Computational study of the potential of phenolic acids berries as an inhibitor of aldose reductase for diabetes mellitus treatment

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Abstract. Berries are known to contain bioactive compounds that can function physiologically to optimise health status. One type of bioactive compound found in berries is phenolic acid. Fruits are known to have activities to inhibit aldose reductase (AR). Inhibition of AR can control microvascular complications of diabetes mellitus, such as blindness, nephropathy and neuropathy. But some AR inhibitors show side effects due to cross-reactions with analogous enzymes, so the purpose of this study is to predict the ability of phenolic acids berries binding to AR and predict selectivity of berries phenolic acids as AR inhibitor through in silico studies. The inhibition activity of twelve berry phenolic acids on AR was analysed using in silico and compared with epalrestat as a commercial AR inhibitor. The phenolic acids were docked to AR using Autodock Vina in PyRx 0.8. 3D molecular interactions visualized using PyMOL were then analyzed with LigPlot+. Chlorogenic acid and neochlorogenic acid show binding energy are higher affinity (-8.0 and -8.3 kcal/mol) than binding energy of epalrestat as a commercial AR inhibitor. The orientation of chlorogenic acid and neochlorogenic acid with the active side of AR showed that the binding of two compounds on the active side of AR occupies the same position as the binding position of epalrestat. Analysis of the interaction of the chlorogenic acid and neochlorogenic acid with AR indicates binding occurs in the pocket specificity of the active side of AR, so indicates that the two compounds have the potential as AR inhibitors.

1. Introduction
Berries are known to contain bioactive compounds, polyphenols, which can functionally optimise health status. Berry polyphenols contain, especially flavonoids (anthocyanins and flavonols), phenolic acids stilbene, lignan and tannins [1]. Some berries are rich in phenolic acids, such as rowanberry (103 mg/100 g), blueberry (85 mg/100 g), chokeberry (96 mg/100 g), Saskatoon berry (59 mg/100 g), sweet rowberry (75 mg/100 g) [2] and D Don berries (2268.1 mg/kg DW) [3]. The major phenolic acids in berries are derivatives of cinnamic and benzoic acid, such as p-coumaric, caffeic acid, ferulic acid,
sinapic acid and cinnamic acid (members of hydroxycinnamic acid) and gallic acid, syringic acid, vanillic acid, protocatechuic acid and p-hydroxybenzoic acid (member of hydroxybenzoic acid). Don berries also contain chlorogenic acid and neochlorogenic acid [3]. Study in vitro showed that chlorogenic acid and tannic acid had a strong inhibition effect at AR [4].

Aldose reductase (AR; AKR1B1; EC 1.1.1.21) is the main enzyme of the aldo-keto reductase family that catalyzes the reduction of excess glucose in a hyperglycemic state into sorbitol via the polyol pathway with the presence of NADPH as a cofactor. Sorbitol causes poor membrane penetration and metabolic causes of various diabetes complications, such as neuropathy, nephropathy, retinopathy and cataract formation [5]. Studies in animal and human show inhibition of aldose reductase acts to prevent or slow the development of DM-related pathologies [6]. However, many AR inhibitors are known to cross-react with other (not selective) analogous enzymes, such as sorbinil and tolrestat which also inhibits aldehyde reductase [7]. So it is necessary to look for inhibitors that are selective for AR, especially from natural sources.

In silico studies in recent years have been developed for predicting the ability of active compounds that have biological effects were then adapted to predictive the ability of active compounds in natural materials to be used as functional foods [8]. Molecular docking is one of the widely used in silico methods. The principle of this technique is to predict the ability of active compounds (ligands) to bind to target proteins to form stable complexes. The ability of a ligand to bind to the active site of the receptor is then tested to assess the strength of activation or strength of the resistance [9]. Therefore, the purpose of this study is to predict the ability of berries phenolic acids to be AR inhibitors through in silico studies. So that the potential for the phenolic acids inhibitory activity can be determined to prevent complications due to chronic diabetes.

