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Research Article

Antihypertensive Effects of Fig Leaves (*Ficus carica* L.) Ethanolic Extract: An In Vitro and In Silico Study

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ABSTRACT

Pathological conditions of hypertension can occur due to contractions of blood vessels. Vascular contraction response occurs when epinephrine successfully binds to adrenergic receptors. Objective: This study aims to determine the antagonistic activity of giving ethanolic extracts of fig leaves (*Ficus carica* L. Folium) in β-adrenergic receptors of isolated aortic organs and to determine the value of the affinity of one of the compounds present in fig leaf extract, which has a strong potential as a vasodilator agent to treat hypertension. This study uses in silico and in vitro methods. The in silico method test used molecular docking, and the in vitro method test used an organ bath on a guinea pig's aortic organs. An in silico docking test was conducted on compounds suspected to have a vasodilator effect, namely Quercetin and β-adrenergic receptor test with native ligand timolol (PDB ID: 6PS6). The in vitro testing of foliar ethanolic extracts was done by administering 0.5 mg of extracts at 100 µL and 200 µL. The resulting data included a percent of aortic contraction data on administering epinephrine concentration series, which was transformed into a pD2 value. The result from the in silico method showed that quercetin binding affinity value (-7.8 kcal/mol) was better than timolol as a comparison (-6.4 kcal/mol). The result from the in vitro method showed that the fig leaf's ethanolic extract could shift the percentage curve of contraction response to the administration of epinephrine series by decreasing the pD2 value. A dose of 0.5 mg volume 200 µL is effective because it gives a significant difference to the control group but does not show a significant difference in the comparison group. Conclusively, the ethanolic extract of fig leaves can be developed as a vasodilator agent.

INTRODUCTION

Hypertension, better known as high blood pressure, is a condition of increasing systolic blood pressure exceeding 140 mmHg and diastolic blood pressure exceeding 90 mmHg on two measurements with an interval of five minutes in a calm state (Destiani *et al*., 2016). The two leading causes of death worldwide in 2016 were ischemic heart disease and stroke, even though hypertension is not contagious (World Health Organization [WHO], 2018). These disorders include stroke, heart attack, and other renal diseases that are known to be kidney diseases. The findings of fundamental medical research in 2018 (Kemenkes, 2018) reported that 34.1% of Indonesian adults under 18 have hypertension. This number grew significantly compared to the Riskesdas figures from 2013, which reached only 25.8%.

The WHO, which oversees global health, has estimated that by 2025, hypertension will affect almost 29% of the global population. According to the WHO, there are 40% more people with hypertension in developed nations than in industrialized ones (35%). Antihypertensive medication side effects may become problematic with continued use. In order to find alternative drugs for treating

hypertension with better safety profiles, researchers have been focusing on anti-hypertension originating from natural resources or herbal medicines (Salve *et al*., 2022). Treatment using plants is a relatively inexpensive drug. Due to its better compatibility with the human body, lower costs than modern pharmaceuticals, and fewer side effects, herbal medicines are used for primary healthcare by roughly 75% to 80% of the world's population, mostly in impoverished nations (Kamyab *et al*., 2021). In Indonesia, many plants have been used traditionally by people to help lower blood pressure (Sukandar *et al*., 2019). *Ficus carica* L. (Moraceae) has been commonly known as 'fig' and is probably a native of South West Asia that rapidly spread to the Mediterranean region. Figs is one of the five plants mentioned in the Quran (Alamgeer *et al*., 2017). Figs are known to have many benefits. One is the leaf part, traditionally used to treat gastrointestinal, respiratory, anti-spasmodic, anti-inflammatory, and cardiovascular diseases (Mawa *et al*., 2013) (Badgujar *et al*., 2014). This is because fig leaves contain many flavonoid compounds such as quercetin, rutin, and luteolin (Ahmad, 2012), which are known to have the potential to reduce blood pressure through the mechanism of reducing oxidative stress, inhibit acetylcholinesterase (ACE), increase endothelial function, and direct activation of smooth muscle blood vessels (Larson *et al*., 2010).

Quercetin has potency as an antihypertensive agent (Grande *et al*., 2016). Quercetin is the most common vegetable and fruit flavonoids (Vicentini *et al*., 2008). It exhibits the highest antiradical properties toward hydroxyl radical, peroxyl, and superoxide anions compared with other flavonoids (Casagrande *et al*., 2006). One of the compounds contained in the fig plant is quercetin (Rao, 2020). Others have claimed that quercetin lowers blood pressure and lessens the severity of hypertension in a variety of rat models, including those that are naturally hypertensive, high-fat, high-sugar diet-fed rats, nitric oxide-deficient rats, angiotensin-infused rats, aortic constriction-prone rats, and Dahl salt-sensitive rats. Additionally, it has been demonstrated that quercetin exhibits in vitro vasodilator effects in isolated rat arteries. These studies offer proof of concept that a quercetin-induced decrease in blood pressure may be the cause of the decreased risk of cardiovascular disease seen in humans with high quercetin diets, even though extra caution should be used when extrapolating results from animal studies to humans (Larson *et al*., 2010). In a previous study, fig leaf extract, when made into tablets at a dose of 100 mg, was able to reduce blood cholesterol levels by 37.98% in rats induced by lard oil and egg yolks (Kurniawan and Audita, 2021). Fig leaves at 5%, 10%, and 15% in mice effectively lower blood glucose levels, induced by alloxan. Fig leaves have the potential to be an additional treatment for diabetes. Lowering blood glucose levels is essential in treating diabetes mellitus, especially type 2, to prevent more serious consequences. An efficient and all-natural therapy approach involves using fig leaves in the proper concentration (Aisyah and Tr, 2023). Besides that, when formulated as a tablet with a dose of 80 mg, fig leaf extract reduced blood glucose levels in rats induced by alloxan 150 mg/kg intraperitoneally (Kurniawan and Yusuf, 2021). Another research showed that a flavonoid suspected to be Quercetin is found in the ethanol extract of fig leaves, which can protect the liver. The analysis findings revealed that there was no discernible difference in the amount of SGPT and SGOT levels reduced across doses, with the dose of 40 mg/200 g BW being the most effective dose with hepatoprotective qualities (Kurniawan and Wardany, 2021). *Ficus carica* L. leaf extract interacts with the nuclear complex to repair DNA strand breaks in MCF10A cells whether diethylstilbestrol (DES) and its oxidative quinine metabolite, 4',4"-diethylstilbestrol quinine (DESQ), are present or not. This leaf extract is physiologically reactive in vitro (Lightbourn and Thomas, 2019). Based on the data above, fig leaf extract has benefits as an antidiabetic, anticholesterol, and anticancer agent, so it does not rule out the possibility that fig leaf extract also has the potential to be used as an antihypertensive. Based on the description above, it is necessary to determine other mechanisms of fig leaf extract to decrease blood pressure through antagonistic activity against β-adrenergic receptors. Testing was done in vitro using isolated guinea pig aortic organs induced with epinephrine as an adrenaline agonist. Epinephrine binds to adrenergic receptors, which can cause vasoconstriction effects, and if it is continuously stimulated, it will result in pathological events such as hypertension. The results of this study are expected to be used as a scientific basis in the development of fig leaf extract as an antihypertensive which has β2 adrenergic receptor antagonist activity and provides new potential in finding natural antihypertensive alternatives with minimal side effects and affordable prices. In addition, this research can be the actual reference regarding

