



ORIGINAL ARTICLE

Hippobroma longiflora L Leaves as a Natural Inhibitor of Cataract Progression: A Comprehensive Study Integrating Ethanol Extract, HPLC, and Molecular Docking Approaches

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Abstract

Cataract remains the highest preventable cause of blindness in the world. The *H. longiflora* plant (*Hippobroma longiflora* L) is a plant that has empirically been widely used to treat a variety of eye diseases including cataract. This plant has high antioxidant activity due to its high phenolic and flavonoid content. The *H. longiflora* plant was extracted using an ethanol solvent and its compound content was analyzed using GCMS and phytochemistry analysis. The extracts obtained were tested with TPC, TFC, antioxidants and quercetin compound with HPLC. From phytochemical and GCMS analysis, it was found that triterpenoid compounds were not found in the sample, and the compound 1-Isobutyl-2,5-dimethyl-4-phenyl-piperidin-4-ol was the most abundant (37.77%), as well as 4-Penten-1-ol, 5-phenyl-3-piperidino (1,30%) were the least abundant in the ethanol extract of the *H. longiflora* leaves extract. The total phenolic, flavonoid and HPLC results of quercetin were 139.21 mg GAE/g; 11.48 mg QE/G and 0.009 mg/G. Meanwhile, in an antioxidant activity test, it was found that the ethanol extract of the *H. longiflora* leaves has very strong antioxidants. This finding revealed the potential of ethanol extracts of *H. longiflora* leaves as a cataract inhibitor through its process as an antioxidant.

Introduction

Cataract is a severe condition of cloudy eye lens that can lead to progressive loss of vision [1,2]. According to age, cataracts are classified as senile, congenital and juvenile cataract [3–5]. The oxidation process plays an important role in the cloudy eye lens in cases of senile cataract. The elderly population will continue to increase, followed by increased prevalence and incidence of senile cataract cases. Senile cataract is one of the leading causes of vision impairment and blindness in the world and is the most common form of cataracts. Currently, the incidence of senile cataracts is 3.9% between the ages of 55–64 and has risen to 92.6% in the age of 80 and over [6]. Cataract cannot be cured with glasses, contact lenses or refractive surgery [6,7]. The primary treatment for cataract is surgical replacement of a broken lens with an implant lens, but surgery is often frightening for most patients and costly. Therefore, the Indonesian government through the Ministry of Health in the last five years has campaigned to optimize the use of

medicines from natural sources to reduce morbidity and mortality of all kinds of diseases including non-communicable disease (NCDs), like cataracts.

Indonesia is a country with a wide range of biodiversity that has about 25.000-30.000 plant species that make up 80% of the world's plant types [8–10]. Prior to the discovery of modern medicine, Indonesians had begun to use natural medicine thousands of years ago, including the use of *Hippobroma longiflora* L or also called madamfate or kitolod. The compounds found in the leaves are antioxidants that neutralize free radicals. This ability can inhibit the progression of severe cataracts or cataractogenesis processes [11]. Previous research has suggested that the *H. longiflora* leaves are widely used as a traditional medicine to treat eye disorders such as itching eyes, red eyes, and even cataracts. In another study mentioned that the leaves of kitolod as an antibacterial for eye treatment [12]. Part of the *H. longiflora* plant that can be used for eye treatment is the leaves and flowers. Other studies also showed that leaf and flower extracts of *H. longiflora* showed positive results as an antibacterial in conjunctivitis disease [13]. *H. longiflora* can fight free radicals through proven antioxidant activity with an IC_{50} of 9.57 $\mu\text{g/mL}$ (ethanolic extract of *H. Longiflora* leaves) [11].

Phytochemical studies show that *H. longiflora* leaves contain active metabolites such as alkaloid, steroid, phenolic compounds and flavonoids [12]. Flavonoid and phenolic are the main components that act as antiproliferative, antibacterial and antioxidants through their role in donating electrons to neutralize free radicals. These compounds are exogenous antioxidants that prevent free radicals through two different mechanisms: direct and indirect. The direct mechanism occurs by donating hydrogen ions that can neutralize the toxic effects of free radicals and indirect mechanism by increasing the expression of endogenous antioxidant genes through several mechanisms. The higher the phenolic and flavonoid content, the higher the ability to donate electrons, the greater the antioxidant ability. Its antioxidant ability is believed to inhibit the progression of cataracts. The most abundant phenolic and flavonoid compounds of the *H. longiflora* plant are found in the leaves [13].

