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Antioxidant Activity of *Aloe vera* and Prediction of Interaction Mechanisms on ROS1 Kinase and Collagenase Receptors

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ABSTRACT: The *Aloe vera* plant has been widely used as a food ingredient, medicine and cosmetics. This research aims to test the gel and ethanol extract of *Aloe vera* leaves as an antioxidant and absorber of UV light in vitro, as well as predicting the interaction mechanism for ROS1 kinase and collagenase receptors in silico.The antioxidant activity test method was carried out in vitro using DPPH (1,1-Diphenyl-2-Picrylhydrazyl-Hydrazine) reagent. Activity as a UV light absorber is carried out by calculating the sun protected factor (SPF) value. The antiaging activity test was carried out by predicting the interaction mechanism of the ROS1 kinase and collagenase receptors in silico using several phenolic compounds that have been found in *Aloe vera*. The total phenolic content of *Aloe vera* ethanol extract was 379.136 ± 0.34 GAE/g sample, while that of *Aloe vera* gel was 0.0619 ± 0.04 GAE/g sample. *Aloe vera* ethanol extract showed moderate antioxidant activity with an IC_{50} of 101.9 µg/mL, and is able to absorb UV light at concentrations of 0.05% and 0.1% with ultra protection criteria. Several phenolic compounds found in *Aloe vera* plants showed high binding energy to ROS1 kinase and collagenase receptors. Isoquercitrin showed the highest binding energy to the ROS1 kinase receptor, while isovitexin showed the highest binding energy to the collagenase receptor. The conclusion of this research showed that *Aloe vera* leaves contain compounds that have potential as antioxidants and antiaging.

Keywords: *Aloe vera,* antioxidant, antiaging, ROS1 kinase, collagenase.

INTRODUCTION

Cosmetics with antiaging content have been trusted as the right skin care products to prevent and fight premature aging. Anti-aging ingredients are active ingredients in cosmetics that are used to slow down or reduce signs of aging on facial skin. Retinoids are vitamin A-derived molecules that work more deeply on collagen to help break down collagen, increase skin cell turnover, and improve discoloration. On the other hand, using retinol on sensitive skin can cause irritation (Zasada & Budzisz, 2019). Based on these problems, it is necessary to obtain anti-aging products from natural ingredients that are high quality and safe for the skin.

Ones of the factors that causes the aging process is the presence of ROS (reactive oxygen species) produced in cells. ROS is a byproduct of aerobic respiration which is involved in several modifications of cellular reactions such as exposure to heavy metals, ionizing radiation and oxidants. An increase in ROS and a decrease in antioxidants from the body can cause cell damage and will also affect skin aging. Because excessive ROS will accelerate the activation process of the elastase enzyme, which will trigger shrinking and aging of the skin (Kim, 2016). ROS1 kinase is a receptor tyrosine kinase that has a role in several cellular processes such as apoptosis, survival, cell migration, and transformation in various malignancies including colorectal cancer, inflammatory myofibroblast tumors, ovarian cancer, and lung cancer. Therefore, ROS1 kinase has become a potential drug target (Vanajothi et al., 2020). Several studies show a relationship between compounds that act as antioxidants and have anticancer activity (Milella et al., 2023). Collagenase enzyme is a metalloproteinase that can degrade molecules such as aggrecan, elastin, fibronectin, gelatine, laminin, and collagen (Raffetto JD, 2012).

The *Aloe vera* plant and its use as medicine has been around since 6000 BC. Ancient records dating back to the Sumerian period during 2200 years BC, indicate the use of this plant as medicine. Currently, *Aloe vera* gel is an active ingredient in various skin lotions, sunscreens and cosmetics.The gel's use in cosmetics has been boosted by claims that it has similar anti-aging effects to vitamin A derivatives (Moghaddasi & Verma, 2016). *Aloe vera* gel is 99% water with a pH of 4.5 and is a common ingredient in many non-prescription skin salves. The gel contains glucomannan and is moisturizing, so it is widely used

in cosmetics. Twenty-five phenolic compounds were identified, including cinnamic acids and other derivatives (e.g., cafeic and chlorogenic acids), chromones (e.g., aloesin and isoaloeresin D), anthracene compounds and derivatives (e.g., aloin A/B and emodin), and several flavonoids (e.g., orientin and isovitexin), among others (Cardarelli et al., 2017); (Quispe et al., 2018). Several studies have proven that compounds found in *Aloe vera* exhibit antioxidant activities (Hęś et al., 2019).

Furthermore, research on the interaction mechanisms of several receptors related to aging has not been widely studied. In this study, we report the phytochemical characteristics of *Aloe vera* gel and ethanol extract, their potential as antioxidants and UV light absorbers based on in vitro SPF analysis, as well as their antiaging activity by predicting the interaction mechanisms of compounds with phytochemical properties. has been reported in *Aloe vera* against ROS1 kinase and collagenase receptor in silico. This research was conducted to add to previous information about the efficacy of *Aloe vera* leaves, as well as to determine compounds that have high activity against several receptors that play a role in maintaining skin health computationally using molecular or in silico models.