the benefits of the fig plant, which is often discussed in reducing blood pressure.

MATERIAL AND METHODS

Materials

Before organ preparation tools, vortex, magnetic stirrer thermostat (Cimarec), transgender isotonic, organ bath (Ugo Basil), bridge amplifier, micro pipet 100µl, 1000µl, 5000µl, Discovery Studio Visualizer BIOVIA 2020 software, Autodock Vina 1.2.0 software (to prepare ligands and target proteins for docking needs in PDBQT format), MarvinSketch 20.14 software, fig leaves ethanolic extracts, aortic organ Cavia porcellus age three months, adrenergic receptor β agonist, aquadest (Brataco), Buffer Krebs solution, epinephrine injection, timolol solution, carbogen gas (Samator), quercetin raw file, timolol raw file, and adrenergic β2 receptor raw file PDB ID: 6PS6).

Methods

Extraction research paths

Fig leaves Simplicia powder was extracted using a modified maceration method with maceration with a 70% ethanol filter ratio of 1:10. Based on the previous method, extracting fig leaves using 70% ethanol will produce a greater yield than using 96% ethanol (Kurniawan and Wardany, 2021).

Identification Test of Fig Leaf Ethanolic Extract Using Thin Layer Chromatography

The identification test with thin layer chromatography (TLC) used the silica gel GF254 stationary phase and the mobile phase in the form of Ethyl Acetate: Methanol: Water namely 6.5: 2.85: 3. Extract and quercetin standard was dissolved with 70% ethanol, then dotted on a silica gel plate measuring 2x10 cm with the help of a capillary tube and elucidated in a TLC vessel which previously had the mobile phase through the saturation process. The spots on the plates were observed in visible light and ultraviolet (UV) light with a wavelength of 254 nm and 366 nm (Prayoga and Rahmawati, 2019).

In Silico Test with Molecular Docking Method

Marker compounds were created using the Marvin Sketch application on the Windows operating system. The marker compound in this study was quercetin, one of the compounds present in fig leaves and thought to have a vasodilatory effect on the aortic organ of guinea pigs (Cavia porcellus). In this study, the protein to be used is β2 adrenergic receptors. This protein can be downloaded from the official website of the protein data bank, namely, www.rcsb.org. This β2 adrenergic protein can be searched using the 6PS6 code, then downloaded in .pdb format. The ligand used in this research is a marker compound from quercetin. These ligands can be downloaded from the significant ligand database, namely Pub Chem (http://pubchem.ncbi.nlm.nih.gov/) in 3D SDF format. Downloaded files can be opened in the Discovery Studio Visualizer application and saved in PDB format (*.pdb). Ligands and proteins as targets for docking were prepared in PDBQT format. At this stage, the AutoDock Tools program is used for target protein preparation in the same application. The addition of hydrogen atoms in the target protein serves to provide partial charges. Then, to adjust the docking area, which includes the position and size of the grid box, the AutoDock Vina application is used. The docking area corresponds to the RMSD value for each conformation. The selected RMSD value was <2Å. Finally, visualization is carried out using the DS Visualizer application to determine the position of the bond between ligands and proteins in two dimensions.

In Vitro Test using an Organ Bath.

Preparation of a buffer solution

Krebs Buffer Solution consists of 2 formulas that must be prepared: A and B. The composition of each formula, A and B, can be seen in Table 1.

Table 1. Composition of Krebs Buffer Solution

Formula A (1,00 L)		Formula B (1,00 L)	
Materials	Quantity	Materials	Quantity
NaCl	68.70 g/L	$NaHCO_3$	21.00 g/L
KCl	4.20 g/L	-	-
$MgSO_4.7H_2O$	2.90 g/L	-	-
$CaCl_2.2H_2O$	3.70 g/L	-	-
$NaH_2PO_4.2H_2O$	2.00 g/L	-	-

The results of the preparation of the two formulas are then stored in different volumetric flasks, and mixing is carried out only when they are to be used. The mixing process was done by taking 100 mL of solution B and dissolving it in 800 mL of distilled water, then taking 100 mL of solution A and dissolving it into one solution B, which has been dissolved with distilled water. Finally, 1 gram / L glucose was added, and the Krebs buffer solution was ready to be used as a substitute for the physiological fluids of the test animals.