The research aims to achieve two primary objectives: firstly, to discover the secondary metabolites present in *H. longiflora* leaves using phytochemical screening, high-performance liquid chromatography (HPLC), and gas chromatography-mass spectrometry (GCMS) analysis; secondly, to assess the potential anti-cataract properties of *H. longiflora* through antioxidant testing, molecular docking, and analysis of malondialdehyde levels, superoxide dismutase, catalase, and glutathione peroxidase levels.

Materials and Methods

Collection and Characterization of H. longiflora Leaves

Determination of *H. longiflora* leaves (*Hippobroma longiflora* L.) is carried out by the Institute of Sciences of Indonesia (LIPI), Bogor, Indonesia. The leaves samples were taken from the village of le Ilie, Ulee Kareng district (Banda Aceh, Aceh, Indonesia) and dried for 14 days. The simplisia was reduced in size and macerated using 70% ethanol (1:10) for 3 days at room temperature. Compound analysis on *H. longiflora* leaves extract was conducted using the Gas Chromatography-Mass Spectrometry (GCMS) method at the Environmental Quality Testing Engineering Laboratory, Faculty of Engineering, Syiah Kuala University using the Shimadzu GCMS-QP2010 instrument. Meanwhile, the quercetin content analysis was conducted using the High-Performance Liquid Chromatography HPLC method at the Biopharmaceutical Research Center, Bogor Agricultural Institute, Bogor, Indonesia.

Phytochemical Screening, Total Phenolic Compound, Total Flavonoid Compound and Antioxidant Activity

Samples of *H. longiflora* leaves extract were tested for their secondary metabolite content (alkaloids, steroids, terpenoids, saponins, phenolics and flavonoids and their total phenolic

content (TPC) and total flavonoid content (TFC). In alkaloid test, we used Meyer, Dragendorff, and Bouchardat reagents. The positive reaction of alkaloid indicated by white (Meyer), brown (Dragendorff), and reddish-brown precipitate (Bouchardat) in solution. In steroids and terpenoids test, we used Liebermann-Burchard reagent. The positive reaction of steroid indicated by green or blue colors and the positive terpenoid reaction indicated by red or purple colors in solution. In saponin test, we used hot aquadest by measuring foam height after 30 minutes [14]. The phenolic compounds were tested using 70% ethanol and 3% FeCl_3 solution. The positive reaction of phenolic indicated by green, red, purple, blue or black colors in solution. And, in the flavonoid test, we used Mg powder and concentrated HCl with positive reaction resulted in a brick red-coloured solution.

In the Total Phenolic Compound (TPC) test, we used gallic acid as a standar solution with concentration series of 20, 30, 40, 50 and 60 ppm. All series concentration were added to the folin-Ciocaltaeau and Na_2CO_3 reagent, and their absorbance was measured at 772 nm wavelengths. Meanwhile, the *H. longiflora* leaves extract was dissolved in an ethanol solvent and given the same procedure as a standard solution of gallic acid. In the Total Flavonoid Content (TFC) test, quercetin was used as a standard solution with concentration series of 2, 4, 6, 8 and 10 ppm. All series concentration were added ethanol solvent, AlCl_3 reagent, potassium acetate and aquadest in sequence and measured their absorption at 440 nm wavelengths. And for the *H. longiflora* leaves extract, we dissolved in ethanol solvent and gave the same procedure as the standard solution of quercetin.

In antioxidant test, we used vitamin C as positive control and 2,2-difenil-1-pikrilhidrazil (DPPH) as a standard solution with concentration series of 3.125; 6.25; 12.5; 25; 50; and 100 ppm and then its wavelengths were measured at 517 nm. The sample solution and vitamin C with various concentration series were reacted with DPPH solutions, incubated for 30 minutes at 37°C and the absorbance measurement was performed using a visible spectroscopic photometer at 517nm. The absorbance value of each concentration was recorded and counted as % inhibition and the IC_{50} value with three repetitions.

Molecular Docking

In this study, quercetin was selected as a ligand and the protein γ D-crystallins (HDGC) as a macromolecule. The ligand structure was downloaded from the website (<https://pubchem.ncbi.nlm.nih.gov/>) with the PubChem ID 5280343, and the protein structure was downloaded from the Protein Data Bank website (<http://www.rcsb.org/structure/2G98>) with the PDB ID 2G98. Protein structures are removed from water molecules and ligands that are attached using the BIOVIA Discovery Studio Visualizer 2021 application and saved as .pdbqt format. Meanwhile, the structure of the ligands was changed to .pdbqt using Open Babel. At the docking process of the ligands and receptors, the gridbox is adjusted to the center_x = 14.1722; center_y = 11.8118 and center_z = 18.8647 with size_x = 57.8499; size_y = 50.0026 and size_z = 73.5689. The docking results are shown in the form of binding affinity and Root Mean Square Deviation (RMSD) with 8 repetitions. The docking results was saved in .pdb format. The type and structure of the bond between the HDGC protein and the ligand is shown in 2 and 3 dimensional using the BIOVIA Discovery Studio Visualizer 2021.