EXPERIMENTAL SECTION

Equipments

This research uses several equipment, including digital analytical balance, rotavapor R114, hotplate with magnetic stirrer, refrigerator, vortex, incubator, particle size analysis Horiba SZ-100, pHmeter, UV-VIS spectrophotometer (UV-Vis 722N), a set of glassware. Computer with Intel Xeon CPU specifications, 32 GB RAM, ten cores, 500 GB SSD. The software used AutoDock Tools 1.5.7, Pymol, Avogadro 2.0, LigPlot+2.2.8, and GIMP 2.0. The receptor proteins ROS1 Kinase (PDB 3ZBF) and collagenase (PDB ID 966C) were each downloaded from the RCSB PDB Database page (www.rcsb.org).

Reagents

2,2-Diphenyl-1-picryl hydrazine (DPPH, Merck), ethanol 96%, chloroform (Merck), H_2SO_4, $FeCl_3$ 0.1%, ammonia, Wagner reagent, Salkowski reagent, $FeCl_3$ reagent, Na_2CO_3, Folin-Ciocalteu, ascorbic acid (Merck), gallic acid (Merck), filter paper, and aluminum foil.

Preparation of Gel and Ethanol Extract of *Aloe Vera* Extract

The leaves of the *Aloe vera* plant were collected from the Yogyakarta area, Indonesia in September 2023, and identified at the Plant Systematics Laboratory, Faculty of Biology, Gadjah Mada University. The results of the determination of the *Aloe vera* leaves used in this research were leaves from the *Aloe vera* type (L.) Burm. f. Next, 12 kg of *Aloe vera* plant leaves were collected and washed clean. A total of 2 kg of *Aloe vera* leaves had the gel removed from the middle of the leaf, while 10 kg was cut into small pieces and dried in the open air exposed to direct sunlight. Dried *Aloe vera* was added with 96% ethanol and then macerated for 24 hours. The filtrate obtained was separated, the residue obtained was macerated again twice. The filtrate obtained was collected and evaporated using a vacuum evaporator until a thick extract was obtained. Each thick ethanol extract and *Aloe vera* gel were used for subsequent experiments.

Qualitative and Quantitative Analysis of Phytochemical Gel and Ethanol Extract from *Aloe Vera*

Each of the gel and ethanol extracts of *Aloe vera* obtained was then subjected to a qualitative phytochemical test which included the sterol and triterpenoid test using the Salkowski method, the alkaloid test using the Wagner method, the tannin and phenolic test with the addition of 0.1% $FeCl_3$ reagent, and the saponin test by observing the formation of foams (Harborne, 1998).

Total phenolic content was determined using folin-ciocalteu (Hagerman, et al., 2000). Each extract was made into a 1% solution with ethanol as a solvent. Each dilute extract was taken as much as 0.1 mL into a test tube and then added with 0.2 mL of folin-ciocalteu, 2 mL of distilled water, and 1 mL of Na_2CO_3 solution. The solution was homogenized and incubated in a water bath at 50 °C for 10 minutes and allowed to stand at room temperature for 2 hours. Three replications (n=3) of the data were collected for the absorbance was measured at a wavelength of 760 nm. The total phenolic content was expressed as mg/g gallic acid.

Antioxidant Activity Test by DPPH Method

The antioxidant activity test used the DPPH (2,2-diphenyl picryl hydrazine) method (Atun et al., 2018), carried out by making a solution of each gel and extract and *Aloe vera* ethanol at several concentrations using ethanol solvent. Each test solution sample was mixed with 0.24 mM DPPH solution (1:1), and homogenized using a vortex. Each test sample was made three times. The samples were left for 30 minutes in a dark room at room temperature. Three replications (n=3) of the data were collected for the absorbance was measured using a spectrophotometer at a wavelength of 516 nm and compared with the absorbance of the control solution. Inhibitory activity is calculated as the percentage decrease in DPPH concentration in the sample solution compared to the control using the formula:

$$\% \text{ Inhibition} = \frac{[\text{Average Absorbansi control} - \text{Average Absorbansi sample}]}{[\text{Average Absorbansi control}]} \times 100\%$$

The % inhibition at various concentrations was then graphed and the regression equation obtained was used to calculate the IC_{50} value for each sample.

Activity Test as an Absorber Of UV Rays *in Vitro*

The activity test as a UV rays absorber *in vitro* using the Walter method. The sample is dissolved in ethanol, its absorption is measured at the optimum wavelength

(290-400 nm). The SPF value is the ratio of the MED (minimum Erythema dose) if a person uses sunscreen at a dose of 2 mg/cm^2 to the MED if they don't. If Io is the intensity of light reaching the skin without sunscreen and I is the intensity with sunscreen present, then the SPF value can be determined through a relationship as follows:

$$A = - \log [I/Io]$$
$$A = - \log [1/SPF]$$
$$A = \log SPF$$

The SPF value is used to determine the maximum type of protection according to Wilkinson and Moore criteria, minimum protection (SPF value 2-4); medium protection (SPF value 4-6); extra protection (SPF value 6-8); maximum protection (SPF value 8-15); and ultra protection (SPF value > 15) (Walters et al., 1997; Cefali et. al.,2019).

