. Landomycin A of A. vera, through hydrogen bond interactions, binds Ser484 and Ser493 in the mutation region of the Omicron RBD. It has been hypothesized that variants carrying multiple mutations in the RBD will probably exhibit an even higher stabilizing effect on the interaction between the viral spike and the host angiotensin-converting enzyme 2 (ACE2) receptor. Hence, landomycin A is predicted to alter this stabilizing effect of the Omicron RBD. Ortega et al. (2022) addressed several further analyses employing computational study which are needed to gain a novel perspective of the SARS-CoV-2 Omicron variant’s detailed structure, residue formation, and changes that can be potentially inhibited as a druggable binding domain (Ahmad, 2021; Mengist et al., 2021; Ortega et al., 2021, 2022).

The previous study also found that residues 301 and 430 in the main receptor-binding motif have a critical mechanism due to their role as the antibody recognition region of the viral spike. The antibody recognition site is critical for efficiency. However, the Omicron variant’s viral spike carries several mutations located in this region. Therefore, the current assumption is that the antibody will be less effective against the Omicron variant, and further study needs to be done to evaluate the immune response elicited by vaccines against recent emerging SARS-CoV-2 variants (Ewer et al., 2021; Harvey et al., 2021; Ravichandran et al., 2020).

Aloe vera metabolites show great docking scores in the Omicron RBD binding domain, with the value ranging from −6 kcal/mol to −10 kcal/mol. In terms of the binding affinity with ACE2, a significant influence of the Omicron variant RBD was found. Residues 468–473 were reported to play a role in this significant change (Kumar et al., 2021). In comparison with the wild-type RBD, the Omicron variant has a better ACE2 binding capacity. Residues T478K and N501Y are associated with increased ACE2 binding capacity (Omotuyi et al., 2022). Neither vitamin C nor landomycin A interacts with these residues. However, the B501Y mutation did not enhance Omicron infectivity and epidermal growth factor receptor (EGFR) binding due to the mutations of the charged to the uncharged polar side chain on its interface of interaction. Therefore, N501Y failed to deliver immune disruption and did not lower vaccine efficiency (Back et al., 2021; Kazybay et al., 2022; Liu et al., 2021).

**Nitric oxide synthase inhibitor**

The release of ROS induces oxidative stress. In terms of improving the health conditions of COVID-19 patients, several clinical approaches have been used in order to lower ROS levels and production, as well as to repair the oxidative stress condition (Derouiche, 2020).

There are a number of studies showing that ROS plays a role in SARS-CoV-2 infections. The infections will generate a sustaining cycle of hematological damage, cytopathic hypoxia, and ROS production (Cecchini and Cecchini, 2020). During disease progression, patients with COVID-19 were shown to undergo hematological damage (Chen et al., 2020; Huang et al., 2020; Lippi and Mattiuzzi, 2020) and cytopathic hypoxia. Disseminated intravascular coagulopathy, sepsis, and decreased oxygen transport to the tissues are some of the main elements presented by COVID-19 patients. In the state of hypoxia, the body generates superoxide, such as hydrogen peroxide, that triggers inductible nitric oxide synthase (iNOS) activation resulting in an increased level of NO and peroxynitrite, which are toxic to mitochondria. Mitochondria impairment leads to cytopathic hypoxia. This creates a self-sustaining cycle and can progress to a
| Metabolite | Structure |
|-----------|-----------|
| **1** Triacetyl-bromo-emodin |
| **2** 6-O-(Alpha-L-rhamnopyranosyl)(1->6)-beta-D-glucopyranosyl)emodin |
| **3** 8-Demethyl-8-(alpha-L-rhamnosyl)tetracenomycin C |
| **4** (10R)-1,8,10Alpha-triacetoxy-3-acetoxymethyl-10-(2-O,3-O,4-O,6-O-tetraacetyl-beta-D-glucopyranosyl)anthrone |