Preparation of Goat's Eye Lens

Goat eyes lens was collected from a cutting house and prepared using Durgapal method [15]. This research has been approved for animal ethics under number 165/KEPH/VI/2023. The lens was extracted using extra-capsular extraction techniques and incubated in artificial aqueous humour (sodium chloride: 140 mM, potassium chloride: 5 mM; magnesium chloro: 2mM; sodium bicarbonate: 0.5 mM, sodium dihydrogen phosphate: 0.5 mM); calcium chlorides: 0.4 mM and glucose: 5.5 mM) at room temperature with an average pH level of 7.8. Streptomycin and penicillin solutions was added to the media to prevent the growth of microbes. All lenses are divided into several groups like Table 1.

This test followed the Ohkawa method using MDA that reacts with thiobarbituric acid to form a pink chromogen. The lens sample was homogenized in potassium chloride solution. In the test

tube, we added 0.2 ml of 8.1% sodium dodecyl sulfate; 1.5 ml of 20% acetic acid (pH 3.5) and 1.5 ml of 0.81% thiobarbituric acid solution successively. Then, in the same test tub, we added 0.2 ml of homogenate lens and heated it in boiling water for 60 minutes. In this sample, we also added butanol/pyridine (15:1v/v) and centrifuge for 15 minutes. The organic top layer is breathed, and the intensity of the pink color was read at 532 nm using tetramethoxypropane as an external standard [16].

Table 1. Goat's eye lens treatment group.

No	Group codes	Treatment
1	I (n=5)	Normal lens
2	II (n=5)	Cataract lens (<i>Induced</i> H ₂ O ₂ 10 mM)
3	III (n=5)	Cataract lens + ethanol leaves extract of <i>H. longiflora</i> (100 µg/ml)
4	IV (n=5)	Cataract lens + ethanol leaves extract of <i>H. longiflora</i> (200 µg/ml)
5	V (n=5)	Cataract lens + ethanol leaves extract of <i>H. longiflora</i> (300 µg/ml)
6	VI (n=5)	Cataract lens + commercial quercetin

Rate of the Malondialdehyde (MDA)

Superoxide Dismutase Test (SOD)

In this test, the homogenate was added to chloroform ethanol solvents and then centrifuged for 60 minutes. The supernatant was separated, the aquadest was added and placed in a water heater. We also added xanthin oxidase into the sample and incubated for 20 minutes. The reaction was stopped by adding 0.8 mmol/L CuCl₂ solution and its absorbance was measured at 560 nm. The inhibition percentage was calculated using Equation 1.

$$\% \text{ inhibition} = \frac{(\text{Blanko absorption} - \text{sample absorption})}{\text{Blanko absorption}} \times 100\% \quad (1)$$

At the standard inhibition curve, the x-axis was set as protein connection, and the y-axis was set as percentage value of inhibitions [17].

Catalase Test (CAT)

This test followed the Ozmen method by incubating homogenate and substrate at 37°C for 60 seconds. This reaction was stopped by adding ammonium molybdate + molybdate complex (32.4 mM/L) and hydrogen peroxide, and then measured the absorbance of 3 blanks (blank 1: 1.0 ml substrate + 1.0 ml molybdate + 0.2 ml sample; blank 2: 1.0 ml substrate + 1.0 mL molybdate + 0.2 ml buffer; Blank 3: 1.0 ml buffer + 1,0 ml molybdate + 0.0 ml buffer) at 405 nm: Catalase activity (kU/l) was calculated using Equation 2 [17].

$$\% \text{ inhibition} = \frac{(\text{Blanko absorbance 1} - \text{sample absorbance})}{(\text{blanko absorbance 2} - \text{blanko absorbance 3})} \times 271 \quad (2)$$

Glutathione Peroxidase Test (GPx)

In this test, the homogenate was added 2 - 50 µL sample in to plate 96-well, and the volume of mixture was added until 50 µl with an assay buffer. The GPx value in this test was expressed as the lens protein nmoles/ min/ mg.

