Antihyperlipidemia and Antihyperglycemic Studies of *Arcangelisia flava* (L.) Merr. Phenolic Compound: Incorporation of In Vivo and In Silico Study at Molecular Level

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Abstract

Yellow root had been extensively studied its potential as herbal medicines in many pharmacologic activities due to its alkaloid content namely berberine. To date, there is no study of the yellow root as source of flavonoid to have anti-hyperglycemia and anti-hyperlipidemia effect. This present study focuses on flavonoid fraction being investigated its in vivo potential to reduce blood glucose level (165 mg/dL at 28 mg/kg of a fraction), peroxidase inhibition, and catalase activation. The extract was obtained from the fresh yellow root, using the re-maceration method. The evaluation of anti-hyperglycemic and anti-hyperlipidemia activity was done using the fraction form of yellow root. The isolation of phenolic compounds was done by radial chromatography (chromatotron). The isolated compound was then structurally and computationally characterized using NMR and molecular docking. The fraction reduced the activity of peroxidase (6.33%) at 14 mg/kg and increase catalase activity (15.74%) at 28 mg/kg of body weight of fraction, respectively. An effort to isolate the flavonoid in the fraction reveals a rutin-like structure, a flavonoid glycoside. This result guides the utilization of rutin and its aglycon as the model of lipid peroxidase inhibitor and catalase activator upon in silico molecular docking.

Keywords: Anti-hyperglycemia, anti-hyperlipidemia, molecular docking, phenolic compound, yellow root

Studi Antihiperlipidemia dan Antihiperglikemik Senyawa Fenolik dari *Arcangelicia flava* (L.) Merr. : Studi Kombinasi *In Vivo* dan *In Silico* pada Tingkat Molekular

Abstrak

Akar kuning telah banyak diteliti sebagai obat herbal dalam banyak kegiatan farmakologis karena kandungan alkaloidnya yaitu berberin. Sampai saat ini, tidak ada penelitian tentang kandungan flavonoid pada akar kuning yang memiliki efek anti-hiperglikemia dan anti-hiperlipidemia. Penelitian ini berfokus pada fraksi flavonoid yang diteliti potensinya secara in vivo untuk mengurangi kadar glukosa darah (165mg/dL pada fraksi 28mg/kg), penghambatan lipid peroksidase dan aktivasi katalase. Ekstrak diperoleh dari akar kuning segar, menggunakan metode remaserasi. Evaluasi aktivitas anti-hiperglikemia dan anti-hiperlipidemia di lakukan dengan menggunakan fraksi akar kuning. Isolasi senyawa fenolik dilakukan dengan kromatografi radial (kromatotron). Senyawa yang diisolasi kemudian dikarakterisasi secara structural dan komputasi dengan menggunakan NMR dan docking molekuler. Fraksi dapat mengurangi aktivitas peroksidase sebesar 6,33% pada 14 mg/kg dan meningkatkan aktivitas katalase sebesar 15,74% pada 28 mg/kg berat badan fraksi. Upaya untuk mengisolasi flavonoid dalam fraksi menunjukkan struktur seperti rutin, glikosida flavonoid. Oleh karena itu hasil ini memandu pemanfaatan rutin dan aglikonnya sebagai model lipid peroksida sedan penghambat katalase pada docking molekul in silico.

Kata Kunci: anti-hiperglikemia, anti-hiperlipidemia, docking molekuler, senyawa fenolik, akar kuning

1. Introduction

Most of the natural flavonoids are a phenolic compound have been well studied its potential as antioxidant, antibacterial and antiviral agent.[1] These actions were due to the flavonoid's capacity to scavenge the free radical atom or molecule, which causes oxidative stress into macromolecule building blocks such as protein, carbohydrate, lipid and nucleic acid leading to the metabolic disorders.[2] A metabolic disorder is indicated when the level of blood glucose and or lipid being increased, which are medically termed as hyperglycemia and hyperlipidemia, respectively. Two other indicators for the metabolic disorder are high blood pressure (hypertension) and obesity, which could complicate two previous disorders leading to a higher risk in diabetes and cardiovascular diseases.[3]

One of the tropical plants belonging to the family of Menispermaceae has been utilized for an herbal remedy is yellow root (*Arcangelisia flava* (L.) Merr.). Several studies on biological activities on this plant such as antibacterial, antifungal, antioxidant, anticancer have been conducted demonstrating its potential for natural drug source. The main chemical constituent had been reported in this species is an isoquinoline alkaloid, named as berberine. This compound has been known for having important biological activities such as choleretic, spasmolytic and hemostatic. Berberine was also studied its pharmacological activity as anti-diabetes due to its inhibition towards α-amylase as well as α-glucosidase, enzymatic targets in the pathogenesis of diabetes mellitus. Besides, other compound had been identified in the yellow root is furanoditerpene reported as an antifungal and antibacterial agent. Although berberine has been quantified its presence in the stem of the yellow root as 5% of dried powder, however, flavonoid was also identified in this plant.[4] Therefore, the next exploration of the yellow root is directed to the particular fraction, which is proposed to be the source of flavonoid broadly studied it's a good scaffold for drug design.