Prediction of The Mechanism of Their interaction with ROS1 Kinase and Collagenase in Silico

The ligand used is a compound that has been reported to be found in the *Aloe vera* plant and a ligand comparator retinol and ascorbic acid as an anti-aging commonly used. The 3D structure was obtained from Pubchem (https://pubchem.ncbi.nlm.nih.gov/) and stabilized using Avogadro 2.0 software. Next, the 3D structure of each compound was created in a pdb file using the Pymol software. Then, Next, the AutodockTools-1.5.7 software was used to convert pdp file to pdbqt file. Docking simulations were carried out using model receptors ROS1 kinase (PDB 3ZBF) and collagenase (PDB ID 966C). Receptors were downloaded from the RCSB PDB database page (www.rcsb.org). Method validation was carried out by redocking using AutoDockTools-1.5.7 software. Re-docking between receptors and natural ligands is carried out on a grid box, which can produce an RMSD (Root Mean Square Deviation) value < 2 Å, which shows that the method used is valid (Bagaria et al., 2012). Then, the test compound is attached to the receptor binding site following the grid box used in validation. The results obtained from this docking process are in the form of compound or ligand binding affinity. This method is used to predict the mechanism of activity of chemical compounds that act as ligands tethered to target receptors in the form of enzymes or proteins, which can be studied using computational approaches. The binding energy can be determined by the Gibbs free energy value (ΔG kcal/mol) (Bajorath, 2015). A Gibbs free energy value of less than zero (0) indicates that the bond between the compound and the target protein occurs spontaneously and is stable (Hill, AD & Reilly, 2008). Next, to determine the interaction between the ligand and the active site on the receptor, ligPlot+ 2.2.8 software was used and visualized using the GIMP 2.0 software and saved in jpg format (Forli et al., 2016).

RESULTS AND DISCUSSION

Preparation for making gel and ethanol extract from the leaves of the *Aloe vera* plant is as showed in **Figure 1**. *Aloe vera* gel is taken directly from the center of the leaf which is white and transparent. A total of 2 kg of leaves produces 500 g of gel. Ethanol extract of *Aloe vera* leaves is made from 10 kg of whole leaves, dried and made into powder. The dry powder (300 g) was then macerated using 96% ethanol, then after removing the solvent, 30 g of thick brown extract was obtained.

Each gel and ethanol extract from *Aloe vera* was subjected to phytochemical tests, phenolic content analysis, antioxidant activity using the DPPH method, and UV light absorption by calculating the SPF value. Phytochemical test shows that each gel and ethanol extract of *Aloe vera* contains phenolic compounds, flavonoids, and saponin. Data on total phenolic content, antioxidant activity expressed in IC_{50}, and UV light absorption from *Aloe vera* gel and ethanol extract are in **Table 1**. Gallic acid was used as a standard phenolic solution. Analysis of total phenolic content uses a gallic acid standard, so that the total phenolic content is expressed as GAE (Gallic Acid Equivalent)/g sample. Gallic acid was dissolved in ethanol at various concentrations, and absorbance was measured at the same wavelength. The calibration curve results obtained have a linear regression equation $y = 0.0043x + 0.0339$ ($R^2 = 0.993$). The total phenolic content ethanol extract of *A. vera* was 379.136 ± 0.34 GAE/g sample, while that gel of *Aloe vera* was 0.0619 ± 0.04 GAE/g sample. Thus, the total phenolic compound content in *Aloe vera* gel is much smaller than in the ethanol extract. This is because *Aloe vera* gel consists of 99% water, polysaccharide and glucomannan(Hęś et al., 2019).

Antioxidant activity was carried out by making solutions from each gel and ethanol extract from *Aloe vera* at various concentrations. DPPH reagent was then added to each solution. The absorbance of each solution was measured at a wavelength of 516 nm. Antioxidant activity is expressed in the IC_{50} μg/mL value of each sample. These data show that the ethanol extract of *Aloe vera* has medium activity with an IC_{50} of 101.9 μg/mL obtained from the regression equation $y = 0.3531x + 6.7112$, with $R^2 = 0.9688$. This shows that the antioxidant activity test observations are valid. However, *Aloe vera* gel contains more than 99% water, so the total phenolic content in the gel is relatively small when compared to ethanol extract. The content of phenolic compounds in *Aloe vera* gel is relatively small so that its antioxidant activity is inactive (> 1000 μg/mL). Apart from that, in gel form it is relatively difficult to react with DPPH, and its activity can be measured at a concentration of 1.25% showing an inhibitory activity of 18%, but at higher concentrations the inhibitory activity becomes smaller. Previous research shows that the highest antioxidant activity is found in the skin of *Aloe vera*

leaves which contain lots of phenolic compounds (Quispe et al., 2018). Several previous studies have shown that *Aloe vera* has a high content of phenolic compounds so it has antioxidant properties (Hęś et al., 2019; Quispe et al., 2018).