Results and Discussion

Gas Chromatography-Mass Spectrometry (GCMS) and High Performance Liquid Chromatography (HPLC)

GCMS are gas chromatographic techniques that used in shared with mass spectrometry. Gas chromatography was used to find vaporizing compounds. The compounds will be separated according to the motion phase partition and the stationary phase. Mass spectrometry was used to determine molecular weight, molecule formula and produced charged molecules. Components that have been separated were ionized, so the gas molecule will be fragmented

into positive ions. The ion fragments produced will be captured by the detector. Each molecule takes a different time to get out of a gas chromatography and its called retention time.

Table 2. Metabolite compounds of ethanolic extract of *H. longiflora* leaves.

No	Retention Time (Min)	Relative Area (%)	Similarity Index	Compound names
1	10.765	13.07	930	Acetophenone
2	31.423	5.1	750	Hexadecanoic acid, methyl ester
3	32.222	28.64	864	n-Hexadecanoic acid
4	35.484	14.13	909	cis-Vaccenic acid
5	46.282	1.30	673	4-Penten-1-ol, 5-phenyl-3-piperidino
6	47.296	37.77	661	1-Isobutyl-2,5-dimethyl-4-phenyl-piperidin-4-ol

Based on Table 2, we found 6 different compounds contained in *H. longiflora* leaves extract. Therefore, it was concluded that 1-Isobutyl-2,5-dimethyl-4-phenyl-piperidin-4-ol is the most compound (37.77%) and 4-Penten-1-ol, 5-phenyl-3-ppiperidino (1.30%) is the least compound contained in the ethanol extract of *H. longiflora* leaves. The n-hexadecanoic acid has antibacterial and antioxidant activity with DPPH methods in the range (100-500 μ g/mL) and reduces the effects of free radical compounds Nitric oxide (0.02-0.16%) and superoxide (17-81.21%).

In HPLC test, it was found that *H. longiflora* leaves extract contains quercetin (0.009 mg/g). HPLC is a liquid chromatographic technique that utilizes high pressure on its analysis. The instrument has a sensitive detector, short analysis time, requires a relatively small sample volume and can analyze compounds both organic and non-organic. Quercetin is a flavonoid group that plays an important role in inhibiting cataract progression through antioxidant activity. This group activates nuclear factors such as nuclear factor erythroid 2-related factor 2 (Nrf2) that involved in the regulation of cytoprotective genes and activates cellular protection against cataract formation [18]. Structure quercetin ditunjukkan pada Figure 1.

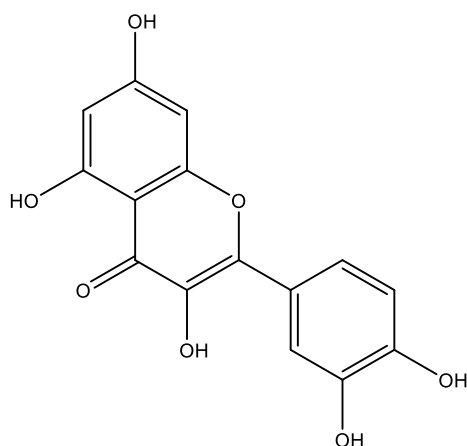


Figure 1. Quercetin structure (making by ChemDraw 19.1 version).

Phytochemical screening, TPC, TFC and antioxidant activity

The results of phytochemistry analysis on *H. longiflora* leaves ethanol extract can be seen in Table 3. Alkaloids, steroids, saponins, phenolics and flavonoid compounds potentially contain antioxidant molecules that can neutralize the presence of free radicals in the human body. In addition, the bioactive compounds also act as an additional electron and reduce metal ions in the body. The previous studies showed that *H. longiflora* leaves extract contained active compounds such as alkaloids, saponins, steroids and flavonoids. Other study also reported results of phytochemical test on *H. longiflora* leaves extracts and showed the presence of active

compounds such as flavonoids, saponins and tannins. Alkaloids compound can be identified by the replacement of iodine ions by nitrogen atoms in the alkaloid structure. The saponins compound can be identified because the hydrophilic group of these compound binds water and the hydrophobic group (aglycon/sapogenin). Meanwhile, the flavonoid compound can be identified by the nucleus reduction of benzopyrone in flavonoid structure forming red flavylum salts.

Table 3. Phytochemical analysis of ethanolic extract of *H. longiflora* leaves.