To date, there is no study of the yellow root as the source of flavonoid used as herbal medicine to treat metabolic disorders. Herewith, our present studies were carried out on phytochemical screening and flavonoid characterization, followed by in vivo studies of yellow root fraction wherein the flavonoid compound being identified as anti-hyperlipidemia and anti-hyperglycemia. The determination of mechanism on how flavonoid compound acts as anti-hyperlipidemia and anti-hyperglycemia was in silico approached using molecular docking against diverse protein targets which contribute to the pathogenesis of hyperglycemia and hyperlipidemic status.

2. Methods

2.1. Material

The instruments used are photometer (AbxPentra 400.), plethysmometer (Memmert), chromatotron (Ser.no. HR5239), and nuclear magnetic resonance (Bruker 400 MHz).

2.2. Methods

The yellow root (*Arcangelisia flava* (L.) Merr) was collected from the Indonesian Institute of Science, Bogor, Indonesia with no. of specimen: 2587/IPH.1.02/If.8/XI/2012. All reagents and solvents for extraction and phytochemical screening are analytical grade (Merck) and used without further purification. Thin Layer Chromatographic (TLC) Silica F_{254} (Merck) was used in chromatographic separation. Reagents for in vivo studies such as glibenclamide, alloxan, physiological NaCl, gemfibrozil, CMC-Na, purified cholesterol, propylthiouracil, high cholesterol dietary food, peroxide lipid kit, glucose kit, and lipid reagent kit. The white rats with Wistar strain were supplied by Mister Tiput (The house of Rats and Mice's Farming) with the specifications: male, age 2-3 months.

2.3. Procedure

2.3.1. Extraction

The fresh yellow root was sliced, covered with black fabric and dried up under sun's exposure. The dried material was pulverized in 177 microns (mesh no 80) of

particle size and then macerated with ethanol 80% at room temperature for 24 hours. The macerate was filtered and the solid phase was re-macerated using the new same solvent. The re-maceration was quadruplicated and the filtrates were combined to be concentrated under reduced pressure. The ethanol residue contamination was tested by adding a mixture of sulphanilic acid and sodium nitrite to the little amount of the concentrated extract. The extract was indicated to be free of ethanol residue contamination when there was no pink color after adding the reagent. The second test for the same purpose was carried out by heating a little amount of the extract after adding a mixture of acetic acid and sulphuric acid. The extract was confirmed to be free of ethanol when there was no banana-like smell.[5]

2.3.2. Phytochemical Screening
a. Alkaloid identification
The extract (500 mg) was dissolved into hydrochloric acid, and then filtered out. The filtrated was dropped using two reagents, i.e. Mayer and Dragendorff. The filtrate was confirmed containing alkaloid when it showed white precipitates and red precipitates upon Mayer reaction and Dragendorff reaction, respectively.[6]

b. Flavonoid identification
The extract (100 mg) was dissolved into ethanol, and then added with magnesium powder and amyl alcohol to be mixed up. The mixture was agitated and the flavonoid content was identified when the amyl alcohol phase showed a red color.[6]

c. Saponin identification
The extract (100 mg) with was dissolved into 20 mL of water and strongly shaken. The presence of saponin was detected when the solution formed a foam phase.[6]

d. Tanin identification
The extract (100 mg) was dropped with iron (III) chloride and the tannin was present when the solution showed a dark-green or dark-blue color.[7]

e. Quinone identification
The extract (100 mg) was dropped with a sodium hydroxide solution and shaken until form a red color indicating the presence of quinone.[7]

2.3.3. Chromatographic determination
The chromatographic method including thin layer chromatography (TLC) and gas chromatography (GC), was used to isolate and identify the presence of flavonoid in the extract either by qualitative or quantitative methods.[8]

2.3.4. Fractionation
The fractionation of the extract was gradually performed using three different solvents including n-hexane, water, and ethyl acetate. An amount of 10 g of extract was dissolved into 100 mL of the mixture of methanol-water (1:1) and then transferred into separating funnel. The mixture was then added with 100 mL of n-hexane and shaken until two layers being formed. The water phase was collected and warmed up at 60°C to remove the residue of methanol and then fractionated using ethyl acetate. The ethyl acetate phase was then collected and concentrated under reduced pressure for further isolation and purification.[9]