The activity test as an absorber of UV light in vitro was carried out by dissolving each sample of gel and ethanol extract of *Aloe vera* at various concentrations, then measuring its absorption in the UV area (290-400 nm). Next, the absorption obtained at the maximum wavelength is used to calculate the SPF value (Walters et al.,1997). These data showed that *Aloe vera* ethanol extract at a concentration of 0.05 and 0.1% have ultra protection.

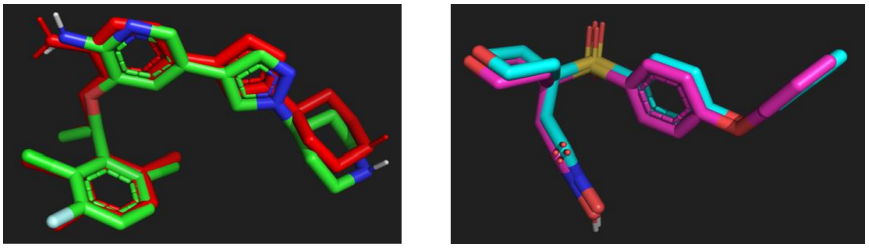
Figure 1. Preparation for making gel and ethanol extract from the leaves of the *Aloe vera* plant

Table 1. Results of analysis of total phenolic content, antioxidant activity against DPPH, and UV light absorber of *A. vera* extract

No	Sample	Total Phenolic content (GAE/ g sample)	Antioxidant activity			UV light absorber		
			Concen-tration (μg/mL)	%In-hibiti-on	IC_{50} (μg/mL)	Con-centrati on (%)	Wavelength (nm)	SPF
1	Ethanol extract of *Aloe vera*	379.136 ± 0.34	250.00	90.49		0.1	295.5 (UV-B)	734.5
			125.00	58.94	101.9 (R^2: 0.9588)	0.05	293.5 (UV-B)	17.95
			62.50	33.67	(moderate activity)	0.025	292.5 (UV-B)	4.53
			31.25	13.77		0.0125	290 (UV-B)	2.16
			15.625	7.71				
2	*Aloe vera* gel	0.0619 ± 0.04	50,000	18.12	>1000 (not active)	6.25	387 (UV-A)	1.02
			25,000	16.42			318 (UV-B)	1.05
			12,500	16.30				
3	*Ascorbic acid* (positive control)		5.00	95.59	1.27 ± 0.02 (R^2: 0.8830 (high activity)			
			2.50	79.82				
			1.25	51.45				
			0.625	32.28				

Table 2. Results of validation of the redocking method

No	Reseptor Protein	Native Ligan	Grid box	RMSD
1	ROS1 Kinase (PDB 3ZBF)	3-[(1R)-1-(2,6-dichloro-3-fluorophenyl)ethoxy]-5-(1-piperidin-4-yl-1H-pyrazol-4-yl)pyridin-2-amine (cfg) C_{21} H_{22} Cl_2 F N_5 O	x = 42.521 y = 19.649 z =19.649 box size x=y =z =30 A°	0.687
2	Collagenase (PDB 966C)	N-Hydroxy-2-[4-(4-phenoxy-benzenesulfonyl)-tetrahydro-pyran-4-yl)-acetamide (rs-2) C_{19} H_{21} N O_6 S	x = 9.166 y = -10.353 z = 38.398 box size x=y= z = 20 A^0	0.414



(**a**) (**b**)

Figure 2. The overlaid image of the crystal structure native ligan cfg (green) and re-docking result (red) at receptor PDB 3ZBF (**a**) and native ligan (rs-2) (blue) and re-docking result (pink) at receptor PDB 966C (**b**)