Continued
| Metabolite                                                                 | Structure                                                                 |
|---------------------------------------------------------------------------|---------------------------------------------------------------------------|
| Aloe-emodin dianthrone diglucoside                                        | ![Aloe-emodin Dianthrone Diglucoside](image1.png)                         |
| (9R)-9-[[(9S)-2-Carboxy-5-[[2S,3R,5S,6R]-4,5-dihydroxy-6-(hydroxymethyl)-3-oxaloxyoxan-2-yl|oxy-4-hydroxy-10-oxo-9H-anthracen-9-yl]-4-hydroxy-10-oxo-5-[[2S,3R,4S,5S,6R]-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl|oxy-9H-anthracene-2-carboxylic acid] | ![Aloe-emodin Dianthrone Diglucoside](image2.png)                         |
| Emodin-8-0-beta-gentiobioside                                              | ![Emodin-8-0-beta-Gentiobioside](image3.png)                              |
| L-Rhodinose-2-deoxy-L-fucose-2-deoxy-L-fucose-10-decarbomethoxy-epsilon- | ![L-Rhodinose-2-Deoxy-L-Fucose-2-Deoxy-L-Fucose-10-Decarbomethoxy-Epsilon-]|
| Metabolite                  | Structure |
|----------------------------|-----------|
| 13 Aloe-Emodin-d5          | ![Structure 13](image13) |
| 14 1-Hydroxyanthrone       | ![Structure 14](image14) |
| 15 12-Demethyl-elloramycin | ![Structure 15](image15) |
| 16 Landomycin X            | ![Structure 16](image16) |
| 17 Landomycin A            | ![Structure 17](image17) |
Table 2. Molecular docking result of vitamin C and A. vera peel and gel metabolites against omicron RBD and ROS-producing enzymes. The lowest docking score values are bolded.

| Metabolite                                                                 | Docking score (kcal/mol) |
|---------------------------------------------------------------------------|--------------------------|
|                                                                           | RBD (7P9L)               |
| Vitamin C                                                                 | −5.1                     |
| Triacetyl-bromo-emodin                                                    | −7.3                     |
| 6-O-(Alpha-L-rhamnospyranosyl)-(1->6)-beta-D-glucopyranosyl) emodin        | −8                       |
| 8-Demethyl-(alpha-L-rhamnosyl)tetraenomycin C                             | −7.4                     |
| (10R)-1,8,10Alpha-triaacetox-3-acetoxymethyl-10-(2-O,3-O,4-O,6-O-tetraacetyl-beta-D-glucopyranosyl)anthrone | −6                       |
| Aloe-emodin 1,8-anthrancarboxy-2-carboxylic acid                           | −8.3                     |
| Emodin-8-o-beta-gentiobioside                                             | −8                       |
| L-Rhodinose-2-deoxy-L-fucose-2-deoxy-L-fucose-10-decarboxamethoxy-epsilon-rhodomycinone | −8.4                     |
| Adxanthromycin A                                                          | −7.5                     |
| Landomycin B                                                              | −9.3                     |
| Landomycin W                                                              | −9                       |
| Aloin 8-O-alpha-D-glucopyranoside                                         | −6.9                     |
| Aloe-emodin d5                                                            | −6.4                     |
| 1-Hydroxyanthrone                                                         | −6.7                     |
| 12-Demethyl-elloramycin                                                  | −7                       |
| Landomycin X                                                              | −9.5                     |
| Landomycin A                                                              | −10                      |

|                                                                           | INOS (4UX6)              |
|                                                                           | eNOS (6CIF)              |
|                                                                           | nNOS (6PMY)              |
|                                                                           | COX-1 (5UX6)             |
|                                                                           | COX-2 (6V3R)             |
|                                                                           | 5-LOX (6N2W)             |

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| Metabolite                                                                 | Docking score (kcal/mol) |
|---------------------------------------------------------------------------|--------------------------|
|                                                                           | RBD (7P9L)               |
| Vitamin C                                                                 | −5.1                     |
| Triacetyl-bromo-emodin                                                    | −7.3                     |
| 6-O-(Alpha-L-rhamnospyranosyl)-(1->6)-beta-D-glucopyranosyl) emodin        | −8                       |
| 8-Demethyl-(alpha-L-rhamnosyl)tetraenomycin C                             | −7.4                     |
| (10R)-1,8,10Alpha-triaacetox-3-acetoxymethyl-10-(2-O,3-O,4-O,6-O-tetraacetyl-beta-D-glucopyranosyl)anthrone | −6                       |
| Aloe-emodin 1,8-anthrancarboxy-2-carboxylic acid                           | −8.3                     |
| Emodin-8-o-beta-gentiobioside                                             | −8                       |
| L-Rhodinose-2-deoxy-L-fucose-2-deoxy-L-fucose-10-decarboxamethoxy-epsilon-rhodomycinone | −8.4                     |
| Adxanthromycin A                                                          | −7.5                     |
| Landomycin B                                                              | −9.3                     |
| Landomycin W                                                              | −9                       |
| Aloin 8-O-alpha-D-glucopyranoside                                         | −6.9                     |
| Aloe-emodin d5                                                            | −6.4                     |
| 1-Hydroxyanthrone                                                         | −6.7                     |
| 12-Demethyl-elloramycin                                                  | −7                       |
| Landomycin X                                                              | −9.5                     |
| Landomycin A                                                              | −10                      |

|                                                                           | INOS (4UX6)              |
|                                                                           | eNOS (6CIF)              |
|                                                                           | nNOS (6PMY)              |
|                                                                           | COX-1 (5UX6)             |
|                                                                           | COX-2 (6V3R)             |
|                                                                           | 5-LOX (6N2W)             |

more severe condition (Connors and Levy, 2020; Mantzarlis et al., 2017; Ottolenghi et al., 2020). In contrast with the NO-induced mitochondria impairment, the production of NO by the eNOS at the proper dose might intriguingly elevate the COVID-19 patient condition. Therefore, selectively targeting NOS is critical (Guan et al., 2020; Fang et al., 2021; Pieretti et al., 2021).