2.3.5. *In vivo* Studies
Anti-hyperglycemic evaluation
The anti-hyperglycemic activity was conducted by determining the blood glucose level after inducing the rat using fructose 1.8 g/kg of body weight, high-fat food (lard 15% and yolk of duck egg) for 50 days. On the 50th day, the total blood glucose was then measured using oral glucose tolerance test and the hypoglycemic effect of glibenclamide as the indicator that the rat had been conditioned as the type 2-diabetes mellitus subject. The rats were divided into five group, i.e. : positive control (orally treated with metformin 126 mg/kg of body weight), negative control (orally treated with 2.5 mL of CMC-Na 0.5%), treatment 1, 2 and 3, which were orally treated with ethyl acetate fraction 7 mg/kg, 14 mg/kg and 28 mg/kg of

body weight, respectively.

2.3.6. Anti-hyperlipidemia evaluation

The anti-hyperlipidemia activity was carried out by determining lipid peroxidase activity and its catalase after inducing the rat using propylthiouracil 10 mg/kg of body weight, the yolk of common quail 1.50 mL, and lard 10% for 14 days. On the 15th day, the total blood cholesterol was then measured as hyperlipidemia. The rats were divided into five group, i. e. : positive control (orally treated with Vitamin E 7.2 UI/200 g of body weight), negative control (orally treated with 2.5 of CMC-Na 0.2%), treatment 1, 2 and 3, which were orally treated with ethyl acetate fraction as done in the previous anti-hyperglycemia activity study. The results were observed as lipid peroxidase inhibition and catalase activity activation after 7 days of fraction exposure.

2.3.7. Isolation of Phenolic Compounds

The isolation of phenolic compounds was carried out using radial chromatography (chromatogram). The eluent used is methanol 100%. An amount of 100-200 g of silica was used as the stationary phase and packed inside the radial column, whereas the mobile phase was flowed down to elute the phenolic compounds using gradient technique. The isolated compound was then structurally characterized using NMR.[10]

2.3.8. *In silico* Studies

Two crystal structures from protein data bank with PDBID 2F8A and 1QQW with regard to human glutathione peroxidase and human catalase, respectively was used as the protein models. According to the published paper, these two enzymes were studied as a protein with an unbound ligand, thereby the active site was located based on the searching of the binding site using Q-site finder. In this study, we utilized the native bound ligand's binding site of both proteins, i. e., heme and malonic acid for 1QQW and 2F8A, respectively. The proteins were prepared using AutoDockTools 1.5.6 by adding polar hydrogen and Kollman charge. The control

docking of heme and malonic acid was carried out to the center of the ligand with a number of grid points = 40, 40, 40 and 50, 50, 50 for 2F8A and 1QQW, respectively, with its spacing was set to 0.375. The grid center of ligand was managed as followed: x = -8.467, y = 27.236, z = 32.091 for 28FA and x = 7.351, y = 41.228, z = 85.75 for 1QQW. The searching parameters were set to the default values (Population size =150, the maximum number of evals = 2500000, maximum of generations = 27000, the maximum number of top individuals that automaticalysurfives = 1). The number of GA run was set to 250. Docking parameters such as random number generation, energy parameters, and step size were also set to the default values. The results were analyzed by checking the RMSD values, ligand-protein interactions, free energy of binding (FEB) as well as the number of conformations exist in a population cluster. For the subsequent molecular docking, rutin and quercetin were sketched using ACD Chemsketch (www.acdlabs.com) and energetically optimized using Marvin Sketch (www.chemaxon.com). The visualization of ligand-protein interaction was done using Discovery Studio 3.5 (www.accelrys.com).

3. Results

3.1. Extraction and Fractionation

The phytochemical screening indicated the presence of alkaloid, flavonoid, saponin, tannin, and quinine.

In the basic-TLC method, a common basic flavonoid; rutin, was detected its presence in the ethyl acetate fractions as shown by the same Rf between ethyl acetate fraction and rutin spots under UV_{254} illumination and coloring agent.

3.2. *In vivo* Studies

Anti-hyperglycemic activity

The antihyperglycemic activity was demonstrated by reducing the blood glucose level in the treatment groups (see Figure 1).