Preparation of Fig Leaf Ethanolic Extract Solution
Fig leaf ethanolic extract stock solution was prepared at a 0.5 mg / mL concentration. The stock solution was weighed by 10 mg of the extract and then dissolved in 10 mL DMSO. The stock solution was taken 1 mL and added 1 mL of DMSO to get a concentration of 0.5 mg / mL.

Preparation of Concentration Series Epinephrine
The preparation of the epinephrine concentration series (MW: 183.204 g / mol) begins with preparing a $2x10^{-3}$M concentration of epinephrine stock solutions in distilled water. Then carried out stratified dilution of the stock solution of $2x10^{-3}$M epinephrine to obtain a solution of $2x10^{-3}$ concentration of epinephrine; $2x10^{-4}$, $2x10^{-5}$, $2x10^{-6}$, $2x10^{-7}$, and $2x10^{-8}$ M.

Preparation of Timolol Solution
The stock solution of timolol (MW: 316.421 g/mol) was made with a concentration of $2x10^{-3}$M by weighing 6.33 mg of timolol powder and then dissolving it in distilled water up to 10.0 mL. The preparation used in this study was in the form of drops of eyes with a concentration of 5 mg/ml. Then, the solution taken in these preparations is 1.26 mL, which is then diluted with distilled water to 10 mL. The 10 µM (10^{-5}M) timolol sets were obtained by taking 100µL of the $2x10^{-3}$M timolol solution, which was put into an organ bath containing 20 mL of Krebs buffer solution. The 50 µM ($5x10^{-5}$M) timolol solution was obtained by taking 500 µL of the $2x10^{-3}$M concentration of timolol solution, which was put into an organ bath containing 20 mL of Krebs buffer solution.

Preparation of Aortic Organs
Handling of tested animals (Cavia porcellus) is approved with Ethical Approval number 037/EP-FKIK-UMY/V/2020 from Ethics Committee Faculty of Medicine and Health Sciences, Universitas Muhammadiyah Yogyakarta. Cavia porcellus was euthanized by dislocation of the spine from the head (cervix), then surgery was performed from the lower abdomen to the chest to make it easier to extract the aorta. The aorta was cleaned of adherent tissue such as fat and taken through a gentle incision and cut into a 6-7 cm size (according to the length of the bath organ size of 20 mL). Once successfully removed, the aortic ring is immediately inserted into a fixation plate containing a buffer solution. Then, the two ends of the aorta are tied using a thread; the lower end is tied to the lever of the bath organ, and the upper end is tied to the part that is connected to the transducer.

Activity Test of Figs leaves Ethanolic Extract Compounds Against Physiological Receptor Agonists
The activity test of fig leaf ethanolic extract compounds against β-adrenergic receptor agonists in the aortic organ of Cavia porcellus isolated using an organ bath begins with the introduction of the highest level of agonist (epinephrine) first. After going through the process of introducing the highest levels of agonists then equilibration until the test organ is in stable condition, it usually takes 30 minutes with 5x replacement of the Krebs buffer solution (washing). Then, a series of agonist concentrations are given in stages from the lowest to the highest concentrations, and the contraction response measures up to achieve the maximum contraction response (100%). After the response to the administration of the agonist series is recorded, washing is again done every 5 minutes for 30 minutes, and measuring the reaction response to the ethanolic extract of figs leaves after going through the second washing process, the organs are immediately administered with figs leaf extract with a concentration of 0.5 mg with the volume of administration. 100 µL and 200 µL, then the next 1 minute was immediately given the agonist in stages from the lowest to the highest concentration. The results of measuring the first contraction response (after giving the agonist only) were then compared with the results of the second contraction response measurement (after giving fig leaf ethanolic extract + agonist administration). If the contraction response has decreased or relaxed, it can be stated that the ethanolic extract of fig leaves can provide a vasodilating effect on agonist-induced guinea pig aortic organs (epinephrine). Results from the in vitro test: The data obtained were data on

contraction or relaxation of the aortic smooth muscle of the Cavia porcellus, which was obtained through recorder data. The data obtained were converted into a percentage (%) response to the maximum response achieved by the antagonist. Then, the data were made in the form of a relationship curve between the logarithm of antagonist concentration and % response.

$$E = \frac{E_{max} \times C}{C + EC_{50}} \quad \text{..} (1)$$

Note:
E : The effect produced at the concentration of C
C : Drug concentration
E_{max} : Maximum response produced by the drug
EC_{50} : The drug concentration that produces 50% of the maximum effect.

EC_{50} can be used to find the affinity parameter for the agonist for the pD2 receptor, where the pD2 value is obtained from the results - Log EC_{50}. The affinity value of an agonist is directly proportional to its pD2 value. The greater the pD2 value, the stronger the affinity of the agonist for a receptor. The EC_{50} value of the receptor agonist, with or without the influence of ethanolic extract compounds, was calculated based on the concentration-% response curve. The EC_{50} value is calculated based on equation 1, which is then transformed into pD2, where pD2 is the value of –Log.EC_{50} (equation 2). Then, the data are presented in the agonist treatment group table with or without the influence of fig leaf extract compounds) and the average pD2 agonist ± Standard Error (pD2 ± SE).

$$LogEC_{50} == \left[+ \frac{50 - Y1}{Y2 - Y1} \times (X2 - X1) \right] + X1 \quad \text{..............................} (2)$$

X1 : Logs. Concentrations with a response just below 50%
X2 : Logs. Concentrations with a response just above 50%
Y1 : % response just below 50%
X2 : % response just above 50%
pD2 : -Log.EC_{50}