NOS, a homodimer nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) binding enzyme, is regulated by several modulatory interactions with the function of converting L-argnine to nitric oxide, one of the ROS. According to the localization or the regulation, NOS is available in three isoforms, namely, nNOS, iNOS, and eNOS. In the state of excessive NO production, this enzyme regulation may lead to cellular or tissue damage (Casas et al., 2020; Stuehr and Vasquez-Vivar, 2017).

The peel and gel of A. vera were shown to have metabolites that bind to NOS with a stable interaction due to the great docking scores and number of bond formations in the binding complex with landomycin X, landomycin A, and landomycin B. This indicates that these compounds can act as the most potent inhibitors. Our finding on the inhibition of NOS, the enzyme that can induce cellular damage in the state of excessive NO production, may explain the result of a study conducted by Moriyama et al. (2016), where they found that A. vera treatment increases cell number and wound healing.

Cyclooxygenase (COX) inhibitor

Viral infections have been associated with molecular processes that promote inflammation and oxidative damage during the infection (Komaravelli and Casola, 2014). COVID-19 pathogenesis is closely related to the development of oxidative stress (Chernyak et al., 2020). COXs are a part of ROS-producing enzymes (Liu et al., 2018). COX produces ROS in the intestinal mucosa. In the presence of free arachidonic acid, COX is activated in the pathway which will result in the production of IL-8 as an end-product and ROS as a byproduct (Alzoghaibi, 2013). The production of ROS leads to oxidative stress, one of the characteristics found in severe COVID-19 patients, that contributes to a complex cellular signaling process in COVID-19 disease progression.

In a severe condition, COVID-19 patients were also shown to have increased fatty acid levels along with inflammatory lipid storms. COX produced inflammatory lipids that were involved in these characteristics (Archambault et al., 2020). Activation of the COX pathway plays a pivotal role in the development of inflammation. It leads to the formation of prostaglandins like PGE2
and PGI2 which are involved in various aspects of inflammation, including the increase of IL-6 production, migration of leukocytes, and fever (McCarthy and Weinberg, 2012).

*Aloe vera* metabolites can inhibit COX (COX-1 and COX-2) according to the molecular interactions formed in the complex in our finding. The most potential ligands as COX inhibitors are landomycin B and landomycin W. However, there is no clear line on whether COX-1 or COX-2 is more important in lowering ROS levels or in the progression of the viral infection. Therefore, the selectivity of the inhibition needs to be taken into consideration for further analysis.

**5-Lipoxygenase inhibitor**

The COVID-19 disease can also be characterized by alveolar and endothelial damage, thrombosis, pulmonary embolism, and inflammatory cell infiltration (Barton et al., 2020; Damiani et al., 2021; Huang et al., 2020; Zhang et al., 2020). The infiltrating inflammatory cells release ROS in order to clear the infection, leading to the production and accumulation of oxidized phospholipids (OxPLs), oxidized phospholipids, locally in the lungs. OxPLs, which were also detected in the lungs of SARS-CoV-2 patients, signal the activation and release of macrophages, TNF-α, and IL-1β (Merad and Martin, 2020; Poeckel and Funk, 2010). Respiratory symptoms are the hallmark manifestations of SARS-COV-2 infection (Terpos et al., 2020).

ROS, along with proinflammatory leukotrienes, are released via the lipoxygenase (LOX) pathway. The release can be derived from arachidonic acid or eicosapentaenoic acid or docosahexaenoic acid (Häfner et al., 2019; O’Flaherty et al., 2012). 5-LOX is one of the enzymes that mediate the LOX pathway. Other enzymes mediating the LOX pathway are 12-LOX, 15-LOX, and the epidermal LOX (Rådmark et al., 2015).

![Figure 3. Binding interaction of vitamin C with (A) Omicron RBD, (B) iNOS, (C) eNOS, (D) nNOS, (E) COX-1, (F) COX-2, and (G) 5-LOX.](image-url)
**Figure 4.** Binding interaction of ligands with the lowest binding energy (landomycin A, PubChem CID: 9988748) against SARS-CoV-2 Omicron RBD. (A) The pose of interactions of the ligand (cyan) and the RBD (gray) in a 3D representation. (B) 2D representation of the pose shows the amino acids of the RBD that interact with the ligand.