3.3. Anti-hyperlipidemic activity

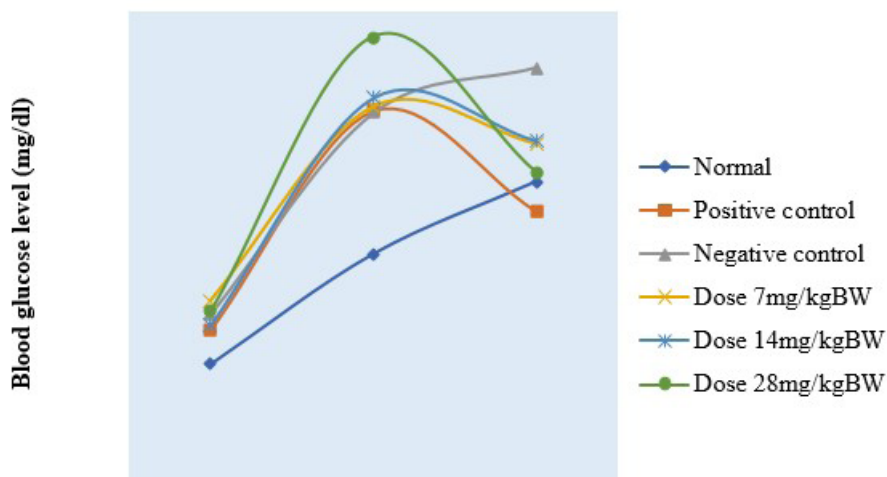The antihyperglycemic activity was also demonstrated by the treatment groups in

Figure 1. Calibration curve The graph plotting blood glucose level upon periodic treatment of yellow root's ethyl acetate fraction doses for formaldehyde standards

inhibiting the lipid peroxidase and catalase activity. Flavonoid is suggested acting as lipid peroxidase inhibitor by scavenging the peroxide radical. This radical production attacks the cell membrane by reacting with a polyunsaturated fatty acid.

3.4. Characterization of a single compound

in ethyl acetate fraction

As presented in Table 1, the pattern of chemical shift and proton multiplicity resembled without being identical with rutin.

3.5. *In silico* study

Further consideration to involving quercetin as the second ligand to be docked

Table 1. The comparison between 1H-NMR profile of rutin and isolate

Rutin	Isolate
12.27 (1H, s, 5-OH)	Not observed
10.51 (1H, brs, 7-OH)	10.30 (1H, brs)
9.35 (1H, brs, 4'-OH)	8.46 (1H, brs)
8.81 (1H, brs, 3'-OH)	8.19 (1H, brs)
7.67 (1H, d, J = 2.1 Hz, H-16)	7.90 (1H, d)
7.62(1H, dd, J = 2.4, 2.4 Hz, H-15)	7.80 (1H, dd)
6.90 (1H, d, J = 8.4 Hz, H-12)	7.10 (1H, d)
6.40 (1H, d, J = 2.1 Hz, H-8)	Not observed
6.23 (1H, d, J = 2.1 Hz, H-6)	6.08 (1H, s)
5.38 (1H, s, aliphatic-OH)	Not observed
5.09 (1H, d, J = 7.2 Hz)	4.27 (d)
4.90 (2H, s, aliphatic-OH)	4.05 (1H, s)
4.52 (1H, s, aliphatic-OH)	Not observed
4.24 (2H, s, - aliphatic-OH)	Not observed
4.08 (1H, q, J = 7.2 Hz, H-5''')	3.90 (1H, q)
3.77 (1H, d, J = 10.8 Hz)	3.78 (1H, s)
3.64 (1H, s)	3.55 (1H, s)
3.34 (9H, brs, aliphatic-Hs)	3.38 (brs)
2.02 (1H, s)	Not observed
1.24 (1H, t)	1.25 (s)
1.12 (3H, d, J = 6.3 Hz, -CH3)	0.86 (m)

those enzymes was associated with the possibility of flavonoid glycoside to be hydrolyzed into its aglycone and glycone during pharmacokinetic steps in in vivo study. Table 2 presented the docking results which were evaluated by its free energy of binding (ΔG_{bind}), hydrogen bonds (H-bonds) interaction with amino acid residues and H-bonds distances.

The docking pose of heme and malonic acid can be seen in Figure 2. In human catalase, rutin interacts with ARG72, HIS75, GLY147, ILE332, ARG354, ALA357, TYR358, HIS362, and ARG365 (Figure 3) in combined with van der Waals and desolvation energy contributed approximately -13.13 kcal/

mol. Quercetin as the metabolite of rutin also showed binding interactions with catalase as well as peroxidase (Figure 4).

4. Discussion

4.1. Extraction and Fractionation

Re-maceration was selected as the method of extraction to avoid the chemical decomposition upon method utilizing heat such as soxhlet. The product was characterized as a sticky dark brown extract with 14.41% of yield.