Timolol Comparative Test
The comparative test for timolol was carried out following the procedure for testing the activity of the ethanolic extract compound of fig leaves against β-adrenergic receptor agonists in the aortic organ of Cavia porcellus isolated using an organ bath. Where the aortic organ is contracted with epinephrine solution before being treated with timolol, which then results in a curve of the relationship between the amount of epinephrine concentration and the percentage of relaxation response aortic organs after treatment with timolol were compared with the percentage of aortic organ relaxation responses treated with fig leaf extract. The fig leaf extract compound was determined as a β receptor antagonist. In the in silico test, the data obtained are validation RMSD values and docking scores or binding scores. If the binding score is lower than the ligand binding score of the comparison, the ethanolic extract of fig leaves has the potential to be used as a β-adrenergic antagonist agent. The fig leaf extract compound was determined as a β receptor antagonist adrenergic if incubation of the isolated Cavia porcellus aortic organ with fig leaf extract compounds resulted in a decrease in the pD2 value of adrenaline. The distribution of adrenaline pD2 data was analyzed using the normality test (Kolmogorov-Smirnov method). Then the decrease in the pD2 value was analyzed using parametric statistical methods, namely one-way ANOVA followed by the LSD test with a 95% confidence level.

RESULTS AND DISCUSSION

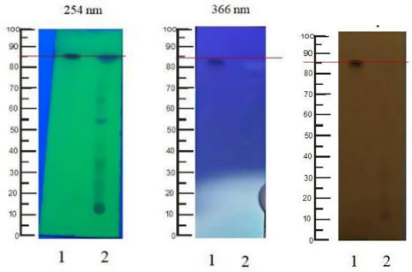
Identification Test of Fig Leaf Ethanolic Extract Using TLC

The results of TLC showed that the sample and standard compound (Quercetin) had the same spot with a value of Rf = 0.9375. In addition, the results also show that the spots can glow under UV light 366 nm and the appearance of a blackish brown color after spraying with FeCl3. Phenol compounds, when sprayed with FeCl3 color reagent, will cause a strong brown or black color (Syamsudin *et al*., 2022). The results of the analysis state that the ethanolic extract samples from fig leaves contain quercetin compounds. The description of the results of the identification of quercetin compounds using the TLC method can be seen in Figure 1.

In Silico test

Molecular docking protocol validation is one of the steps to provide confidence in the truth of in silico molecular docking test results. Molecular docking protocol validation using the post-selection stage testing of a molecule whose conformation and orientation are known to the receptor active site used (Diallo *et al.,* 2021). The molecule used can be a native ligand or other compound that is known to have pharmacological activity when it binds to the receptor or protein used. The protocol validation

After sprayed with $FeCl_3$



Note :
1. Quercetin standard
2. Figs leaves extract

Figure 1. The results of the identification of quercetin compounds using the TLC method

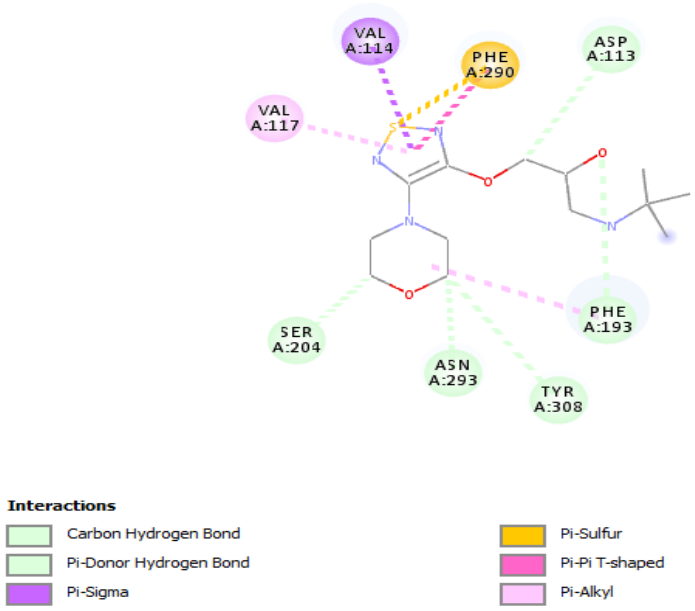


Figure 2. 2D visualization results of timolol on β2 adrenergic receptors. The image above shows the linked amino acids with the ligand and the type of bond that occurs.

process is considered successful if the program used can produce a Root Mean Square Deviation (RMSD) test value below 2Ă. After that, post-selection was carried out to select the best affinity from the conformation of the molecule, which is less than 2μ. In this in silico test, the PDB ID used was 6PS6 with β2 adrenergic receptors and native ligand timolol. After the protein was cleaned of interfering molecules (H2O and other native ligands) and optimized, the protein was run 5 times using AutoDock Vina software. The docking test of the native ligand timolol produced several affinity values with an RMSD < 2Ă, which indicated that the docking validation had been carried out successfully. The best affinity obtained from timolol for β2 adrenergic receptors is -6.4 kcal/mol. In addition, the docking of the quercetin molecule was also successfully carried out with a docking RMSD value of less than 2Ă and the best affinity with a value of -7.8 kcal/mol. Timolol 2D visualization results for β2 adrenergic receptors are shown in Figure 2.