2. Materials and Method
Analytical-Descriptive experimental model was used to assess the ability of the phenolic acids of berries and to measure the binding affinity of the phenolic acids of berries to the AR protein. Berries phenolic acids, as ligands, are collected from a literature survey [3, 4], namely p-coumaric acid (4-hydroxyxycinnamic acid), caffeic acid (3,4-dihydroxycinnamic acid), chlorogenic acid (3-O-cafeoylquinic acid), neochlorogenic acid (trans-5-O-caffeoyl-D-quinic acid), ferulic acid (4-hydroxy-3-methoxycinnamic acid), sinapic acid (3,5-Dimethoxy-4-hydroxycinnamic acid) and cinnamic acid (trans-cinnamic acid) (members of hydroxycinnamic acids) and gallic acid (3,4,5-trihydroxybenzoic acid), syringic acid (4-hydroxy-3-methoxybenzoic acid), vanillic acid (3,4-dihydroxybenzoic acid), protocatechuic acid (4-hydroxy-3,5-dimethoxybenzoic acid) and p-hydroxybenzoic acid (4-hydroxybenzoic acid) (member of hydroxybenzoic acids). The berries phenolic acids were obtained with a recorded CID from the PubChem (https://www.ncbi.nlm.nih.gov) server (Table 2).

The three-dimensional structure of each phenolic acids is obtained from the database of the PubChem compound (https://www.ncbi.nlm.nih.gov) in SDF format. Twelve phenolic acid structures were minimized with Open Babel which was integrated with PyRx 0.8. While the three-dimensional structure of AR which is conjugated with NADP+ and epalrestat, as a receptor, is obtained from protein data bank (http://www.rcsb.org/pdb/home/home.do) database with ID 4JIR. Modification of AR (eliminating α-D-G-6-P and water molecules) was carried out using AutodockTool integrated with PyRx 0.8 [10]

Protein-ligand process docking and virtual screening were performed using Autodock Vina in PyRx 0.8 open source software (http://pyrx.sourceforge.net) [10]. Twelve phenolic acids of berries are subjects (ligands) that bind to AR as the target protein. The docking method is used to evaluate binding affinity. Docking is done by regulating proteins (receptors) as rigid molecules and ligands as flexible molecules on the active side. Result docking was carried out using PyMOL [11].

The interaction between phenolic acid and AR and hydrophobic bonds and hydrogen bonds formed in the complex are visualized by LigPlot [12]. The inhibitory potential can be seen from the interaction of each of the phenolic acids gave to the amino acid residues which comprise the active side of AR. The active side of AR has three pocket, namely anion binding pocket, hydrophobic pocket
and specificity pocket. The selectivity of the phenolic acid gave to AR is determined by the interaction of phenolic acid with the amino acid residues that make up the specificity pocket [13].

3. Results and Discussion

3.1. Analysis of potency berries phenolic acids as aldose reductase inhibitor with in silico method

Phenolic acid is classified in non-flavonoid polyphenols which have one carboxyl group that is bound to the benzene ring (phenolcarboxylic acid). Based on its structure, phenolic acids are divided into two classes, namely benzoic acid derivatives and cinnamic acid derivatives (Table 1). The second difference in the derivative of this phenolic acid compound lies in the pattern of hydroxylation and its aromatic ring methoxylation [14].

| Table 1. Structure of berries phenolic acids |
|---------------------------------------------|
| Hydroxybenzoic acid derivative | R | R1 | R2 | R3 | R4 | R5 |
| Gallic acid | H | OH | OH | OH | OH | H |
| Vanillic acid | H | H | OCH3 | OH | H | H |
| Protocatechuic acid | H | H | OH | OH | H | H |
| Syringic acid | H | H | OCH3 | OH | OCH3 | H |
| p-hydroxybenzoic acid | H | H | H | OH | H | H |

| Hydroxycinnamic acid derivative | |
|---------------------------------|-----------------------------------|
| Cinnamic acid | H | H | H | H | H | H |
| p-coumaric acid | H | H | H | OH | H | H |
| Caffeic acid | H | H | OH | OH | H | H |
| Ferulic acid | H | H | OCH3 | OH | H | H |
| Sinapic acid | H | H | OCH3 | OH | OCH3 | H |
| Chlorogenic acid | QA* | H | H | OH | OH | H |
| Neochlorogenic acid | QA* | H | H | OH | OH | H |