Therefore, the ethyl acetate fraction was selected as the sample for further in vivo experiment and followed by structural characterization for a single compound.

Table 2. The docking results of rutin and quercetin as the model for peroxidase and catalase inhibitors.

| Ligand	Peroxidase (2F8A)			Catalase (1QQW)		
	ΔG_{bind}	H-bonds	distances	ΔG_{bind}	H-bonds	distances
Heme				-19.69 (RMSD 1.16 Å)	ARG72	1.56
					ARG112	1.81
					SER114	2.03
					PHE334	2.40
					TYR358	1.88
					HIS362	2.13
					ARG365	2.15
Malonic acid	-6.04 (RMSD 1.82 Å)	THR143	1.87			
		ARG179	1.78			
		ARG180	1.83			
Rutin	-3.66	GLY48	1.68	-9.39	ARG72	2.04
		GLN82	1.97		HIS75	2.22
		THR143	2.38		GLY147	1.67
		ASP144	2.18		ILE332	1.68
		TRP160	2.16		ARG354	2.38
		ARG179	2.10		ALA357	2.25
		ARG180	1.73		TYR358	1.89
					HIS362	2.45
					ARG365	2.36
Quercetin	-5.05	GLY48	1.73	-8.89	VAL73	2.16
		GLN82	2.03		ARG112	1.98
		ASP144	1.91		SER114	2.28
		TRP160	2.24		HIS362	2.18
		ARG179	2.38		ARG365	2.06
		ARG180	1.89		PHE334	2.22
					THR361	1.95
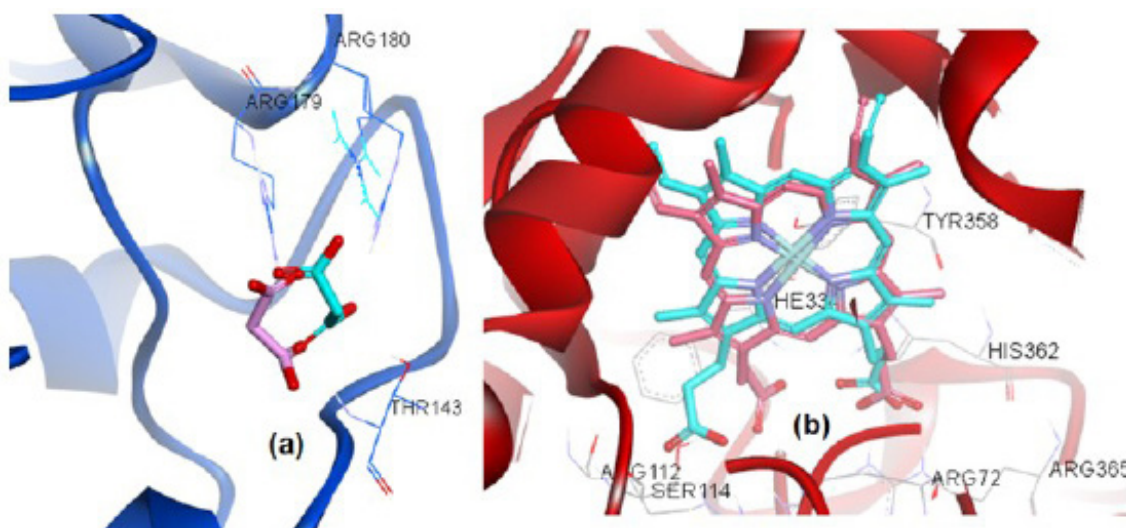
Figure 2. The superposition of initial and control docking pose of (a) malonic acid in human glutathione peroxidase (PDBID 2F8A) and (b) heme in human catalase (PDBID 1QQW). The initial pose was colored by cyan, whereas the control docking pose was pink. Discovery Studio 3.5 (www.accelrys.com) was used as the visualizer.

4.2. *In vivo* Studies

4.2.1. Anti-hyperglycemic activity

At the initial day, the blood glucose level of the treatment group is still maintained as the negative control showing the blood glucose level as 89.8 mg/dL. There was only treatment 2 showing the blood glucose reduction down to 83.8 mg/dL, which is close to the positive control. Two other treatments (1 and 3) are slightly higher than the negative control but these values were not significantly different as shown by its ANOVA test. On day 51, the blood glucose was re-measured and the results showed no blood glucose reduction.