From the results of visualization using the DS Visualizer, the docking score of the timolol compound showed the best affinity value during the 2nd run, namely -6.4 with a lower bond RMSD value of 1.277 (<2Ă) and an upper bond of 2.092Å. Timolol compound is known to be bound to several protein residues, namely, TYR (308),

ASN (293), SER (204), VAL (117), VAL (114), PHE (290), ASP (113), and PHE (193). Timolol is a propanolamine derivative and a nonselective β-adrenergic receptor antagonist which has antihypertensive properties. Timolol competitively binds to β1 adrenergic receptors in cardiac and vascular smooth muscle. The action of β1 adrenergic receptor blockade results in a decrease in heart rate and cardiac output, a decrease in systolic and diastolic blood pressure, and a reduction in reflex orthostatic hypotension. In addition, timolol also competitively binds β2 adrenergic in bronchial and vascular smooth muscle, which results in a decrease in β-adrenergic stimulation, so that the effect of β2 adrenergic receptor blockade in the form of an increase in peripheral blood vessels and the end result of blockade are both capable of providing a vasodilatory effect. From the 2D visualization results of the quercetin compound, *Ficus carica* L folium shows the best affinity value after the -8th running, namely -7.8 kcal/mol with a lower bond RMSD value of 0.625 Å and an upper bond of 1.579 Å. The visualization results also show that the fig leaf quercetin binds to 11 residues of the target protein, namely, THR (110), PHE (289), VAL (114), PHE (290), THR (118), VAL (117), SER (204), ASN (293), TYR (308), ASP (113) and PHE (193). The results of the 2D visualization of Quercetin against β2 adrenergic receptors are shown in Figure 3.

Molecular docking method based on the results of docking performed on the drug timolol and quercetin using a voltage-gated β2 adrenergic agonist (PDB ID: 6PS6). It is known that the binding affinity value of quercetin (-7.8 kcal/mol) is better than that of timolol (-6.4 kcal/mol). These results predict that Quercetin has a stronger binding to β2 adrenergic receptors than timolol; this is because the smaller the value of the affinity, the more stable the receptor bond with the ligand (Altuntaş *et al*., 2020). In addition, the docking results also show that there is a protein residue that binds the ligand so that the event can provide pharmacological activity. When viewed from the table above, the quercetin ligand has the same 7 amino acid residue residues as timolol. Thus, the results of the molecular docking test between timolol and quercetin showed that they both have antagonistic activity against β2 adrenergic receptors. The score of docking ligands against β-adrenergic receptors is shown in Table 2. Quercetin and timolol have the same type of amino acids in binding to the original ligand.

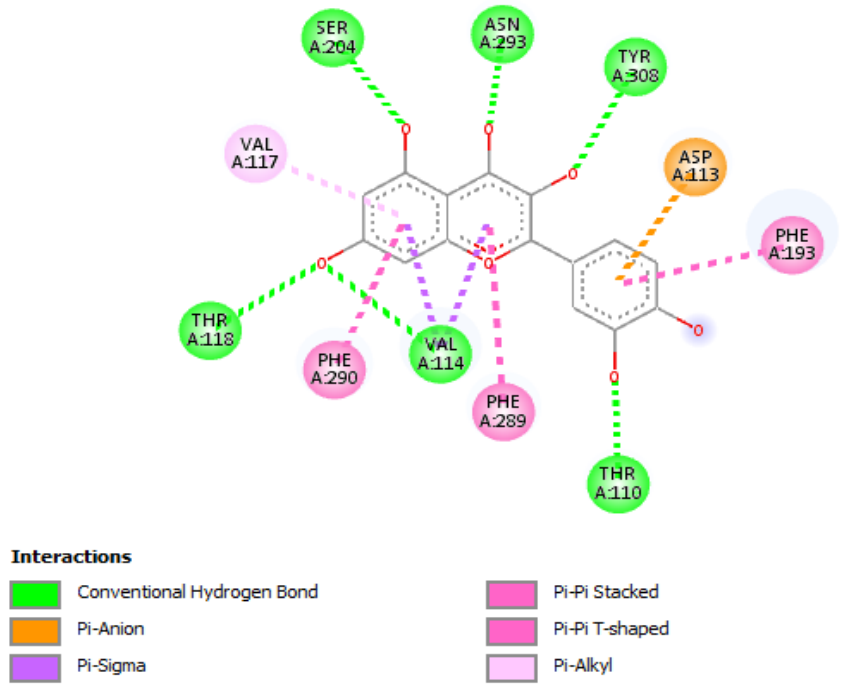
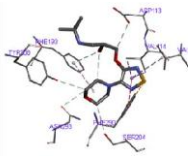
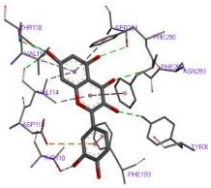


Figure 3. 2D visualization results of Quercetin on Adrenergic β2 Receptors. The image above shows the linked amino acids with the ligand and the type of bond that occurs.

Table 2. Scores of docking ligand against β-adrenergic receptors

Ligand	Energy (kcal/mol)	RMSD(Ă)	Interacting amino acid residue	Binding affinity (kcal/mol)
Timolol	-6.4	1.277	THR(308)*, ASN (293)*, SER(204)*,VAL(117)*, VAL(114)*,PHE(290)* ,ASP (113)*	
Quercetin	-7.8	0.625	THR(110), PHE(289), VAL(114)*, PHE(290)*, THR(118), VAL(117)*, SER(204)*, ASN(293)*, TYR(308)*,ASP(113)*, PHE(193)	

Note: (*) between Quercetin and timolol have the same type of amino acid in binding to the original ligand.