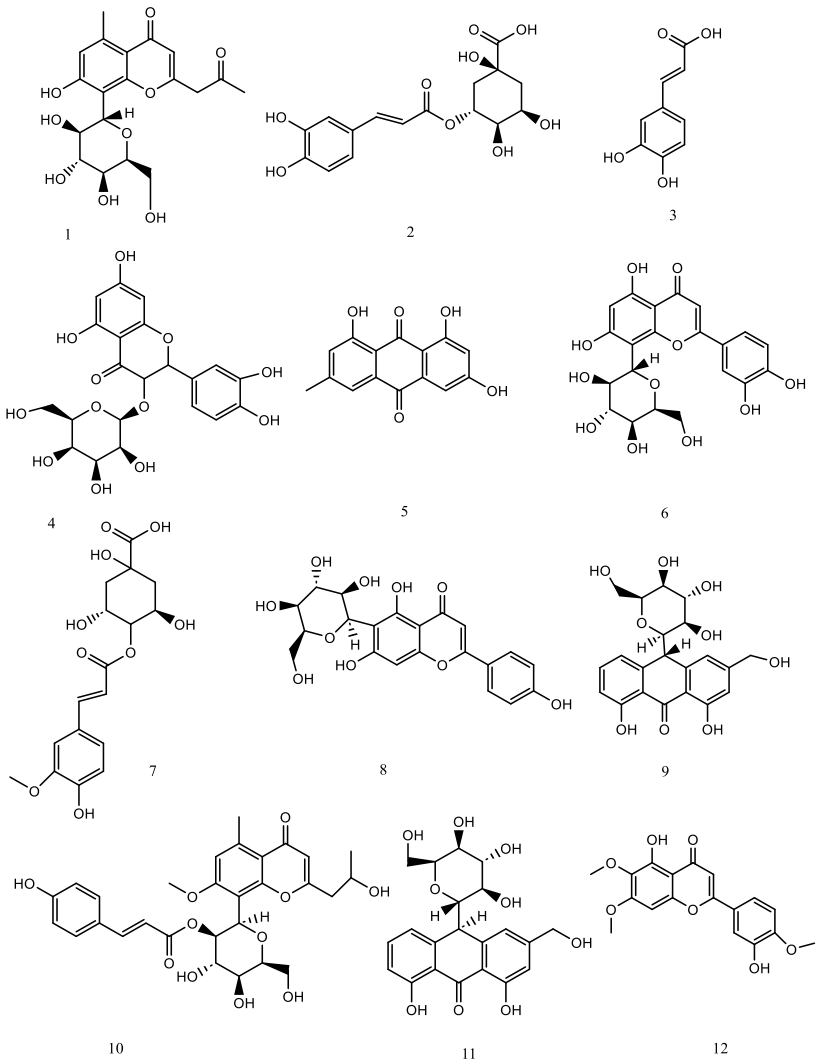


Figure 3. Structure of phenolic compounds used as ligands of *Aloe vera* plants

Docking simulation was carried out by molecular docking of the ligands to the ROS1 kinase (PDB 3ZBF) and collagenase (PDB ID 966C) receptors were downloaded from Protein Data Bank (www.pdb.org). Validation of the re-docking data obtained is in Table 2. Re-docking between receptors and natural ligands is carried out on a grid box which can produce an RMSD (Root Mean Square Deviation) value < 2 Å which shows that the method used is valids (Bagaria et al., 2012). The overlaid image of the crystal structure native ligan cfg and rs-2 before and after re-docking at **Figure 2**.

Aloe vera plants are reported to contain several phenolic compounds (Hęś et al., 2019; Quispe et al., 2018). However, in the in silico antiaging activity test, only twelve phenolic compounds were selected which showed the best activity. These compounds are aloesin (1), chlorogenic acid (2), caffeic acid (3), isoquercitrin (4), emodin (5), luteolin (6), feruloylquinic acid (7), isovitexin (8), aloin-A (9), isoaleoresin-D (10), aloin B (11), eupatorine (12). The structure of this compound is in **Figure 3**.

The results of the docking analysis of 12 compounds found in *Aloe vera*, ascorbic acid (positive control), retinol (positive control), and native ligand (cfg) against the ROS1 kinase receptor (PDB 3ZBF) are in **Table 3**, while for the collagenase receptor (PDB 966C) is in **Table 4**. The data obtained includes binding energy (ΔG kcal /mol), hydrogen bonds, and hydrophobic interactions.