The treatment 2 and 3 increased the blood glucose but it was still considered a slight. The blood glucose was finally re-measured on day 58 demonstrating the decline values along with the incline doses of ethyl acetate fraction indicating it's in vivo antihyperglycemic effect. The lowest blood glucose level (165 mg/dL) was achieved by the highest dose of fraction (28 mg/kg of body weight), which is close to the positive control (144.28 mg/dL).[11]

As well studied, how the phenolic compounds especially flavonoid reduce the blood glucose level, involves two mechanisms. The first mechanism is via its
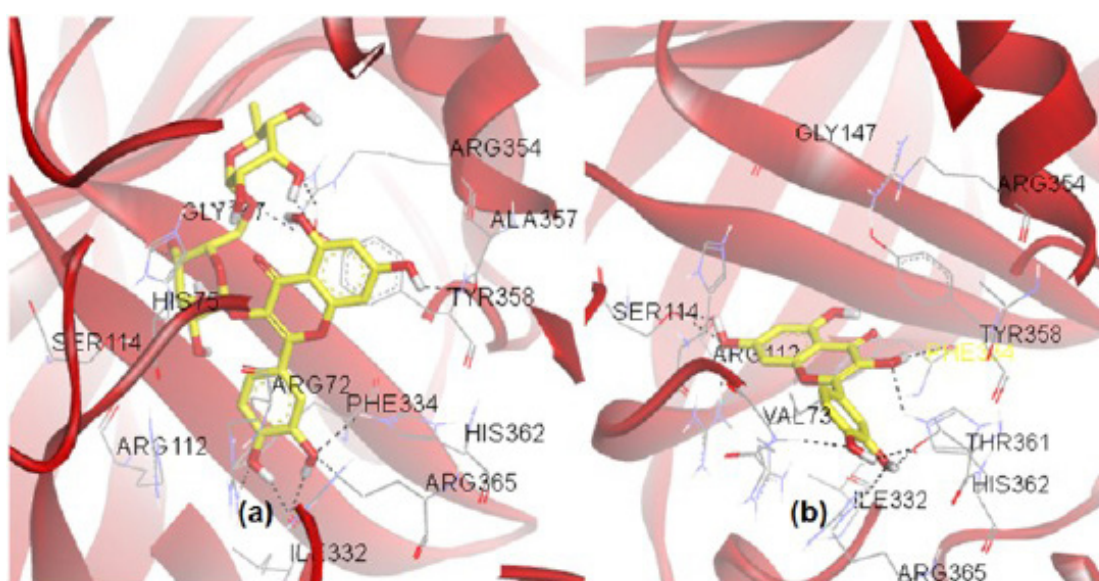

Figure 3. The docking pose of (a) rutin and (b) quercetin in human glutathione peroxidase (PDBID 2F8A). Discovery Studio 3.5 (www.accelrys.com) was used as the visualizer.
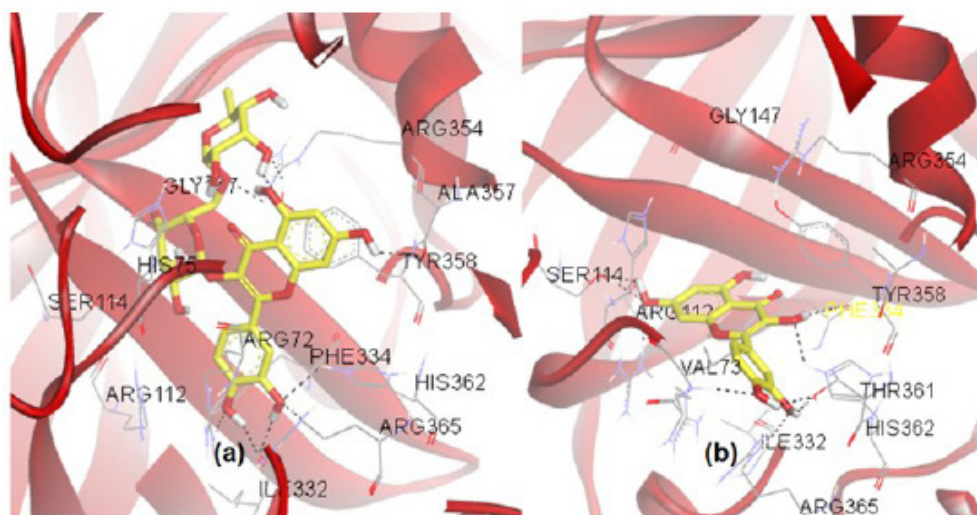
Figure 4. The docking pose of (a) rutin and (b) quercetin in human catalase (PDBID 2F8A). Discovery Studio 3.5 (www.accelrys.com) was used as the visualizer.