No	Secondary metabolite	Ethanolic extract of <i>H. longiflora</i> leaves
1	Alkaloids	+
2	Steroids	+
3	Triterpenoids	-
4	Saponins	+
5	Phenolics	+
6	Flavonoids	+

(+) present; (-) absent

In the quantitative analysis of total phenolic content (TPC), phenol reacts with the Folin–Ciocalteu reagent to form a blue-coloured complex compound. The standard curve is determined by defect acid with absorption measured at a wavelength of 762 nm (Table 4).

Table 4. Measurements of standard absorption of gallic acid.

No	Concentration (ppm)	Absorbance (762 nm)
1	20	0.41
2	30	0.49
3	40	0.71
4	50	0.78
5	60	0.80

Calibration curves are calculated using Microsoft Excel and obtained R^2 values of 0.9158; intercepts of 0.204; and gradients of 0.0108 (Table 5); thus obtaining the standard curve equation of $y = 0,0108x + 0,204$ (Figure 2). The R^2 value indicates that the calibration curve has a good correlation between the standard concentration and the response of the instrument. The standard Y curve equation is used to determine the concentration of *H. longiflora* ethanol extract samples using the LINEST function in Microsoft Excel. In this study, a total phenolic content of 139.21 mgGAE/g was obtained with an absorption value of 1.71. The higher the absorption value, the higher the concentration of TPC in the sample. In addition, the use of ethanol solvents resulted in more identified and soluble polar compounds, so it could be concluded that the phenolic compound in the sample was polar.

Table 5. Statistical data values and uncertainties obtained based on calibration curves

Name	Results
Slope	0.010
Intercept	0.204
Standard deviation of slope (Sm)	0.002
Standard deviation of intercept (Sb)	0.080
Coefficient of determination (R^2)	0.915
Standard deviation of regression (Sr)	0.059
Sum of square regression	0.117
Sum of square residual	0.010

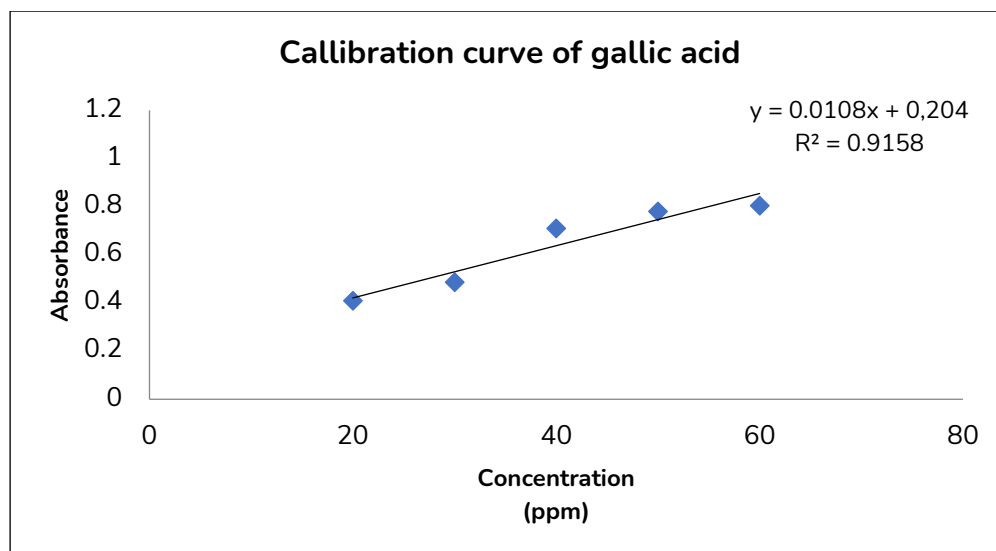


Figure 2. Gallic acid calibration curve.

Quantitative analysis of total flavonoid content (TFC) using quercetin as a standard solution measured at a wavelength of 440 nm (Table 6). Calibration curves are calculated using Microsoft Excel and obtained R^2 values of 0.9457; intercepts of 0.033; and gradients of 0.007 (Table 7); thus obtaining the standard curve equation of $y = 0,00795x + 0.0337$ (Figure 3).

Table 6. Measurements of standard absorption of quercetin.

No	Concentration (ppm)	Absorbance (440 nm)
1	2	0.05
2	4	0.06
3	6	0.07
4	8	0.10
5	10	0.12

Table 7. Statistical data values and uncertainties obtained based on calibration curves.