Table 3. Results of docking analysis of the ROS1 kinase (PDB 3ZBF) receptor

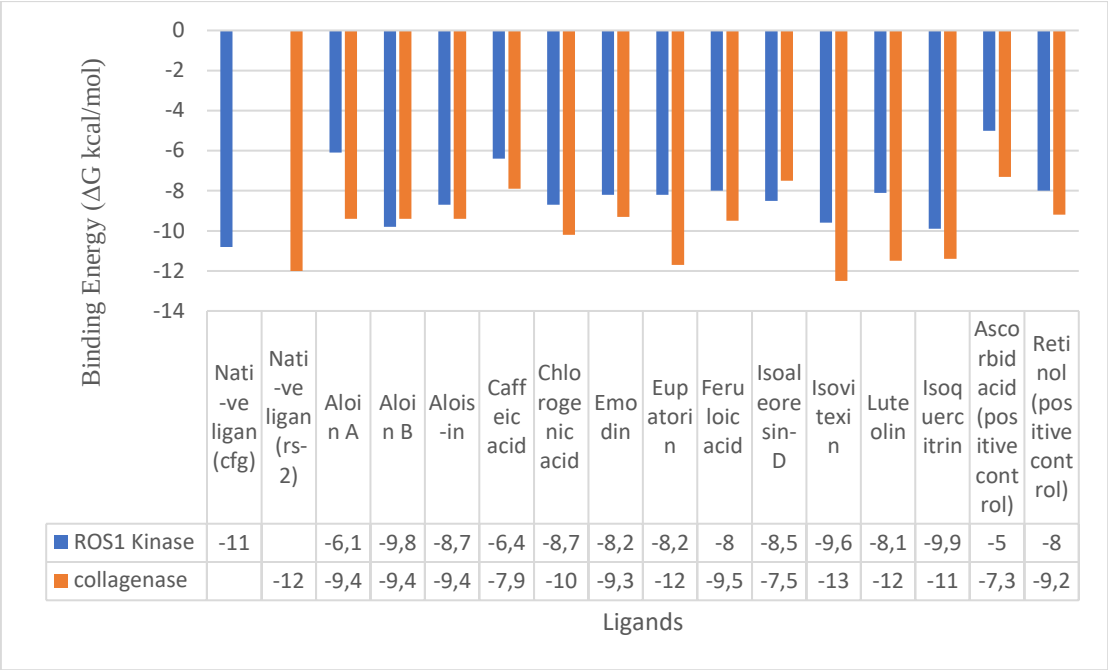
No	Ligan/Compounds	Binding energy, (ΔG Kcal/mol)	Hidrogen bond	Hydrophobic interation
1	Native ligan (cfg)	-10.8	Glu2027	Arg2083; Leu2086; Leu2010; Ala1978; Lys1980; Leu2026; Met2029; Leu1951; Gly2032; Leu2028
2	Aloin A	-6.1	-	Asn2224; Lys2228; Leu2217; Asn2220; Phe2218; Phe2221
3	Aloin B	-9.8	Asp2033; Glu2030; Glu1961	Thr2036; Lys2040; Gly2031; Gly2032; Leu2028; Met2029; Leu1952; Met2029
4	Aloisin	-8.7	Asp2033	Lys1980; Gly1957; Val1959; Leu2026; Gly2032; Leu2086; Leu2010; Leu1951; Ala1978
5	Caffeic acid	-6.4	Lys1980; Glu2027; Met2029	Val1959; Ala1978; Leu2086; Leu2028
6	Chlorogenic acid	-8.7	Met2029; Asp2102	Gly2101; Leu2086; Arg2083; Asn2084; Val1959; Glu2030; Leu1951; Gly2032
7	Emodin	-8.2	Glu2027; Met2029	Leu2086; Leu2026; Ala1978; Val1959; Leu1951; Gly2032; Leu2028; Glu2030
8	Eupatorin	-8.2	Met2029	Asp2102; Lys1980; Leu2028; Glu2030; Leu1951; Gly2032
9	Feruloic acid	-8.0	Glu2027	Lys1980; Val1959; Leu2026; Leu2010; Met2020; Ala1978; Gly2032; Leu2086; Leu2028; Leu1951; Glu1961; Leu1958
10	Isoaleoresin-D	-8.5	Arg2083; Leu1958	Gly1957; Ser1953; Lys1980; Val1959; Asp2102; Gly1952; Gly2032; Gly2101; Leu2086; Asp2033; Asn2084; Leu1951; Lys1980
11	Isovitexin	-9.6	Arg2083; Asp2033	Val1959; Leu2086; Leu1951; Met2029; Leu2028; Glu1961; Glu2030
12	Luteolin	-8.1	Asp2102; Lys1980; Met2029	Leu2026; Leu2086; Gly2032; Leu1951; Ala1978; Leu2028
13	Isoquercitrin	-9.9	Asp2033	Met2029; Gly2032; Glu2030; Leu1951; Leu2086; Asp2102; Gly2101; Leu2010; Leu2026; Val1959
14	Ascorbid acid (positive control)	-5.0	Glu2027; Met2029	Gly2032; Val1959; Leu2086; Leu2026; Leu2028; Ala1978
15	Retinol (positive control)	-8.0	-	Asp2102; Leu2026; Val1959; Leu2086; Leu2010; Leu1951; Met2029; Leu2028; Glu2030

Table 4. Results of docking analysis of the collagenase (PDB 966C) receptor

No	Ligan/Compounds	Binding energy, (ΔG Kcal/mol)	Hidrogen bond	Hydrophobic interation
1	Native ligan (rs-2)	-12.0	Glu219; Leu181; Ala182; His228	Thr241; Arg214; Val215; Ser239; Leu235; Tyr240; His218; His222; Asn180; Pro238
2	Aloin A	-9.4	Glu219; Leu181; Ala182; Ser239; Tyr237; Asn180	Tyr240; Gly179; Pro238; His218; His228
3	Aloin B	-9.4	Ser239; Pro238; Gly179; Al182; Asn180	Tyr240; Leu181
4	Aloisin	-9.4	Ser239; Tyr240; Tyr237; Ala182; Asn180	His218; Val215; Glu249; Leu181; Pro238; His228
5	Caffeic acid	-7.9	Ala182; Thr241	Val215; Leu181; Glu219; His248; Tyr240; Arg214; Ser239
6	Chlorogenic acid	-10.2	Thr241; His228; Asn180	Tyr240; Arg214; Leu181; His218; Val215; Leu235; Glu219; Gly179
7	Emodin	-9.3	Tyr237; Ser239	Asp245; Ser243; Gln247; Gly233; Val246; Arg214; Ala234; Leu235; His218; Val215; Thr241
8	Eupatorin	-11.7	Leu181; Ala182; Tyr237	Asn180; Gly179; Val215; Pro218; Ser239; Arg214; Leu235; His218; Thr241
9	Feruloic acid	-9.5	Asn180; Ala184	Tyr240; Leu181; Tyr23; Val215; His228; His222; His218; Ser239; Leu235; His183; Ala182
10	Isoaleoresin-D	-7.5	Asp175; Asn180	Gly179; Tyr240; His228; Pro238
11	Isovitexin	-12.5	Ala182; Glu219; Tyr237; Pro238	Ala234; Val246; Leu235; Arg214; Thr241; Tyr240; Ser239; Val215; His218
12	Luteolin	-11.5	Asn180; Leu181; Ala182; Tyr237	Glu219; Pro238
13	Isoquercitrin	-11.4	Ser239; Tyr237; Tyr240; Asn180; Pro238	Val215; His218; His222; His228; Glu219; His183; Leu181; Ala182
14	Ascorbic acid (positive control)	-7.3	Leu235; His218; Arg214; Thr241; Tyr237; Ile232; Ala234	Val215; Ser239
15	Retinol (positive control)	-9.2	Leu181	Glu219; Ser239; Tyr240; Arg214; Tyr237; Ala234; Leu235; His218; Thr241; Ile232; Ser243