intra pancreatic antioxidant activity, thus it prevents the pancreatic beta cell damage.[12] The second one is via extrapancreatic mechanism by inhibiting α-glucosidase activity leading to the reduction of glucose absorption.[13] Beside, flavonoid was also known to increase the intestinal mucous layer, thus the glucose intake into the intestine will be inhibited. Another mechanism worked on protein kinase C modulation causing the increase of GLUT 4 activity.[14] The presence of flavonoid in blood will significantly increase the solubility of glucose in the blood advancing the glucose excretion via urine.[15]

4.2.2. Anti-hyperlipidemic activity

In general, the activity of lipid peroxidase was decreased along with the increase of the fraction dose although there was a slight fluctuation due to the uncontrolled distracting factors. In contrast, the activity of catalase was increased up in line with the increasing dose. The activity of lipid peroxidase was reduced down to 6.33 % at the moderate dose (14 mg/kg of body weight), which is close to the positive control (3.73%), whereas the activity of catalase was significantly activated at the maximum dose (28 mg/kg of body weight) up to 15.74%.[16] These results indicate that the yellow root ethyl acetate fraction could be the source of antihyperlipidemic agent.

Antioxidant plays a role in preventing the auto-oxidation, thus it distracts the free radical propagation via a few mechanisms,

i.e., blocking the chemical that initiates the peroxidation, forming chelate with metal to stop the peroxidase catalysis, preventing the entry of $\cdot O_2^-$ into the reaction, breaking down the auto-oxidation reaction chain and reducing the concentration of $\cdot O_2^{-}$[17]. A structure-activity relationship study suggests that blocking of the free radical chain was affected by the presence of aromatic rings in flavonoid via hydrogen radical donation to the free radical. This reaction forms an intermediate state, which is stable and does not cause the surrounding cell damage18. The activity of the catalase is also important due to its activity in breaking down the peroxide lipid into water and oxygen, therefore reducing the lipid peroxidase level in blood.[19]

4.3. Characterization of a single compound in ethyl acetate fraction

More exploration was managed on the isolation of single compound which could contribute to the activity of flavonoid fraction as antihyperglycemic and antihyperlipidemic. With regard to the chromatogram showing a single peak, the isolate was then identified using 1H-NMR. As results, the spectrum showed crowded signals at 3-4 ppm which indicates sugar protons as commonly presented in flavonoid glycoside. This incorporates with the preliminary phytochemical screening in which one of the spots was in line with rutin, a flavonoid glycoside which was used as the reference. Further characterization was

concerned at 6-7 ppm which also showed a signal multiplicity indicating the substituted pattern of aromatic proton associating with the presence of the flavonoid aglycon. The presence of hydroxyl proton was also detected supporting the prediction that the compound could be flavonoid glycoside. Unfortunately, the ^{13}C-NMR did not show any strong signal regarding to the very limited sample. As well practiced, ^{13}C-NMR needs a high concentration of sample due to its low magnetic moment as well as abundance in nature. We also could not observe the molecular weight of the isolate via mass spectroscopy due to the limited simple, therefore the isolate only could be suggested as flavonoid glycoside without knowing the actual structure. However, we tried to compare the ^{1}H-NMR profile of our isolate with rutin as reported in a published article. As presented in Table 6, the pattern of chemical shift and proton multiplicity resembled without being identical with rutin. The isolate was about 71.23% of similarity with rutin by means six protons were missing in the isolate. Apparently, seven different proton signals (3.97, s; 5.2, m; 6.80, s; 7.79, dd; 7.51, s; 7.43, s; 7.34, s) were observed indicating the different compound between the isolate and rutin. Further study was required to confirm the real structure of the isolate using 2D-NMR, MS as well as X-Ray crystallography.

4.4. *In silico* study

With aim to elucidate the molecular mechanism on how the proposed compound in the flavonoid fraction being active as antihyperlipidemic. Fortunately, the antihyperlipidemic in this study was determined according to the lipid peroxidase inhibition as well as catalase activation. Therefore, the binding mode of the proposed compound could be suggested via in silico study using molecular docking. The proposed compound used as the ligand was supposed to be the isolate. Unfortunately, we could not confirm the real structure of the isolate as discussed in the previous section. However, the isolate has 71.23% of similarity to rutin, thus, we decide to utilize rutin as the model of

the proposed compound to be docked to the corresponding enzymes. Further consideration to involving quercetin as the second ligand to be docked those enzymes was associated with the possibility of flavonoid glycoside to be hydrolyzed into its aglycone and glycone during pharmacokinetic steps in in vivo study as illustrated in Figure 4. Table 7 presented the docking results which were evaluated by its free energy of binding (ΔG_{bind}), hydrogen bonds (H-bonds) interaction with amino acid residues and H-bonds distances.