Notes: QA* = quinic acid

The docking results between AR and twelve phenolic acids contained in the berries showed that the derivative of cinnamic acid have higher free binding energy compared to the benzoic acid derivative. However, only two berry phenolic acids, namely chlorogenic acid and neochlorogenic acid have higher free binding energy, -8.0 and -8.1 kcal/mol, respectively, than epalrestat (-7.9 kcal/mol) as commercial AR inhibitors (Table 2). Free binding energy is a measure of the ability of the active compound to bind to the target protein. In every spontaneous process, protein-ligand binding only occurs when free energy changes (ΔG) of the system are negative when the system reaches a constant equilibrium of pressure and temperature. Because the protein-ligand association extension is defined by the magnitude of the Gibbs free energy (ΔG) is negative, it can be assumed that the Gibbs free energy (ΔG) determines the stability of any given protein-ligand complex, or, alternatively, the binding affinity of the ligand to the accepter [15]. Based on that, chlorogenic acid and neochlorogenic acid predicted spontaneous bond to active side of AR form stable protein-ligand complex compared to other phenolic acids.

A further analysis evaluating the orientation of chlorogenic acid and neochlorogenic acid with the active side of AR showed that the binding of two compounds on the active side of AR occupies the same position as the binding position of commercial AR inhibitors (epalrestat) (Figure 1). This indicates that the two compounds have the potential as AR inhibitors.
3.2. Analysis of interaction and selectivity between ligand phenolic acids berries and aldose reductase with in silico method

The interaction of most inhibitors and AR on the active side was carried out after NADPH binds to AR and induces conformational changes, namely the formation of a "closed" conformation of the "seat belt" loop. At least there are three binding pockets on the active side of AR. The first is anion binding pocket, usually occupied by an anion ligand head. Anion binding pocket consists of residues of Tyr48, His110, Trp20 and Trp111 as well as the positively charged nicotinamide portion of the NADP$^+$ cofactor. The second is the hydrophobic pocket, known as pocket specificity and is limited by Leu300, Cys298, Cys303, Trp111 and Phe122 residues. Specific pocket display high flexibility and the residues that arrange this pocket are not conserved in other aldo-keto reductases, such as aldehyde reductase. The third is another hydrophobic pocket formed on Trp20 and Trp219 [13].

Chlorogenic acid is predicted to interact with Aldose Reductase-NADP$^+$ by forming six hydrogen bonds and eight hydrophobic interactions (Table 2). Chlorogenic acid ligands interact with the active side of AR through anion binding pocket, specificity pocket and hydrophobic pocket. The carboxyl groups and hydroxyl groups in the aromatic quinic acid rings interact respectively with His110 polar residue, Trp111 and Nap404 aromatic residues in the anion binding pocket and with Cys298 polar residues at specific points through hydrogen bonds. Interactions in pocket specificity also occur with Phe122 aromatic residues and Leu300 apolar residues through hydrophobic interactions. Another hydrophobic pocked is also formed on Trp20 and Trp219 aromatic residues. In addition, the interaction between chlorogenic acid and AR was also formed by hydroxyl groups on aromatic caffeic acid rings with Val297 through hydrogen bonds and hydrophobic interactions on Trp79, Ala299 and Leu301. The presence of interactions on residues of Trp111, Phe122, Cys298 and Leu300 [13] shows that chlorogenic acid has a high selectivity for AR. This is in line with the in vitro study of AR inhibitory activity by several phenolic acids which showed chlorogenic acid had a strong inhibitory effect on the activity of AR [4].