Name	Results
Slope	0.007
Intercept	0.033
Standard deviation of slope (Sm)	0.001
Standard deviation of intercept (Sb)	0.007
Coefficient of determination (R^2)	0.945
Standard deviation of regression (Sr)	0.006
Sum of square regression	0.002
Sum of square residual	0.001

Similar to TPC, sample concentrations are calculated using the LINEST function in Microsoft Excel. In this study, a total flavonoid content of 11.48 mg QE/g was obtained with an absorption value of 0.125. Because it is soluble in ethanol solvent, the flavonoid compound is polar. Other report showed that total phenolic and total flavonoid content of ethanol extract of *H. longiflora* leaves consecutive of 2.48 mg GAE/g and 2.62 mg QE/gr.

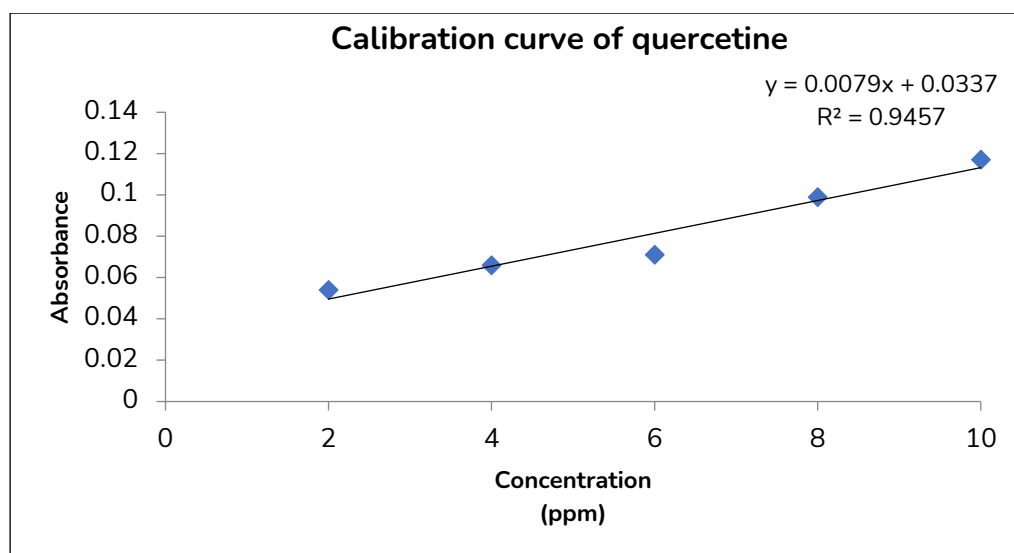


Figure 3. Quercetine calibration curve.

The IC_{50} value describes the level of a compound that can disable 50% of the strength of DPPH free radicals. The IC_{50} value is divided into several categories: the IC_{50} less than 50 ppm is a very strong antioxidant; the IC_{50} in the range of 50-100 ppm is powerful antioxidants; the IC_{50} between 100-150 ppm is moderate; the IC_{50} between 150-200 ppm, and more than 200 ppm are very weak. According to Table 8, an IC_{50} value of 9.11 ppm of ethanol *H. longiflora* leaves extract was obtained. Based on the above categories, the antioxidant activity of ethanol leaves extract (*Hippobroma longiflora* L.) is 9.11 ppm and its very strong.

Table 8. Antioxidant activity of ethanolic extract of *H. longiflora* leaves.

Concentration (ppm)	Absorbance	% Inhibition	Negative control	IC_{50} of ethanolic extract of <i>H. longiflora</i> leaves
100	0.45	53.95		
50	0.47	52.03		
25	0.49	50.42		
12.5	0.49	50.37	0.99	9.11 ppm
6.25	0.49	49.83		
3.12	0.49	49.67		

Molecular Docking

Molecular docking between quercetin and HDGC protein yields 9 conformations with the smallest RMSD value being 0 and binding affinity value is -7.0 kcal/mol. Root Mean Square Deviation (RMSD) is a value that indicates the degree of deviation of the docking result of the ligand. The larger the RMSD value, the greater the deviation that occurs, so the larger is the error of prediction of the ligand-receptor interaction. A very small RMSD value indicates a good ligand conformation because the ligands are in an optimum position on the active site of the protein. Generally, the best RMSD value is 0 and the still accepted RMSD value is $<2 \text{ \AA}$. Exhaustiveness is the level of expertise in the docking process. The degree of rigidity used in this study is 8 repetitions. So, we found 9 conformations of the ligand. Meanwhile, the binding affinity value indicates the bonding energy between the compound and the ligand. The smaller the binding affinity value, the easier and more stable the bonding occurs.