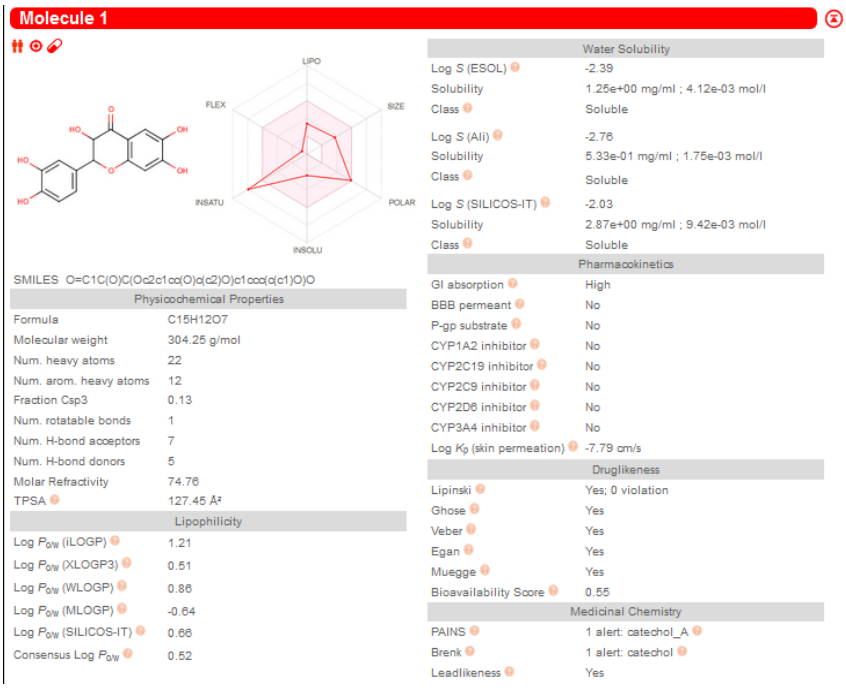


Figure 4. Quercetin ADME In Silico Test.

ADME Quercetin

The in silico ADME test for quercetin compounds using the Swiss ADME online application obtained results in the area of quercetin bioavailability with chemical-physical properties, lipophilicity, water solubility, pharmacokinetics, drug-likeness, and medicinal chemistry. The results in the bioavailability hexagram area show that quercetin is included in the range of compounds that have good bioavailability in the parameters of lipophilicity, size, polarity, water solubility, and flexibility.

Quercetin ADME test results are shown in Figure 4.

In Vitro Test

Effect of Fig Leaf Extract on β2 Adrenergic Receptors

In the analysis process, it must be known that if the administration of epinephrine in the organ bath causes contraction of the aortic smooth

muscle, it can be ascertained that the target that binds to the epinephrine neurotransmitter precisely binds to the β2 adrenergic receptor. If the administration of fig leaf extract can reduce the contraction of aortic smooth muscle due to the administration of epinephrine, it can be said that fig leaf extract has β2 adrenergic receptor antagonism activity, resulting in a vasodilating effect. This antagonistic activity can be measured by the pD2 value of epinephrine without and by administering fig leaf extract. If the pD2 value gets smaller, it shows that the decrease in contraction is getting bigger. Table 3 provides results that shifted the pD2 value due to the effect of giving 0.5 mg of fig leaf extract with a volume of 100 and 200 µL.

The Table 3 shows that the pD2 value after giving the extract has decreased, so it can be stated that fig leaf extract can reduce the response of aortic smooth muscle contraction or, in other words, fig leaf extract has antagonistic activity against β2-adrenergic receptors, which can produce vasodilating effects. The pD2 value of the epinephrine control group in the treatment group was given 0.5 mg of fig leaf extract with a volume of 100 µL and a volume of 200 µL, respectively 7.40, 6.99, and 5.42. The decrease in pD2 value began to be seen from the administration of 0.5 mg of fig leaf ethanolic extract volume of 100 µL. In addition, from the table above, the results show that the type of antagonism of fig leaf extract is a non-competitive antagonist. This finding was indicated by the E-max value, which did not reach 100% in the extract treatment group. The value of E-max that does not reach the value of 100% is due to the addition of agonist concentrations that are not able to shift and overcome the effects of non-competitive antagonists. The non-competitive antagonist has a binding mechanism, not at the place occupied by the agonist. Meanwhile, the logarithmic relationship curve of the ethanolic extract concentration of fig leaves to the response of the smooth muscle contraction of a guinea pig aorta (Fig. 5) shows a shift in the curve. This shows that the control ability of epinephrine in contracting the guinea pig aorta has decreased as a result of treatment by giving fig leaf extract as much as 0.5 grams in a volume of 100 and 200µL. The two concentrations were equally able to shift the curve from the epinephrine control to reduce the contraction effect. However, after going through a statistical test using the Post Hoc Test One-way ANOVA with a 95% confidence level, the results between the epinephrine control and administration of 0.5 grams of fig leaf extract with a volume of 100µL showed no significant difference (P> 0.05). The epinephrine control group with 0.5 grams of fig leaf extract volume 200µL showed a significant difference (p <0.05). The concentration of 0.5 grams of fig leaf extract volume of 100 and 200 µL also showed a significant difference. The concentration of leaf extract, which was able to provide a vasodilating effect with a significant difference, was 0.5 mg with a volume of 200 µL.

Timolol Comparative Test

The comparative test in this study used timolol, whose treatment was made the same as the treatment of fig leaf extract. The timolol drug used was Cendo Timol® eye drops 5 mL. Timolol has been known to have a non-specific β-adrenergic antagonist effect so that it can cause a dilating effect of vascular smooth muscle (Negri *et al*., 2019). In ophthalmic medication, timolol can decrease eye tension through vasodilation of the blood vessels in the eye. In addition to validating the study, the purpose of the comparative test in this study was also to see whether fig leaf extract had the same results as timolol or not. The logarithmic relationship curve of timolol (M) concentration to the response of guinea pig aortic smooth muscle contraction without and with 10 and 50µM timolol administration can be seen in Figure 6.

Table 3. Shift in pD2 values due to administration of 0.5 mg of Figs leaf extract volume of 100 and 200 µL

No	Treatment Groups	pD2 ± SEM	Emax (%) ± SEM
1	*Ephineprine*	7.40 ± 0.17	100 ± 0.00
2	Figs leaves extract 0.5 mg volume 100 µL	6.99 ± 0.07	90.16 ± 2.24
3	Figs leaves extract 0.5 mg volume 200 µL	5.42 ± 0.36	75.45 ± 4.29

SEM, standard error of the mean.