| Ligands                        | CID       | Binding affinity (kcal/mol) on aldose reductase |
|-------------------------------|-----------|-------------------------------------------------|
| Hydroxycinnamic acid derivative |           |                                                 |
| Cinnamic acid                 | 444539    | -6.1                                            |
| $p$-coumaric acid             | 637542    | -6.1                                            |
| Caffeic acid                  | 689043    | -6.5                                            |
| Ferulic acid                  | 445858    | -6.4                                            |
| Sinapic acid                  | 637775    | -6.6                                            |
| Chlorogenic acid              | 1794427   | **-8.0**                                        |
| Neochlorogenic acid           | 5280633   | **-8.3**                                        |
| Hydroxybenzoic acid derivative |           |                                                 |
| Gallic acid                   | 370       | -5.7                                            |
| Vanillic acid                 | 8468      | -5.6                                            |
| Protocatechuic acid           | 72        | -5.7                                            |
| Syringic acid                 | 10742     | -5.8                                            |
| $p$-hydroxybenzoic acid       | 135       | -5.5                                            |
| Reference ligand              | 1549120   | **-7.9**                                        |

Table 2. Binding energy affinity phenolic acids berries on aldose reductase
Figure 1. The binding orientation of chlorogenic acid (green), neochlorogenic acid (yellow), epalrestat (cyan) on aldose reductase and NADP⁺ complexes (white and blue)

The carboxyl group and the hydroxyl group of the aromatic quinic acid ring and the hydroxyl group aromatic caffeic acid ring in neochlorogenic acid play an important role in the interaction with the amino acid residues on the active side of AR through seven hydrogen bonds and five hydrophobic interactions (Table 3). In anion binding pocked, interactions with Trp20, Tyr48 aromatic residues occur, polar residues Trp111 His110 and Nap404 through hydrogen bonds. Whereas in pocked specificity, interactions occur in Phe122, Trp219 aromatic residues, Cys298 polar residues and Leu300 apolar residues with hydrophobic interactions. In addition, hydrogen bonds in neochlorogenic acids are also formed with Val297 and Ala299 residues. Hydrophobic interactions also occur with Leu301 residues. The high specificity of neochlorogenic acid for AR is indicated by interactions at residues Phe122, Trp219, Cys298 and Leu300 [13].

Table 3. Interaction of chlorogenic acid and neochlorogenic acid of berries and comparative ligands (epalrestat) with aldose reductase

| Ligand          | Amino acid residues                                      |
|-----------------|----------------------------------------------------------|
| Chlorogenic acid| Hydrophobic interaction: Trp20, Val47, Trp79, Phe122, Trp219, Ala299, Leu300, Leu301 |
|                 | Hydrogen bonding: His110, Trp111, Val297, Cys298, Nap404 |
| Neochlorogenic acid| Hydrophobic interaction: Phe122, Trp219, Cys298, Leu300, Leu301 |
|                 | Hydrogen bonding: Trp20, Tyr48, His110, Trp111, Val297, Ala299, Nap404 |
| Epalrestat      | Hydrophobic interaction: Trp20, Val47, Tyr48, His110, Trp111, Trp219, Leu301, Nap404 |
|                 | Hydrogen bonding: Ala299, Leu300                          |

4. Conclusions
Molecular results of docking of phenolic acid berries on AR show that cinnamic acid derivative phenolic acids have higher binding affinity than benzoic acid derivative phenolic acids. Two berry phenolic acids derived from cinnamic acid, namely chlorogenic acid and neochlorogenic acid have a greater binding affinity (-8.0 and -8.3 kcal/mol) than binding energy of epalrestat (a commercial AR
inhibitor) (7.9 kcal/mol). The interaction of chlorogenic acid and neochlorogenic acid at residues Phe122, Cys298 and Leu300 shows that both phenolic acids have selectivity for AR.

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