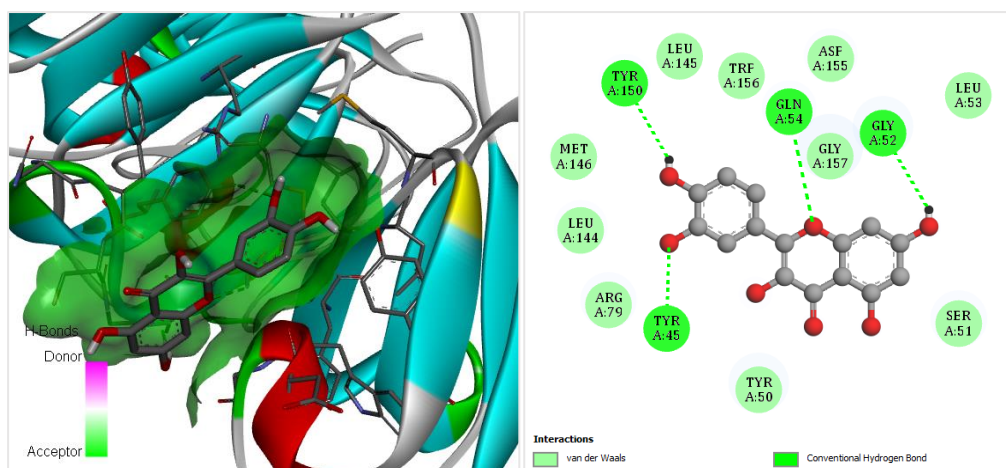


Figure 4. Visualization of the 3 dimensional (left) and 2 dimensional (right) docking result of quercetin against HDGC protein.

Interaction between quercetin and HDGC protein using hydrogen binding and Van der Waals bond with 14 amino residues shown in Figure 4. Hydrogen bond (Tyrosine: 45 and 150; Glycine: 52 and Glutamine: 54) is interaction between hydrogen atoms (H) with electronegative atoms such as fluorine (F), nitrogen (N) and oxygen (O). The more hydrogen bonds are formed with the amino acid residues, the stronger the bonds will be and the lower and more stable the bond value of affinity. Van der Waals bond is included in electrostatic interaction. Electrostatic interaction is interaction between atoms due to polarity differences. This interaction is weak and non-covalent, so it is easy to get rid of. Van der Waals interaction has a relatively weak attraction of electricity due to permanent or induced molecular radiation. Although the strength of this interaction is weak, in large quantities, electrostatic interaction has a major contribution to the formation of protein conformation.

In this study, the docking results were used to predict the potential of ethanol extracts of kitolod leaves as anti-cataracts by looking at binding affinity and bond stability. Based on binding affinity (-7.0 kcal/mol) and Figure 4, it was concluded that the quercetin compound contained in ethanolic extract of *H. longiflora* leaves has the potential to inhibit cataract progression through the inhibition of the HDGC protein that causes cataracts.

Rates of malondialdehyde (MDA), superoxide dismutase (SOD), catalase (COD) and Glutathione peroxidase test (GPx)

In condition of oxidative stress, free radicals interact with bilayer lipid of cell membranes. This process produces the final product of malondialdehyde. MDA may indicate the presence of oxidative stress and affect the cloudy of the lens.

Based on Figure 5, the highest level of MDA was found in group II that did not contain *H. longiflora* leaves extract. It shows that the lens from group II has a highest lens cloudy than the other group. High MDA value indicates high oxidative stress conditions due to the addition of H_2O_2 [19]. The higher the level of MDA, the more the lens is cloudy. Group VI had the lowest cloudy lens due to commercial treatment of quercetin and showed lowest levels of MDA. Meanwhile, the MDA level had decreased in groups III, IV, and V (groups given *H. longiflora* leaves extract 100, 200, and 300 $\mu\text{g/ml}$) with the lowest value found in the group V. This suggests that antioxidant in *H. longiflora* played a role in inhibiting cataractic progressiveness by reducing the oxidative stress characterized by decreasing the MDA level.