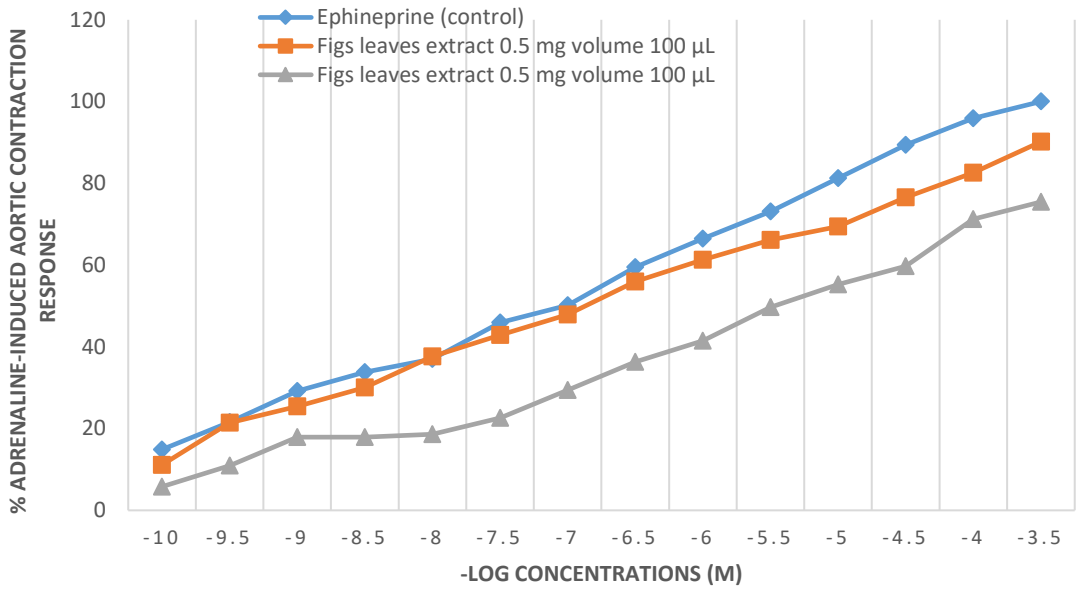


Figure 5. Curve of the Logarithmic Relationship of figs Leaf Extract Concentration Against% Response of Guinea Pig Aortic Plain Muscle Contraction Without and With 0.5 grams of figs Leaf Extract Volume 100 and 200µL

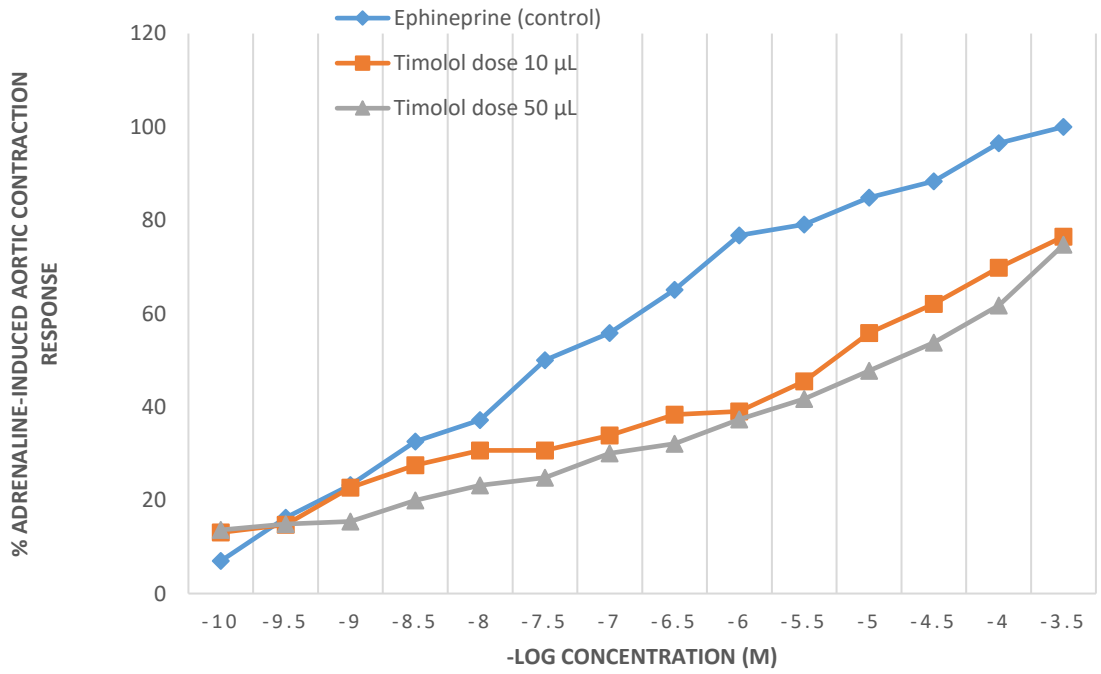


Figure 6. Curve of the Logarithmic Relationship of Timolol (M) Concentration on% Response of Guinea Pig Aortic Smooth Muscle Contraction Without and With 10 and 50µM Timolol

The comparative test results for timolol at doses of 10 and 50 µM showed a decrease in the contraction effect of the aortic smooth muscle of guinea pigs that had been induced by the epinephrine concentration series. These results are characterized by a shift in the curve due to the administration of timolol and also a decrease in the pD2 epinephrine value, which is shown in Table 4. The average value score of pD2 epinephrine for the control treatment, timolol 10 µM and timolol 50 µM, was 7.19, 5.92, and 5.08, respectively. Based on the statistical test of the decrease in pD2 values, the control group with both 10 and 50 µM of thymolol doses showed a significant difference ($p < 0.05$), while the timolol dose group 10 and 50 µM did not show any significant difference. This shows that there is no significant effect related to increasing the timolol dose on the resulting effect. Previous studies found no difference in the decrease of intraocular pressure between timolol 0.25% and 0.5%. Timolol was given twice daily for four weeks in a double-masked, three-period crossover research. At the end of the study, the mean intraocular pressure reduction was 11.313.18 mmHg (34.67%) with timolol 0.25% against 12.033.72 mmHg (35.95%) with timolol 0.5%. When timolol 0.25% and 0.5% were dosed once daily for 8 weeks or twice daily for 6 months, the results were comparable (Shen and Bejanian, 2016).