Control docking was performed to validate the parameters used in the docking protocol and showed the acceptable RMSD (< 2.0 Å) describing that re-docked ligand was located near to its initial pose. Heme was re-docked in a high affinity to human catalase with ΔG_{bind} = -19.69 kcal/mol. This affinity was majorly contributed by a number of hydrogen bond interactions with ARG72, ARG112, SER114, PHE334, TYR358, HIS362 and ARG365 in combined with van der Waals interaction and desolvation (Energy -18.84 kcal/mol). Beside, electrostatic interactions were observed between heme with ARG112 and ARG365 by contributing energy -2.64 kcal/mol. The final total internal energy (-0.02 kcal/mol), torsional free energy (+1.79 kcal/mol) and unbound system's energy (-0.02 kcal/mol) gave less contribution in the ligand's binding. Likewise, malonic acid showed binding into human glutathione peroxidase with acceptable ΔG_{bind} (-6.04 kcal/mol). This was particularly contributed by hydrogen bond interactions with THR143, ARG179, and ARG180 in combined with van der Waals interaction and desolvation energy (-2.88 kcal/mol). Two electrostatic interactions between carboxylate ion of malonic acid with ARG179 and ARG180 makes a major contribution to ligand's binding with energy -3.76 kcal/mol. There have been final total internal energy (+1.13 kcal/mol), torsional free energy (+0.60 kcal/mol) and unbound system's energy (+1.13 kcal/mol) made up the ΔG_{bind} of malonic acid = -19.69 kcal/mol.

Enzyme inhibitor/activator should mimic the binding mode of the reference ligand/substrate but at the same time, they

generate a new binding mode with the enzyme. In this study, rutin was capable to dock at the same binding site with heme in human catalase as well as malonic acid in human glutathione peroxidase. Although the ΔG_{bind} of rutin was significantly higher than heme and malonic acid associating with its lower affinity toward enzymes, however, they demonstrated similar hydrogen bond interactions with both proteins. There was almost no electrostatic contribution (-0.42 kcal/mol), whereas high torsions in glycoside moiety contributed to positive energy (+4.77 kcal/mol) value resulting in a higher ΔG_{bind}. However, there were some new binding region founds in this ligand such as HIS75, GLY147, ILE332 and ALA37 promoting the chance of rutin as human catalase activator. Likewise, in glutathione peroxidase, rutin could cover all binding modes performed by malonic acid (THR143, ARG179, ARG180) due to it's a bigger molecule. The new binding region was found regarding interaction with GLY48, GLN82, ASP144, and TRP160 reflecting its insight activity as peroxidase inhibitor.

The ΔG_{bind} of this ligand is significantly lower than its parent molecule. This was caused by the disconnected glycoside moiety to the flavonoid associating with its lower torsion energy. These were favorable conformations which could be confirmed from its less cluster number in conformations, therefore it could be a good lead structure for further design as catalase and peroxidase inhibitor.

As antihyperglycemics, we did not study the binding prediction of rutin and quercetin as the enzyme inhibition assay was not provided. However, flavonoid has been extensively studying as antidiabetes via α-glucosidase inhibition. Therefore, further works could include the investigation of a flavonoid extract of yellow root on α-glucosidase inhibition assay to study the molecular mechanism on how flavonoid extract of yellow root acts as antihyperglycemics.

5.   Conclusion

Ethyl acetate fraction of yellow root was qualitatively determined its flavonoid presence based on chromatographic as well as spectroscopic methods. The fraction was then investigated its potential for antihyperglycemic and antihyperlipidemic in rats demonstrating significant effect towards a reduced blood glucose level as well as lipid peroxidase inhibition and also catalase activation, respectively. Further characterization of a single compound isolated from the ethyl acetate fraction suggested a rutin-like flavonoid glycoside due to their proton environment similarity especially in the pattern of rutinoside (glycoside) region (3-4 ppm) and aromatic region of quercetin as the aglycon (6-7 ppm) using ^{1}H-NMR spectroscopy. Although the compound could not be purely characterized, however, those similar proton environments with rutin guided us to utilize rutin and its aglycon (quercetin) as the model of human catalase and glutathione inhibitors associating with its activity as antihyperlipidemic. Either rutin or quercetin was able to dock one of the pocket sites that naturally bound to the native ligand revealing an insight molecular mechanism as antihyperlipidemic. In corresponding with this, flavonoid had been studied its potential as anti-diabetes (antihyperglycemic) via α-glucosidase inhibition. Future studies on yellow root would be managed to purely characterize the glycoside flavonoid in ethyl acetate fraction followed by in vitro α-glucosidase inhibition assay to look down its insight mechanism as antihyperglycemic.

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