**Figure 5.** Binding interaction of ligands with the lowest binding energy (landomycin X, PubChem CID: 156580396) against iNOS. (A) The pose of interactions of the ligand (green) and the iNOS (gray) in a 3D representation with 180° rotation views. (B) 2D representation of the pose shows the amino acids of the iNOS that interact with the ligand.
5-LOX, along with COX, can generate ROS as byproducts of its activation. In the presence of free arachidonic acid or NADH or NADPH, 5-LOX produces superoxide, one class of ROS. The activation of 5-LOX mediates the accumulation of intracellular superoxide (Alzoghaibi, 2013; Zhang et al., 2014). Landomycin W, a metabolite localized only in the peel of A. vera, is the most potential metabolite to be developed as a 5-LOX inhibitor due to the high number of bond formations and great docking scores. Landomycin W can possibly act as an inhibitor to stop 5-LOX activation. This inhibition is critical due to the activation of 5-LOX, and the release of leukotrienes may contribute to the severity of the disease (Ayola-Serrano et al., 2021). 5-LOX inhibitors can also stop the disease progression by preventing 5-LOX from inducing TNF-a activation and the translocation of NF-κB into the nucleus (Lin et al., 2014).

In severe disease progression, COVID-19 patients are characterized by robust levels of lipids produced by the COX and LOX pathways. Leukotrienes such as LTC4, LTB4, 20-COOH-LTB4, LTE4, and eoxin E4 are also increased (Archambault et al., 2020). A treatment targeting leukotrienes production has been

Figure 6. Binding interaction of ligands with the lowest binding energy (landomycin A, PubChem CID: 9988748) against eNOS. (A) The pose of interactions of the ligand (cyan) and the eNOS (gray) in a 3D representation. (B) 2D representation of the pose shows the amino acids of the eNOS that interact with the ligand.

Figure 7. Binding interaction of ligands with the lowest binding energy (landomycin B, PubChem CID: 53297405) against nNOS. (A) The pose of interactions of the ligand (yellow) and the nNOS (gray) in a 3D representation. (B) 2D representation of the pose shows the amino acids of the nNOS that interact with the ligand.
Figure 8. Binding interaction of ligands with the lowest binding energy (landomycin B, PubChem CID: 53297405) against COX-1. (A) The pose of interactions of the ligand (yellow) and the COX-1 (gray) in a 3D representation. (B) 2D representation of the pose shows the amino acids of COX-1 that interact with the ligand.

Figure 9. Binding interaction of ligands with the lowest binding energy (landomycin W, PubChem CID: 50993663) against COX-2. (A) The pose of interactions of the ligand (violet) and the COX-2 (gray) in a 3D representation. (B) 2D representation of the pose shows the amino acids of COX-2 that interact with the ligand.
previously offered to treat mild to severe COVID-19 infection (Funk and Ardakani, 2020; Halpin et al., 2020).

CONCLUSION

In conclusion, there are differences in the metabolite composition of the peel and gel of A. vera. These metabolites of A. vera collected from North Sulawesi of Indonesia are found to be different from those collected from different cultivation areas. The docking score and bond formation evaluation of the metabolites in complex with Omicron RBD and ROS-producing enzymes suggest that A. vera can inactivate their function and alter the Omicron RBD-ACE2 complex formation and inhibit ROS production, which contributes to the pathogenesis and severity progression of COVID-19.

ACKNOWLEDGMENTS

The authors would like to thank Sam Ratulangi University for the support of this research through the UNSRAT Excellent Applied Research Scheme, which was funded by the PNBPNational Research Contract No. 821/UN12.13/LT/2021.

AUTHORS’ CONTRIBUTIONS

BJK and F designed the study. BJK and F carried out the laboratory work. BJK, MJK, and TET did the in silico work. BJK, MJK, and TET analyzed the data. BJK wrote the draft of the manuscript. BJK, MJK, TET, and F read and enriched the manuscript discussion. F and TET reviewed the final manuscript. All authors approved the final version of the manuscript.

FINANCIAL SUPPORT

Already given in acknowledgments section.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

PUBLISHER’S NOTE

This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

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How to cite this article:
Kepel BJ, Kalalo MJ, Tallei TE, Fatimawali. Employing an in-silico approach in the evaluation of Aloe vera metabolites as inhibitors of Omicron RBD and the ROS-dependent cellular signaling processes. J Appl Pharm Sci, 2023; 13(03):114–131.
SUPPLEMENTARY MATERIAL

Supplementary data can be downloaded from the journal’s website

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