Increases in concentration from 0.25% to 0.5% did not significantly lower intraocular pressure from baseline in a similar dose escalation study of timolol at 0.25% and 0.5% twice daily. It can be concluded that with a dose of 10 µM, timolol is able to provide a vasodilatory effect.

The antagonistic properties possessed by timolol are the same as the antagonistic properties of fig leaf extract are non-competitive antagonists; this is because the Emax value does not reach 100% in the timolol treatment. The Emax produced by the timolol group concentrations of 10 and 50 µM, respectively, participated at 78.76% and 90.27%. Non-competitive antagonists react irreversibly, where high agonist concentrations can inhibit the effects of certain antagonist concentrations. In addition, based on the results of statistical tests between timolol and figs leaf extract, the value of the difference was not significant ($P > 0.05$), so it can be stated that both timolol and figs leaf extract have a potential vasodilator pharmacological effect on the smooth muscle of the guinea pig aortic aorta. Fig leaves ethanolic extract contains many secondary metabolite compounds. figs contain a similar phenolic profile composed by 3-O- and 5-O-caffeoylquinic acids, ferulic acid, quercetin-3-O-glucoside, quercetin-3-O-rutinoside, psoralen and bergapten, 3-O-Caffeoylquinic acid and quercetin-3-O-glucoside, oxalic, citric, malic, quinic, shikimic and fumaric acids (Oliveira *et al*., 2009). In the previous discussion, quercetin, which is one of the compounds contained in the ethanolic extract of fig leaves, shows a better affinity value compared to timolol (in silico) for β2 adrenergic receptors. These results provide the basis for researchers to predict the vasodilating effects that appear in the in vitro test due to the presence of these compounds. It does not rule out the presence of other secondary metabolites in the ethanolic extract of fig leaves, which may have a vasodilator effect on the guinea pig aorta. This shows the potential for fig leaves to be further developed as an antihypertensive agent. This result is also supported by the results of other research tests that utilize the fruit part of the fig plant.

Blood pressure was evaluated using a non-invasive technique after extract was given to normotensive Sprague Dawley rats in dosages of 250, 500, and 1000 mg/kg (p.o.). Three weeks of oral 10% glucose treatment in rats resulted in the development of hypertension. In normotensive and hypertensive rats administered with glucose, the hypotensive impact of the extract (1000 mg/kg p.o.) was investigated. The impact of crude extract on contraction force and heart rate was evaluated using Langendorff's isolated heart method. In both normal-blood pressure and hypertensive rats given with glucose, the 1000 mg/kg dose dramatically lowered blood pressure.

Table 4. Shift in pD2 values due to the effect of 10 and 50 µM Timolol administration

No	Treatment Groups	pD2 score± SEM	Emax (%) ± SEM
1	Epinephrine	7.19 ± 0.36	100 ± 0.00
2	Timolol 10 µM	5.92 ± 0.35	78.76 ± 5.08
3	Timolol 50 µM	5.08 ± 0.05	90.27 ± 1.70

SEM, standard error of the mean

The study on an isolated heart revealed that the extract had detrimental inotropic and chronotropic effects but was unable to stop the stimulatory effects of CaCl2 and adrenaline. The results of the current study suggest that the cardioinhibitory, antihypertensive, and diuretic effects of the fruit of *Ficus carica* may be attributed to the presence of flavonoids, phenols, and potassium through several pathways. According to experimental evidence, *Ficus carica* fruit may lower the incidence of coronary heart disease in hypertensive individuals, improve endurance in heart failure patients, and affect coronary ischemia and reperfusion injury (Alamgeer *et al*., 2017). In silico tests can be used to find out the interaction between a compound and the target cell protein as a receptor. Computing techniques using PyRx and PyMol that may be used to determine the pharmacophore of a chemical can be used to view the interaction of compounds with receptors. In case the compounds contained noni extracts, the virtual screening technique was employed to identify compounds that had the greatest potential to become medicines. The scopoletin chemicals in noni fruit and the protein NOS3 that is involved in controlling blood pressure in blood vessels were the targets of the insilico test. Compared to antihypertensive medications (captopril), the noni fruit extract considerably lowers blood pressure, according to the in silico test results. Scopoletin in noni fruit has a value of -7.6 and captopril alone has a value of 5.7 (Hijriansyah *et al*., 2020).

CONCLUSIONS

The administration of ethanolic extract of fig leaves (*Ficus carica* L.) with a concentration of 0.5 mg by volume of 200 µL gave a significantly different vasodilation effect to the guinea pig aorta induced by epinephrine. The ethanol extract of fig leaves (*Ficus carica* L.) shows a non-specific antagonism effect profile that causes a vasodilating effect on the guinea pig aorta induced by epinephrine. Quercetin compounds in silico have the ability to inhibit β2-adrenergic receptors better (quercetin affinity: -7.8 kcal/mol) than timolol compounds (timolol affinity: -6.4 kcal/mol). The result from the in vitro method showed that the ethanolic extract of fig leaf was able to shift the percentage curve of contraction response to the administration of epinephrine series by decreasing the pD2 value. A dose of 0.5 mg volume 200 µL is an effective dose because it gives a significant difference to the control group but does not show a significant difference in the comparison group. It is necessary to do further research on secondary metabolite compounds contained in the ethanolic extract of fig leaves as candidates for vasodilator agent compounds.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

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