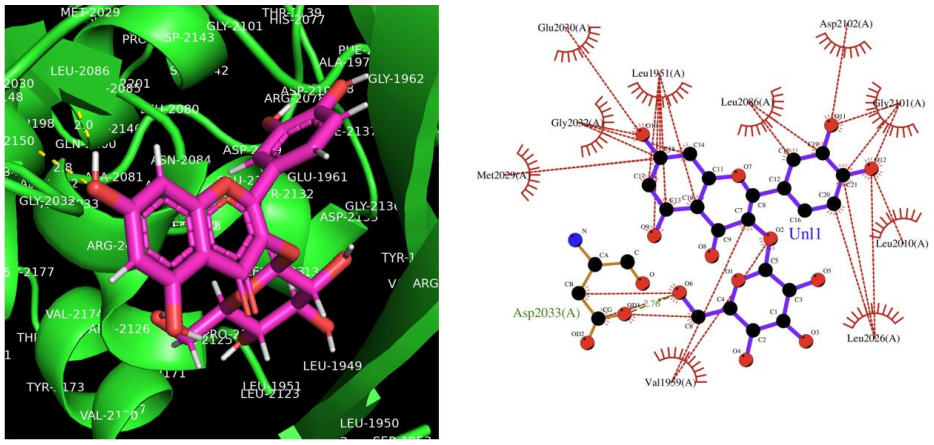
Docking method to predict the activity and mechanism of molecular interactions between twelve compounds found in *Aloe vera* plants, two positive controls (ascorbic acid and retinol). This compound is used as a ligand that will attach to the ROS 1 kinase and collagenase receptors in the same position as the native ligands found on each receptor. The information obtained from this method is data on the binding energy and interaction of the ligand with the amino acid residues of the receptor. Data on binding energy between compounds or ligands is expressed in Gibbs free energy (ΔG kcal/mol (Du et al., 2016). For more details, it is made in graphic form in **Figure 4**.

In this study ascorbic acid and retinol were used as positive controls. Ascorbic acid and retinol are widely used as active ingredients in various cosmetics to maintain healthy skin. Ascorbic acid, as one of the basic exogenous vitamins, is known for its tremendous antioxidant properties. Ascorbic acid interacts with small molecule antioxidants, including tocopherol, glutathione and thioredoxin, but also can stimulate the biosynthesis and activation of antioxidant enzymes, such as superoxide dismutase, catalase or glutathione peroxidase (Gęgotek & Skrzydlewska, 2023). Likewise, retinol or retinoic acid is an active form of vitamin A which functions as a precursor to retinoic acid. It can improve the condition of skin that has aged due to sun exposure by stimulating the formation of new collagen and preventing skin damage (Quan, 2023).



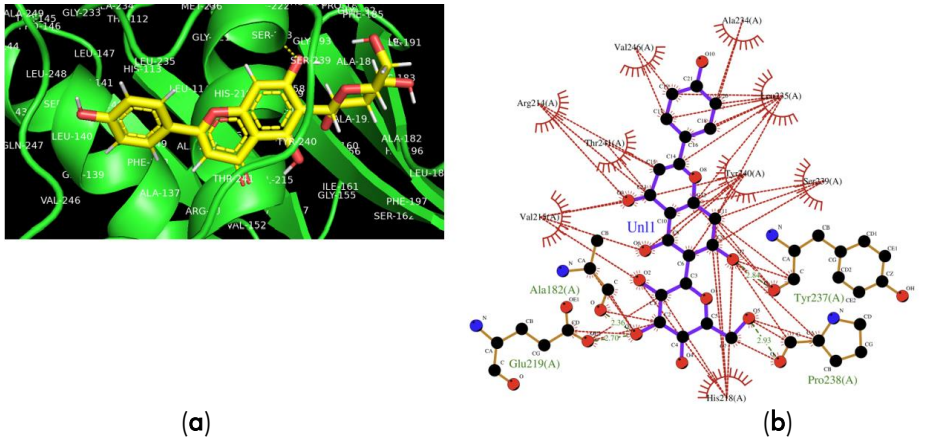
	Native ligan (cfg)	Native ligan (rs-2)	Aloin A	Aloin B	Aloisin	Caffeic acid	Chlorogenic acid	Emodin	Eupatorin	Feruloic acid	Isoaloeresin-D	Isovitexin	Lutеolin	Isoquercitrin	Ascorbid acid (positive control)	Retinol (positive control)
ROS1 Kinase	-11		-6,1	-9,8	-8,7	-6,4	-8,7	-8,2	-8,2	-8	-8,5	-9,6	-8,1	-9,9	-5	-8
collagenase		-12	-9,4	-9,4	-9,4	-7,9	-10	-9,3	-12	-9,5	-7,5	-13	-12	-11	-7,3	-9,2