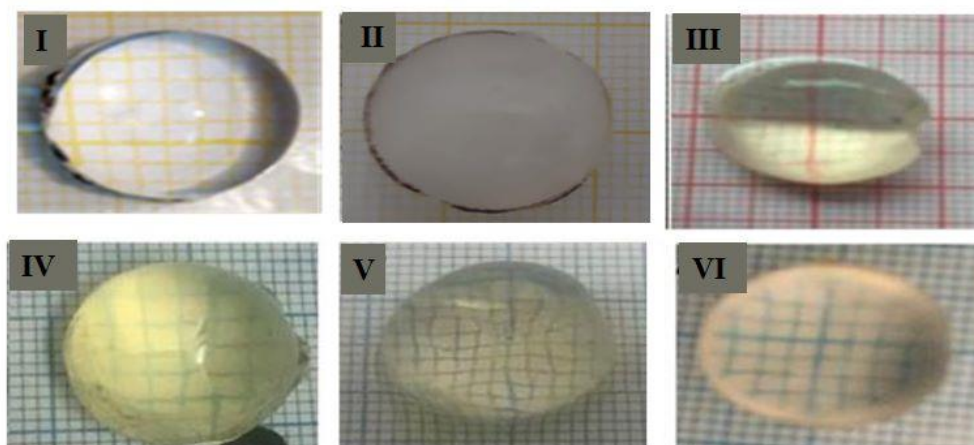


Figure 5. Effect of ethanolic extract of *H. longiflora* leaves on MDA levels on goat's eye lenses. (I) Normal lens cataracts; (II) Cataract lens (Induced H_2O_2 10 mM); (III) Cataract lens + ethanol leaves extract of *H. longiflora* (100 $\mu\text{g/ml}$); (IV) Cataract lens + ethanol leaves extract of *H. longiflora* (200 $\mu\text{g/ml}$); (V) Cataract lens + ethanol leaves extract of *H. longiflora* (300 $\mu\text{g/ml}$); (VI) Cataract lens + commercial quercetin.

Previous study showed that antioxidants can act as a tool that will prevent the formation of ROS. Preventing ROS formation on the antioxidant by acting as a scavenger and removing the ROS or as an ion that binds metal ions into inactive compounds [20]. MDA is known as a dominant damage product resulting from lipid peroxide. In human cortical cataract as well as nuclear cataracts, MDA has been found to be 3.5 times higher than normal, and identified as a 2-thyobarbitrate acid reactive (TBARS).

Meanwhile, SOD, CAT, and GPx activity were also measured in all of the group of goat's eye lenses. The lowest SOD, CAT, and GPx values occurred in group 2 (non *H. longiflora* leaves extract). This is due to the lack of antioxidant that play a role in capturing and inhibiting oxidative stress processes in the body characterized by increased levels of MDA [17]. In groups III, IV, and V (containing *H. longiflora* leaves extract 100, 200, and 300 $\mu\text{g/ml}$), levels of SOD, CAT, and GPx increased as concentration increased. Meanwhile, Group VI has the highest SOD, CAT and GPx, where it's the best compared to the lowest MDA. The addition of 300 $\mu\text{g/ml}$ of *H. longiflora* leaves extract has already given effective results in reducing eye lens cloudy as well as improving SOD, CAT and GPx. This suggests that the antioxidant in the *H. longiflora* extract can reduce the oxidative stress that occurs on the lens and thus inhibit the cataract process.

One of the antioxidant found in *H. longiflora* extract is flavonoid. Flavonoids are secondary antioxidant that can catch free radicals [21]. According to previous research, flavonoid can counteract free radical by suppressing free radical formation or ROS and inhibiting enzymes and the clamping of metal ions involved in free radical production. Flavonoids is high-grade antioxidant that can capture free radicals such as anion superoxide and hydroxyl free radicals [22]. Flavonoids can stimulate the formation of endogenous antioxidants such as SOD, CAT and GPx, blocking propagation reactions and capturing ROS thus reducing the risk of oxidative stress in cells.

Conclusions

This study revealed the potential of the ethanol extract in inhibiting cataract progression through antioxidant activity. This was in line with another study that stated the use of a solvent affects the antioxidant activity of *H. longiflora* leaves extract, and ethanol solvent provided better antioxidant effects than methanol. Additionally, another study supported these findings, stating that the antioxidant activity of the ethanol extract was more potent than the n-hexane

and chloroform fractions but smaller than that of ascorbic acid. The presence of free radicals was closely related to the levels of MDA, SOD, CAT, and GPx. Decreases in MDA levels and increases in SODs, CATs, and GPxs occurred at a concentration of 300 µg/ml *H. longiflora* leaves extract. This suggested that *H. longiflora* leaves extract could act as an inhibitor of cataract progression through its role as an antioxidant.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The data availability statement should state whether data is available and how it can be accessed. It should also provide any necessary details on data sharing policies.

Conflicts of Interest: All the authors declare there are no conflicts of interest that has been reported for this research.

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