Figure 4. Gafik binding energy of phenolic compounds from *Aloe vera*, native ligands, and positive controls (ascorbic acids and retinol) against ROS1 Kinase and Collagenase



(**a**) (**b**)

Figure 5. ROS1 Kinase protein receptor complex with isoquercitrin (**a**), interaction that occur ini the compelx between amino acid residues and isoquercitrin (**b**) (The presence of hydrogen bonds is shown by the green line, while hydrophobic interactions are shown by the red line)



(**a**) (**b**)

Figure 6. Collagenase protein receptor complex with isovitexin (**a**), interaction that occur in the complex between amino acid residues and isovitexin (**b**) (The presence of hydrogen bonds is shown by the green line, while hydrophobic interactions are shown by the red line)

In silico, ascobic acid and retinol showed binding energies to the collagenase receptor (PDB 966C) of -7.3 and -9.2 Kcal/mol respectively, whereas towards ROS 1 Kinase ascobic acid is relatively less stable than retinol and other phenolic compounds. This may be due to the molecular size of ascorbate acid being relatively small compared to the others, so that its interaction with amino acid residues is less strong. The smaller the binding energy obtained, the more stable the bonds formed. The twelve compounds that have been reported to be found in the *Aloe vera* plant used in this research are phenolic compounds. Some compounds have relatively high binding energies when compared to ascorbic acid and retinol, but when compared to native ligands, they are still relatively low. The docking results showed that the binding energy of the native ligand is the highest compared to other compounds. Based on the binding energy data, isoquercitrin showed the highest binding energy to ROS1 kinase compared to the phenolic compounds tested on *Aloe vera*. Furthermore, isovitexin has the highest binding energy to the collagenase receptor compared to other phenolic compounds, native ligand, and positive control.

The interactions formed between receptors and ligands are described using the LigPlot software and visualized using software GIMP 2.0. This interaction is depicted with a LigPlot graph which shows hydrogen bonds (green line) and hydrophobic interactions (red line). for examples in **Figure 5** showed the ROS1 kinase receptor protein complex (PDB 3ZBF) with isoquercitrin (a) interactions that occur in the complex between amino acid residues and isoquercitrin (b). **Figure 6** showed the Collagenase protein receptor (PDB 966C) complex with isovitexin (a), the interaction that occurs in the complex between amino acid residues and isovitexin (b).

During the aging process, collagen, elastin, and hyaluronic acid decrease. It causes strength and flexibility of the skin decreases so that visible wrinkles appear on the surface of the skin. Apart from that, what causes the skin to shrink is an increase in enzyme activity such as collagenase, elastase and hyaluronidase. Collagenase is an enzyme that is able to degrade collagen. The results of this study indicate that isovitexin has a better affinity energy than the native ligand (rs-2). In vivo research shows that isovitexin can improve the properties of keratinocyte stem cells, characterized by a significant increase in stem cell protein levels, so it has the potential to prevent skin damages (Chowjarean et al., 2019). Isovitexin can upregulate antioxidant gene and protein expression, reduce ROS accumulation, and increase the accumulation of the transcription factor skinhead -1 (SKN-1) in the nucleus (Tao et al, 2023).

The presence of radicals or reactive oxygen can react with fibroblasts, thereby reducing collagen production. Phenolic compounds found in the *Aloe vera* plant showed high activity as antioxidants (Benzidia et al., 2019; Hęś et al., 2019). Previous research results showed that isoquercitrin is a compound with antiaging properties (Liu et al., 2023). Thus, the results of this study support previous research which showed that the phenolic compounds found in the *Aloe vera* plant have potential as antioxidants and can prevent collagen hydrolysis reactions or are antiaging.

CONCLUSIONS

In conclusion, in this study we evaluated that the gel and ethanol extract of *Aloe vera* gel contain phenolic compounds, flavonoids and saponins. The total phenolic compound content of the ethanol extract of *Aloe vera* was 379.138 ± 0.335 mg/g GAE sample; Meanwhile, *Aloe vera* gel contained 0.0619 ± 0.038 mg/g GAE sample. Ethanol extract of *Aloe vera* has higher antioxidant activity than *Aloe vera* gel. Gel and ethanol extract of *Aloe vera* can generally absorb UV rays. Several phenolic compounds found in *Aloe vera* plants showed high binding energy to ROS1 kinase and collagenase receptors. Isoquercitrin showed the highest binding energy to the ROS1 kinase receptor, while isovitexin showed the highest binding energy to the collagenase receptor. *Aloe vera* contains phenolic compounds which have potential as antioxidants and antiaging

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